National Institute on Drug Abuse

Research Monograph Series 44

RAUS

RESEARCH ANALYSIS and UTILIZATION SYSTEM

Marijuana Effects on the Endocrine and Reproductive Systems

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

Marijuana Effects on the Endocrine and Reproductive Systems

Editors:

Monique C. Braude, Ph.D.

Jacqueline P. Ludford, M.S.
National Institute on Drug Abuse

NIDA Research Monograph 44

A RAUS Review Report

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

National Institute on Drug Abuse
5600 Fishers Lane
Rockville, Maryland 20857

NIDA Research Monographs are prepared by the research divisions of the National Institute on Drug Abuse and published by its Office of Science. The primary objective of the scenes is to provide critical reviews of research problem areas and techniques. the content of state of-the-art conferences, Integrative research reviews and signifigant original research Its dual publication emphasis is rapid and targeted dissemination to the scientific and professional community

Editorial Advisory Board

Avram Goldstein, M.D.
Addiction Research Foundation
Palo Alto. California

Jerome Jaffe, M.D.
University of Connecticut School of Medicine
Formington, Connecticut

Reese T. Jones, M.D.
Langley Porter Neuropsychiatric Institute
University of California
San Francisco, California

Jack Mendelson, M.D.
Alcohol once Drug Abuse Research Center
Harvard Medical School
McLeon Hospital
Belmont, Massachusetts

Helen Nowlis, Ph.D.
Rochester. New York

Lee Robins, Ph.D.
Washington University School of Medicine
St Louis, Missouri

NIDA Research Monograph Series

William Pollin, M.D.
DIRECTOR, NIDA
Jack Durell, M.D.
ASSOCIATE DIRECTOR, OFFICE OF SCIENCE, NIDA
EDITOR-IN-CHIEF
Eleanor W. Waldrop
MANAGING EDITOR

Parklawn Building, 5600 Fishers Lane Rockville Maryland 20857

Marijuana Effects on the Endocrine and Reproductive Systems

ACKNOWLEDGMENT

This monograph is based upon papers and discussion from the RAUS Review Conference on the Endocrine and Reproductive Effects of Marijuana, held March 1 and 2, 1983, in Rockville, Maryland. The conference was sponsored by the Office of Science and the Division of Preclinical Research, National Institute on Drug Abuse.

COPYRIGHT STATUS

The National Institute on Drug Abuse has obtained permission from the copyright holders to reproduce certain previously published material as noted in the text. Further reproduction of this material is prohibited without specific permission of the copyright holders. All other material in this volume except quoted passages from copyrighted sources is in the public domain and may be used or reproduced without permission from the Institute or the authors. Citation of the source is appreciated.

Opinions expressed in this volume are those of the authors and do not necessarily reflect the opinions or official policy of the National Institute on Drug Abuse or any other part of the U.S. Department of Health and Human Services.

The U.S. Government does not endorse or favor any specific commercial product or commodity. Trade or proprietary names appearing in this publication are used only because they are considered essential in the context of the studies reported herein.

Library of Congress catalog card number 83-,600600

DHHS publication number (HDM)84-1278
Printed 1984

NIDA Research Monographs are indexed in the Index Medicus. They are selectively included in the coverage of American Statistics Index, Biosciences Information Service, Chemical Abstracts, Current Contents, Psychological Abstracts, and Psychopharmacology Abstracts.

Preface

The Research Analysis and Utilization System (RAUS) is designed to serve four functions:

- Collect and systematically classify the findings of all intramural and extramural research supported by the hational institute on Drug Abuse (NIDA);
- Evaluate the findings in selected areas of particular interest and formulate a state-of-the-art review by a panel of scientific peers;
- Disseminate findings to researchers in the field and to administrators, planners, instructors, and other interested persons;
- Provide a feedback mechanism to NIDA staff and planners so that the administration and monitoring of the NIDA research program reflect the very latest knowledge gleaned from research in the field.

Since there is a limit to the number of reseach findings that can be intensively reviewed annually, four subject areas are chosen each year to undergo a thorough examination. Distinguished scientists in the selected field are provided with copies of reports from NIDA-funded research and invited to add any information derived from the literature and from their own research in order to formulate a comprehensive vick of the field. Each reviewer is charged with writing a state-of-the-art paper in his or her particular subject area. These papers, together with a summary of the discussions and recommendations which take place at the review meeting, make up a RAUS Review Report in the NIDA Research Monograph series.

The subject of the effects of marijuana on the endocrine and reproductive systems was chosen for a RAUS review in Fiscal Year 1983 because marijuana use is so widespread among American youth and, therefore, is of great programmatic importance to NIDA. Increased prevalence of marijuana use over the past decade has been accompanied by decreasing age of first use, and there is grave public health concern about its effects on youth who are undergoing maturation of their reproductive systems at about the same time as they are likely to begin using marijuana. Since there is a growing body of research on the subject, it became incumbent upon NIDA to gather the knowledge that was available, evaluate it, and disseminate it. The results of the RAUS review are presented in this monograph.

Dr. Monique C. Braude served as the scientific chairperson for the meeting . Jacqueline P. Ludford is the RAUS coordinator.

Contents

Preface v

Executive Summary
Jacqueline P. Ludford and Monique C. Braude 1

Effects of Cannabinoids on Gene Expression
Gary S. Stein and Janet L. Stein 5

Effects of Cannabis and Natural Cannabinoids on
Chromosomes and Ova
Akira Morishima 25

Endocrine Effects of Marijuana in the Male: Preclinical Studies
Jack Harclerode 46

Endocrine Aspects of Cannabinoid Action in Female Subprimates:
Search for Sites of Action
LeeTyrey 65

Acute, Short-Term, and Chronic Effects of Marijuana
on the Female Primate Reproductive Function
Carol Grace Smith and Ricardo H. Asch 82

Effects of Marijuana on Neuroendocrine Hormones
in Human Males and Females
Jack H. Mendelson and Nancy K. Mello 97

Effect of Marijuana on Pregnancy and Fetal Development
in the Human
Katherine Tennes 115

Discussion and Recommendations
Moniaue C. Braude 124

List of NIDA Research Monographs 130

Executive Summary

Jacqueline P. Ludford, M.S., and Monique C. Braude, Ph.D.

Isolated reports of impaired sexual behavior, lowered hormone levels, and abnormal offspring in animals after administration of marijuana or its active principles, such as delta-9-tetrahydrocannabinol (THC), prompted a review of current findings relevant to the effects of marijuana on genetics and reproduction. A RAUS review meeting was held on March 1-2, 1983, and reviewers were charged with evaluating the state of the art in the following areas:

o Effects of Cannabinoids on Gene Expression	Dr. Gary Stein, University of Florida
o Effects of Cannabis and Natural Cannabinoids on Chromosomes and Ova	Dr. Akira Morishima, Columbia University
o Effects of Marijuana in the Male: Preclinical Studies	Dr. Jack Harclerode, Bucknell University
o Endocrine Aspects of Cannabinoid Action in Female Subprimates: Search for Site of Action	Dr. Lee Tyrey, Duke University
o Acute, Short Term, and Chronic Effects on the Female Primate Reproductive Function	Dr. Carol Smith, Uniformed Services University of the Health Sciences
o Effects of Marijuana on Neuroendocrine Function in Human Males and Females	Dr. Jack Mendelson, McLean Hospital/ Harvard University
o Marijuana: Prenatal Exposure in the Human	Dr. Katherine Tennes, University of Colorado

Dr. Stein first discussed the importance of the preferential expression of specific genes which can be associated with modifications in the organization and/or representation of genetic sequences. To address regulation of eukaryotic genetic sequences, one must consider control at several cellular levels in the nucleus and cytoplasm. Gene expression encompasses an extensive range of cellular structures and biochemical processes starting in the nucleus with DNA and terminating with the RNA molecule. Dr. Stein then reviewed his studies on the effect of cannabinoids on the genome, and on gene expression. To assess more definitively the influences of cannabinoids on gene expression, Stein's group examined the effect of Delta-9-THC on the representation of RNA transcripts from two defined genetic sequences, histone genes and ribosomal genes, in several human cell lines. They found that THC causes a dose-dependent reduction in the cellular representation of histone mRNA sequences at the higher concentrations used in their assay. The extent to which cannabinoids affect the expression of specific genetic sequences other than histone sequences is still an open-ended question. Understanding the manner in which drug-induced alterations in gene expression are brought about should, Stein believes, provide insight into the molecular basis of cannabinoid-related modifications in cellular function.

Dr. Morishima reviewed the various reports on studies of the effects of marijuana and natural cannabinoids on chromosomes. The evidence from the available cytogenetic studies suggests that cannabis and cannabinoids are extremely weak clastogens, i.e., produce little chromosome breakage and that their clastogenic effects become apparent only in appropriately sensitive test systems such as primary spermatocytes and bone marrow cells, whereas the human lymphocyte system is relatively insensitive to their clastogenic effects. He then reported the results of his recent studies on the effects of THC on mice ova, showing that chronic administration of THC to sexually developing mice produced an increase in abnormal ova, although the percentage of increase was small. It appears that this increase in degenerated ova was caused by their inability to successfully undergo first cleavage division, probably affecting the process of meiosis. Following his review of the studies which reported errors of chromosome separation (ECS) and of this recent data, Morishima now proposes a new concept that cannabis and cannabinoids in vitro and in vivo act by disrupting the meiotic as well as the mitotic processes.

Dr. Harclerode focused on the effect of chronic exposure of laboratory animals to cannabinoids, with emphasis on the male reproductive system. The reports in the literature of reduction in reproductive organ weights are accompanied by reports that show that the auality and ouantity of sperm produced by the testis are affected by cannabinoids. Treatment of mice with THC for as little as 5 days resulted in reduction of sperm production and appearance of abnormal sperm. This was often accompanied by a decrease in testicle and seminal vesicle weights. Two gonadotropins, LH and FSH, secreted by the pituitary gland are of major importance to reproduction in the male. A single hypothalamic factor, the

gonadotropin-releasing hormone (GnRH) is believed to be responsible for the release of LH and FSH. THC induces a block of GnRH release which results in lowered LH and FSH, thus reducing testosterone production by the Leydig cells of the testis. Other hormones that might have a synergistic or antagonistic effect upon reproduction in the male are the adrenal cortical hormones, thyroid hormones, growth hormones, and prolactin. THC appears to depress prolactin, thyroid gland function, and growth hormone while elevating adrenal cortical steroids.

Dr. Tyrey reviewed the effects of cannabinoids, primarily THC, on the female reproductive function in subprimates and discussed cannabinoid action on the target organs (uterus and ovary) as well as on the CNS and the hypothalamic-pituitary axis. He concluded that the search for a site of cannabinoid action in subprimates has raised the possibility of cannabinoid effects at each level of the female reproductive system. He feels that the early suggestion that THC may have a direct "estrogen-like action on the uterus" has not been substantiated by later studies which failed to show that THC interacts with the estrogen cytoplasmic receptor. However, there is now evidence that THC alters the secretion of reproductive pituitary hormones (LH, prolactin) and of ACTH through effects in the brain.

Dr. Smith reviewed the acute and chronic effects of THC on the reproductive function of the female primate. She pointed out that studies in these species show that cannabinoids inhibit secretion of LH and FSH as well as prolactin. These changes in pituitary hormones produce decreases in sex steroid hormones and cause changes in ovulation. Dr. Smith also emphasized that the principal site of action of cannabinoids is the hypothalamus. Her recent findings show, however, that these cannabinoid effects are reversible in sexually mature animals when drug treatment is terminated and that there is development of tolerance to the effects of THC after chronic administration. This may explain why evidence of disrupting effects on female reproductive function has been scarce. She observed that, in humans, it is not yet known how much disruption of reproductive hormone levels is necessary for changes in human fertility and sexual function to become apparent, and she emphasized the need for clinical studies in female marijuana smokers.

There are technical problems in obtaining data on the effect of marijuana on human reproductive systems, but Dr. Mendelson reported on the available data and on his own considerable work in this area. In his study of the effects of marijuana on pituitary-gonadal hormones in human males, he found that the use of marijuana alone did not suppress testosterone levels. Similar carefully controlled studies of human female hormone levels are scarce, although reports from animals suggest, as mentioned above, that THC may produce a significant decrease in prolactin and LH. There have been reports that marijuana users had shorter menstrual cycles and lower prolactin levels than age-matched nonusers. A residential ward study in human females is now underway, and some preliminary observations were reported at the meeting.

Dr. Tennes reviewed the current knowledge about the effect of marijuana on human pregnancy and fetal development. Although 10% to 37% of pregnant women report use of marijuana, evidence regarding its effect is confounded by the use of other substances, nutrition, truthfulness of the woman's recall and report of the amount of use, changes in use during pregnancy and the trimester when these changes occurred, and a host of other technical problems. There is suggestive evidence that marijuana may alter the delivery process, reduce intrauterine weight gain by the fetus, or affect visual and neurological excitatory responses. All of these findings need to be confirmed in their relationship to marijuana use, especially since marijuana is freauently used in conjunction with tobacco and alcohol, which have their own deleterious effects on the fetus.

These data will be further discussed in the Discussion and Recommendations Section.

AUTHORS

Jacqueline P. Ludford, M.S.
Research Analysis Branch
Office of Science
National Institute on Drug Abuse
Rockville, Maryland 20857

Monique C. Braude, Ph.D.
Biomedical Branch
Division of Preclinical Research
National Institute on Drug Abuse
Rockville, Maryland 20857

Effects of Cannabinoids on Gene Expression

Gary S. Stein, Ph.D., and Janet L. Stein, Ph.D.

INTRODUCTION

In this article we will consider approaches that have been taken and can be taken to assess the influence of cannabinoids and other abused substances on the genome and on gene expression. This is a problem central to understanding drug-induced effects on a broad spectrum of biological processes since numerous modifications in cell structure and function, which have been reported to be associated with abused substances, either a) affect expression of genetic sequence or b) are a reflection of modifications in gene expression. Within this context we should emphasize that drug-induced perturbations in gene expression can result from alterations in the genome itself or from modifications in the transcription, processing, or translation of genetic information.

This article will be divided into three parts. First, by way of introduction, we will summarize the experimental basis for our current concepts of the eukaryotic genome and eukaryotic gene control. Second, we will review approaches that nave been taken to address the influence of cannabinoids on gene expression. We will then consider approaches, which can be taken and should be pursued, to further define in molecular terms cannabinoid-induced effects on the structure, organization, and regulation of specific genes.

It is our strong conviction that there are many long-standing and to date unresolved questions related to cannabinoid-induced effects on genes and gene control. Answers to these questions are essential to understand the influence of abused substances from the standpoints of immediate health hazards and, perhaps even more important, of hereditary effects. It is encouraging tnat during the past several years our understanding of genes and gene regulation in cells has evolved dramatically, largely through a number of highly innovative cellular and molecular approaches that have been taken to address the organization and regulation of eukaryotic genes. We are therefore now in a position, conceptually and technologically, to apply these approaches to assessing the effects of abused substances on the genome and on gene expression--particularly in human cells.

I. Genes and Gene Regulation

Several of the experimental observations which historically have served as the basis for our current concept of gene expression are summarized in table 1. While in general terms, these classical observations have a direct bearing on the manner in which eukaryotic genes are controlled, a number of subtle qualifications based on recent results provide explanations for long-standing inconsistencies in our understanding of eukaryotic gene regulation.

TABLE 1

Gene Expression in Eukaryotic Cells

1. All diploid cells in an organism contain the same amount of DNA.
2. All diploid cells contain identical genetic information.
3. Limited expression of genes in all cells.
4. Differences and similarities in expression of specific genes in differentiated cells.
5. Ability to modify expression of specific genes.

The initial experimental Observations which led to models for eukaryotic gene control were that all diploid cells of an organism contain the same amount of DNA and that the DNA sequences present in all diploid cells are identical. Equally important were the observations that all cells express only a limited number of genetic sequences and that those genes expressed reflect general metabolic requirements shared by all living cells as well as specialized requirements of differentiated cells. For example, almost all cells express genes encoding enzymes involved in intermediary metabolism while expression of globin genes is restricted to erythropoietic cells. Superimposed upon this preferential expression of specific genes, which permits cells to execute their specialized biological/biochemical functions, is the flexibility to permit variation in those genes expressed in response to modifications of cellular activities or cellular requirements. It was these observations that led to experimental pursuit of the mechanisms by which defined genetic sequences are selectively expressed while others are held in a nontranscribed structure, conformation, and transcriptional state. What we must now additionally take into consideration is that expression of genes can be associated with modifications in the organization and/or the representation of genetic sequences.

Our views of eukaryotic genes and eukaryotic gene regulation are constantly evolving. Structural and functional properties of genes are largely inseparable, as reflected by a functional relationship between the organization and expression of genetic sequences. The eukaryotic genome is a protein-DNA complex, both chromosomal proteins and DNA being essential for genome structure, and alterations in the interactions of chromosomal proteins with DNA in turn affect transcription or the transcriptional potential of specific genes. It is becoming increasingly apparent that the eukaryotic genome is not a static macromolecular complex, but rather is subject to modifications in organization, structure, and conformation which influence expression. There are different types of genes, those which encode proteins and those for which the products are ribosomal or transfer RNAs. Moreover, there are substantial differences in the organization of various genetic sequences, ranging in complexity from genes whose encoded proteins are represented by contiguous nucleotide sequences to genes from which the transcripts must undergo numerous splicing steps to generate functional messenger RNAs. It has been well documented that different genes are under different types of regulation. Likewise, there may be some differences in the structure and regulation of the same genes in different biological situations.

It therefore follows that to address regulation of eukaryotic genetic sequences it is necessary to consider control at several levels, which have been delineated in table 2. By definition gene expression encompasses an extensive range of cellular structures

TABLE 2

Regulation of Gene Expression

NUCLEUS	DNA	TRANSCRIPTION .Deletion-Addition .Rearrangement .Amplification .Methylation
	Nucleoplasm	TRANSCRIPT PROCESSING .Splicing .5' Capping .3" Polyadenylation .Methylation .RNA-Protein complexes
CYTOPLASM		TRANSPORT TO CYTOPLASM TRANSLATION POST-TRANSLATIONAL MODIFICATIONS

and biochemical processes, beginning in the nucleus at the DNA double helix and terminating with a completely processed and functional protein or RNA molecule. This presents a problem of an extremely complex nature, and cannabinoid-induced lesions may reside at any one or a combination of cellular levels.

Within the nucleus key steps in control of gene readout reside at the level of the genome and in the nucleoplasm. Cannabinoids may influence the structure and/or function of DNA nucleotide sequences which constitute structural genes or their components, in which case regions of the genome coding for defined proteins would not be transcribed or the transcripts would not be appropriately processed and translated into functional proteins. In addition, cannabinoid-induced alterations in genetic sequences coding for the synthesis of ribosomal RNAs, tRNAs, or purported "regulatory RNAs" must be considered. Cannabinoio-induced alterations may also become apparent in the nucleotides contained within regulatory sequences or within those sequences involved in punctuating the genetic code. In an overall evaluation of tne mechanisms by which cannabinoids may modify genes, one must bear in mind that there are four general categories of changes in the nucleotide bases which are prevalent--base substitutions, modifications of preexisting bases, base additions, and base deletions. Recent evidence for additions, deletions, and amplification of nucleotide sequences, as well as rearrangements of genetic sequences in conjunction with expression, necessitates serious consideration of quantitative and qualitative modifications in DNA as potential regulatory events, and hence targets for drug-induced perturbations in gene expression. Within this context drug-mediated effects on DNA methylation, which has been implicated in structural/transcriptional properties of genetic sequences, should not be overlooked.

In evaluating the implications of cannabinoid-associated DNA sequence modifications, one must critically determine the influence of these drugs on the capability of the cell to repair its DNA correctly. The repair process may itself introduce or amplify errors.

Cannabinoid-induced modifications in gene expression may also result from changes in macromolecules, principally chromosomal proteins, which interact with DNA and are intimately involved with the structural and transcriptional properties of the genome. Variations of these proteins and their mode of association with other genome components may be attributable to alterations in amino acid sequences as well as to post-translational modifications such as acetylation, methylation, phosphorylation, and ADP-ribosylation. It should be kept in mind that cannabinoid-induced changes in the metabolism of acetate, methyl, phosphate, and ADP-ribose groups may be caused by variations in genetically coded enzymes which are responsible for the addition and removal of these moieties from genome-associated proteins. In addition, some of these post-translational modifications of chromosomal proteins may occur, at least in part, by nonenzymatic mechanisms.

Another class of macromolecules which possess the ability to influence readout as as function of cannabinoid treatment are the RNA polymerases. Here, cannabinoid-induced changes may reside in any one or several of the polymerases, in any one or several of the subunits of the given polymerase, or in "factors" which influence the specificity or efficiency of the enzyme.

A complex system which contains numerous focal points for cannabinoid-induced lesions in the expression of genetic information is that which is utilized in the processing of RNA molecules. This is a multicomponent system consisting of: a) endo- and exonucleases which cleave and degrade ribonucleotide sequences during RNA precursor processing; b) enzymes modifying ribonucleotide bases; c) nucleotidyl exotransferases which utilize the 3' and 5' ends of RNA molecules as primers for addition of nontemplated ribonucleotides; and a) proteins which complex with RNAs or precursors thereof and are involved with enzymatic modifications of transcripts, export of transcripts from the nucleus to the cytoplasm, or assembly of functional translational complexes. Such processing occurs in the three principal classes of RNA molecules--ribosomal RNAs, messenger RNAs, and transfer RNAs. While these reactions generally occur in the nucleoplasm, they have also been reported to take place, to some extent, in the cytoplasm.

Cannabinoid-induced aberrations in gene expression may also result from perturbations in the equally complex cellular protein synthesizing and processing machinery which resides primarily in the cytoplasm. This may involve lesions in ribosomal and transfer RNAs, in ribosomal proteins, in the extensive range of "translational factors," and in enzymes involved in the assembly and/or activation of proteins. Enzymes involved with protein turnover constitute targets often overlooked when considering potentially important sites for cannabinoid-induced lesions in gene expression.

From the preceding discussion it should be apparent that cannabinoid-induced modifications in gene expression may result from perturbations in a broad spectrum of macromolecular, biosynthetic processes in the nucleus as well as in the cytoplasm. Any step in the elaboration and processing of genetic information is a potential target for a drug-induced lesion. Do cannabinoids modify the structure or composition of the genome? Do cannabinoids modify which genes are transcribed and which remain silent? Do cannabinoids affect the efficiency or fidelity of transcription? Are RNA processing steps modified by cannabinoids? Do these drugs act at the translational level? The key to addressing these questions is availability of high resolution procedures for detecting cannabinoid-induced changes in gene expression at various levels, and equally important, for determining if drug-induced perturbations in gene expression are functional or nonfunctional.

446-384 0 - 84 - 2

II. Effect of Cannaoinoids on the Genome

A. Composition of the Genome

The eukaryotic genome exists in the form of a protein-DNA complex (Stein et al. 1974, 1975); hence, an assessment of cannabinoid-induced effects on the composition of the genome requires evaluating the influence of cannabinoids on both DNA and chromosomal proteins. It is also necessary to consider the influence of cannabinoids on both chromatin and on chromosomes since these represent interchangeable modes of genome packaging.

Several laboratories have investigated the effects of cannabinoids on chromosome morphology and on the cellular representation of specific chromosomes. Yet, to date this remains an area where considerable controversy exists. The critical issues are whether cannabinoids exhibit clastogenic activity, that is, induce chromosome breaks, and/or whether cannabinoids act as mitotic poisons. The latter effect would imply drug-induced action, direct or indirect, on the mitotic apparatus or on the region of the chromosome where attachment of spindle fibers occurs--centromeric DNA or centromere-associated chromosomal proteins. The mutagenic nature of cannabinoid-induced chromosomal lesions also remains to be resolved. An indepth review of these chromosome-related effects of cannabinoids is covered in the chapter by Morishima in this volume.

An examination of the influence of cannabinoids on chromosomal proteins indicates that the relative composition of both histones and nonhistone chromosomal proteins is not significantly altered. However, psychoactive and nonpsychoactive cannabinoids appear to bring about a dose-dependent decrease in the synthesis of some chromosomal polypeptides (Mon et al. 1981a,b). These results tend to suggest that while cannabinoids do not affect the relative cellular levels of specific histones, which are the molecules primarily responsible for DNA packaging, these drugs may affect the ability of cells to express genes which code for histone proteins and/or affect histone protein turnover. Nonhistone chromosomal proteins, which are involved in structural, enzymatic, and regulatory action at the level of the genome, may be similarly affected following cannabinoid treatment. Variations observed in the extent to which chromosomal proteins are acetylated following cannabinoid treatment can be related to changes in the nature of chromosomal protein-DNA interaction, which may in turn reflect drug-induced modification in chromatin structure and/or in transcriptional properties of the genome. The studies carried out to date do indeed suggest possible drug-induced changes in genome composition, structure, and function but the data are of a correlative nature. Although as discussed above, cannabinoid-induced alterations in gene organization are not an unrealistic expectation, experimental data to substantiate or eliminate such a possibility are lacking.

B. Gene Expression

Two approaches have been undertaken in several laboratories, including ours, to study cannabinoid-induced effects on gene expression (Blevins and Regan 1976; Carchman et al. 1976a,b; Desoize et al. 1979; End et al. 1977; Green et al. 1983; Nahas et al. 1974a,b, 1977; Lemberger 1973; McClean and Zimmerman 1976; Mon et al. 1978, 1981a,b; Nahas and Desoize 1974; Nahas and Paton 1979; White et al. 1976; Zimmerman and McClean 1973; Zimerman and Zimmerman 1976; Zimmerman et al. 1979). Early *in vivo* studies suggested that cannaoinoid treatment brings about dose-dependent inhibition of ^{3}H-thymidine incorporation into DNA, ^{3}H-uridine incorporation into RNA, and ^{3}H-leucine incorporation into protein. However, these results, particularly the ^{3}H-uridine and ^{3}H-leucine results, are complicated by the influence of cannabinoids on the ribonucleotide and amino acid precursor pools--perhaps in part a reflection of cannabinoid-induced effects on cellular membranes. *In vitro* transcription stuaies carried out using isolated nuclei, DNA, or chromatin suggest that such preparations from untreated control and cannabinoid-treated cells do not differ significantly with respect to their ability to synthesize RNA. Interpretation of the latter studies is not complicated by drug-related effects on precursor pools; however, from these *in vitro* experiments it is possible to conclude only that the overall transcriptional capacity of the genome is refractory to cannabinoid treatment, and no indication of possible cannabinoid-induced effects on the qualitative nature of gene transcription can be gleaned. Furthermore, caution should be exercised in interpreting results from *in vitro* studies because the fidelity of the transcription process and the transcripts by necessity must be carefully evaluated.

Recently, to assess more definitively the influence of cannabinoids on gene expression, we examined the effect of Δ^9-THC on the representation of RNA transcripts from two defined genetic sequences, histone genes and ribosomal genes, in several human cell lines. Levels of cellular histone mRNAs and ribosomal RNAs were assayed by hybridization with cloned genomic human histone and ribosomal genes under conditions where quantitation was not influenced by nucleotide precursor pools. Our results suggest that Δ^9-THC causes a dose-dependent reduction in the cellular representation of histone mRNA sequences. This drug-induced reduction is at least to some extent selective because cellular levels of ribosomal RNAs are not affected. We have also observed that the cannabinoid-induced effect on histone gene expression is less pronounced in human cells with active drug-metabolizing systems.

Human histone and ribosomal genes represent two distinct types of genetic sequences which differ with respect to their organization, regulation, and functions. Human nistone genes are a family of moderately reiterated genetic sequences--approximately 40 copies per haploid genome. Each histone mRNA is transcribed from a set of contiguous nucleotide sequences (unspliced), and histone gene

expression is related to cell proliferation. The gene products, the histone proteins, are required for packaging several yaras of DNA into "nucleosomes" where they are contained in a nucleus only several microns in diameter. These histone proteins are necessary for genome replication (to package newly replicated DNA) and additionally play a role in the control of gene expression. The human ribosomal genes are represented as a reiterated set of sequences and the final gene products are the major structural RNA species associated with large and small ribosomal subunits. In contrast to the histone genes, where the primary transcripts undergo a minimal amount of processing, the 5.8S, 18S, and 28S ribosomal RNAs are derived from a 45S precursor via a series of post-transcriptional cleavages.

Initially, the steady state levels of histone mRNAs were determined in exponentially growing human cervical carcinoma cells, HeLa S3 cells, following treatment with increasing concentrations of Δ^9-THC. Total cellular RNAs were fractionated electrophoretically in 1.5% agarose gels (Rave et al. 1979), transferred to nitrocellulose (Southern 1975) and hybridized with ^{32}P-labeled [nick-translated (Maniatis et al. 1975)] cloned genomic human histone sequences (Sierra et al. 1982). The levels of histone mRNAs were then assayed autoradiographically.

Isolation of total celluar RNA permits greater than 90% recovery, circumventing loss of RNA through nuclease activity and physical manipulations which generally occur during subcellular fractionation. Because the hybridization probe is radiolabeled *in vitro* rather than the cellular RNAs *in vivo*, quantitation of RNAs is not complicated by the intracellular ribonucleotide precursor pools. RNA samples are quantitated spectrophotometrically prior to electrophoretic fractionation and the extent of transfer to nitrocellulose is monitored by ethidium bromide staining and/or ultraviolet shadowing prior to and following diffusion transfer. The efficiency of transfer to nitrocellulose by the procedure used in these experiments has been monitored by transfer of ^{32}P-labeled DNA and shown to be greater than 95%.

The data in figures 1 and 2 clearly indicate that Δ^9-THC brings about a dose-dependent decrease in the representation of mRNAs for the four core histone proteins, H2A, H2B, H3, and H4. Shown in figure 1A is a hybridization signal obtained when 50 μg of nitrocellulose-immobilized, total cellular HeLa cell RNAs from control, and Δ^9-THC treated, cells are hybridized with a cloned human DNA sequence (pFF435) encoding H2A, H2B, and H3 histone mRNAs. While the levels of H2A, H2B, an H3 histone mRNAs isolated from cells treated with 10 μM Δ^9-THC are not below those from nondrug-treated or vehicle-treated controls, a marked inhibition (greater than 80%--see table 3) is observed in cells treated with 30 μM and 40 μM drug concentrations. Verification that equivalent amounts of all' RNA samples were fractionated can be gleaned from figure 1B which shows similar levels of ethidium bromide staining of all RNAs and from figure 1C which shows similar levels of all RNAs by ultraviolet shadowing. It should be

TABLE 3

Effect of ^{9}-THC on Cellular Levels
of Human (HeLa) Histone mRNAs

Treatment	Drug Conc.	% Inhibition
Δ^9-THC	10 μM	0.0
Δ^9-THC	30 μM	78.1
Δ^9-THC	40 μM	81.0
Vehicle Control	0	0.0
Control	0	0.0

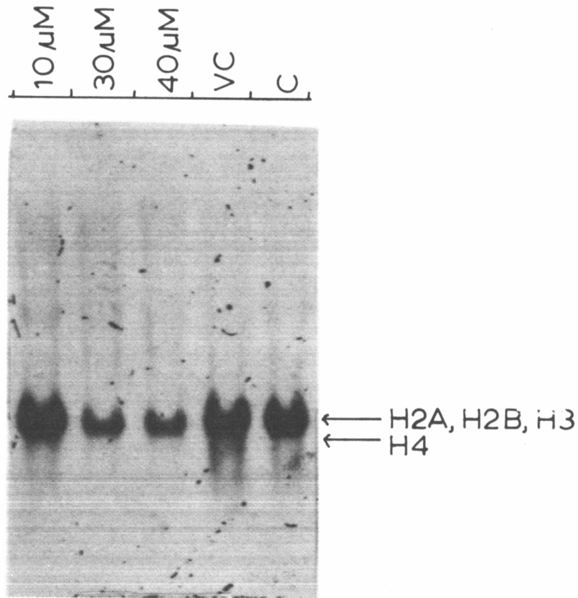


FIGURE 1A

A) Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of mRNAs for three of the four core histone proteins, H2A, H2B, and H3. The signals shown were obtained when 50 μg of electrophoretically fractionated nitrocellulose-imobilized total cellular HeLa cell RNAs were hybridized to a cloned human DNA sequence (FF435) encoding H2A, H2B, and H3 histone mRNAs.

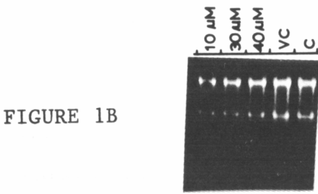
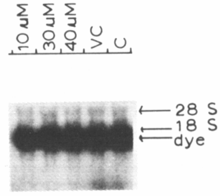


FIGURE 1B

FIGURE 1C



B.) Ethidium bromide stain of 1.5% (w/v) agarose gel with 6% (w/v) formaldehyde, containing 10 μg of each of the Δ⁹-THC treated and control samples of total cellular RNAs from HeLa cells. The gel was stained for one hour in 0.1 M anmonium acetate containing 0.1 μg/ml ethidium bromide and destained overnight in water. The gel was placed on a shortwave ultraviolet transilluminator and photographed with Polaroid type 57 film using an orange filter.

C) Ultraviolet shadowing of 1.% (w/v) agarose gel with 6% (w/v) formaldehyde, containing 50 μg of each of the Δ⁹-THC-treated and control samples of total cellular RNAs from HeLa cells. The gel was placed on a cellulose-fluorescent thin layer chromatography plate and illuminated from above by shortwave ultmviolet ligkt. The gel was photographed with polaroid type 57 film using an orange filter.

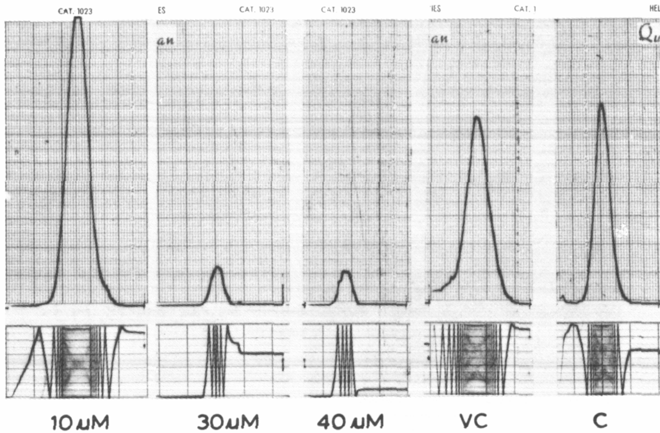


FIGURE 1D

D) Densitometric scan of autoradiographic hybridization signals obtained when 50 μg of electrophoretically fractionated nitrocellulose immobilized total cellular RNAs from HeLa cells treated with varying concentrations of Δ⁹-THC were hybridized to a cloned human DNA sequence (pFF435) encoding H2A, H2B, and H3 histones. The top portion of the scan meusures the absorbance of the signal which is determined electronically within the densitometer based on the measured optical density. The lower portion is the Zig-Zag time base integrator and is used to quantitate the area under the curve and thus, the concentration of the sample.

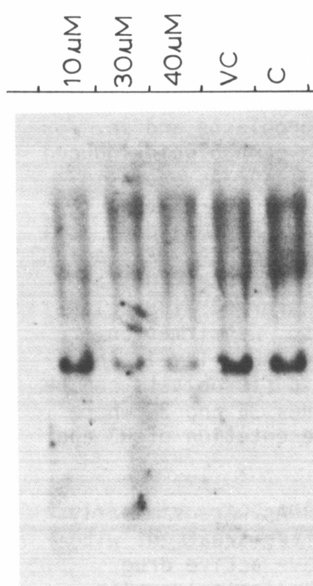


FIGURE 2A

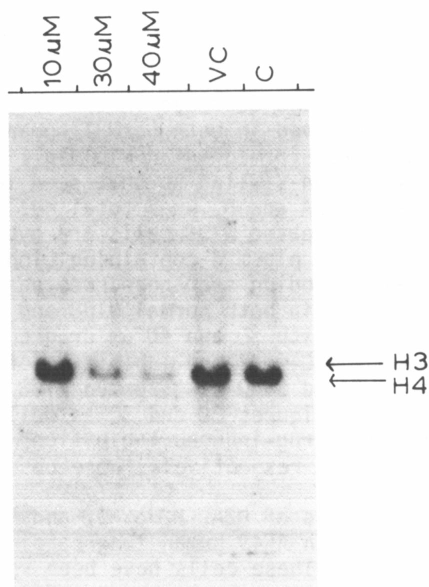


FIGURE 2B

Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ9-THC on the representation of mRNAs for histones H3 and H4. The signals shown were obtained when 5 0 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular HeLa cell RNAs were hybridized to cloned hwnan DNA sequences encoding: A) H3 histone (pF0422) and B) H4 histone (pF0108A).

noted that because equivalent amounts of RNA from control and drug-treated cells were analyzed, the data in figure 1A reflect a dose-dependent, Δ9-THC-mediated inhibition in the relative representation of three core histone mRNA species. A dose-dependent inhibition of the absolute amounts of H2A, H2B, and H3 histone mRNA/cell, with pronounced inhibition evident at 30 and 40 uM drug concentrations, was also observed when equivalent aliquots (by volume) of RNA extracts from equivalent numbers of control and Δ9-THC-treated cells were similarly analyzed (see figure 1D). The data in figure 2 are results from experiments in which total cellular RNAs from control and Δ9-THC-treated exponentially growing HeLa S3 cells were analyzed by hybriaization with cloned genomic H3 (pFO 422) (figure 2A) or H4 (pFO 108A) (figure 2B) histone sequences. Consistent with the results shown in figure 1, a greater than 80% inhibition in the representation of H3 and H4 histone mRNAs was observed following treatment with 30 and 40 μM drug concentrations.

The influence of Δ^9-THC on the levels of histone mRNAs was then studied in normal human diploid cells (WI38 human diploid fibroblasts) and in SV40-transformed WI38 cells. A dose-dependent, drug-induced decrease in the levels of all four core nistone mRNAs was observed in both normal human diploid fibroblasts and in SV40-transformed human diploid fibroblasts--a cannabinoid-induced inhibition similar to that seen in HeLa S3 cells. As shown in figures 3A and B, when total cellular RNAs from control and Ag-THC-treated WI38 cells are hybriaized with ^{32}p-labeled pFF435, a plasmid containing cloned human genomic H2A, H2B, and H3 histone coding sequences, decreased levels of histone mRNAs are observed in both normal WI38 and in SV40-transformed WI38 cells treated with 30 and 40 µM drug concentrations. Confirmation of the Δ^9-THC-mediated inhibition of core histone mRNA levels in normal and SV40-transformed WI38 human diploid fibroblasts can be seen in figures 3C and 3D as well as in figures 3E and 3F where similar drug-induced inhibitions in the representation of H3 and H4 mRNAs, respectively, were observed.

The levels of H2A, H2B, H3, and H4 histone mRNAs were similarly assayed in A549 human lung carcinoma cells after treatment with A^9-THC. These cells have been reported to have active drug metabolizing systems and to efficiently metabolize polycyclic hydrocarbon-containing carcinogens. A pronounced decrease in the inhibitory effect of Δ^9-THC on the representation of core histone mRNAs was observed in A549 cells compared With HeLa S3 cells and WI38 cells (normal and SV40-transformed). It is unlikely that the reduced sensitivity of A549 cells to cannabinoid treatment is attributable to changes in drug uptake. Tne intracellular levels of Δ^9-THC in SV40-transformed WI38 cells and in A549 cells, when monitored by intracellular incorporation of ^{3}H-Δ^9-THC (table 4) do not reflect the differences seen in histone mRNA levels (figures 3 and 4).

TABLE 4

Cellular Uptake and Subcellular Distribution of ^{3}H-Δ^9-THC

cell type	cpm/10^7 cells	% nucleus	% cytoplasm
SV-40-WI-38	1.2×10^5	32.6%	67.4%
A549	1.3×10^5	37.9%	62.1%

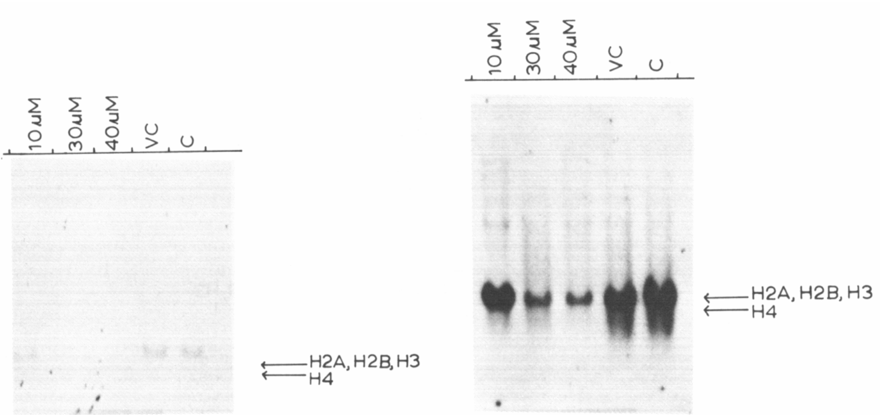


FIGURE 3A

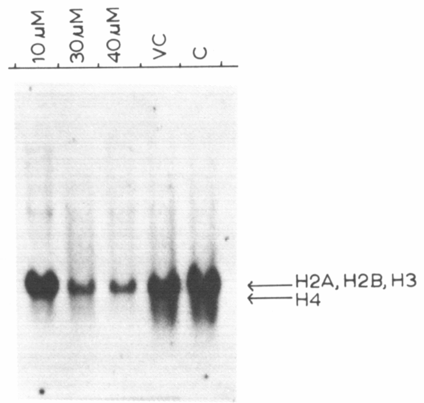


FIGURE 3B

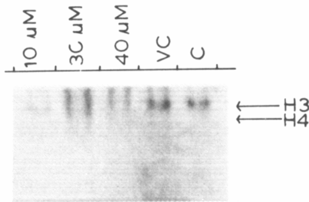


FIGURE 3C

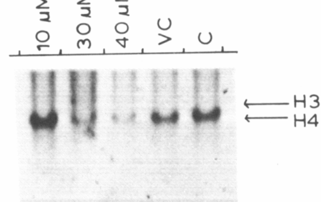


FIGURE 3D

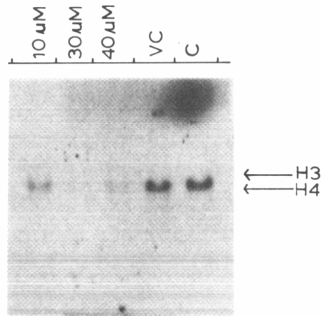


FIGURE 3E

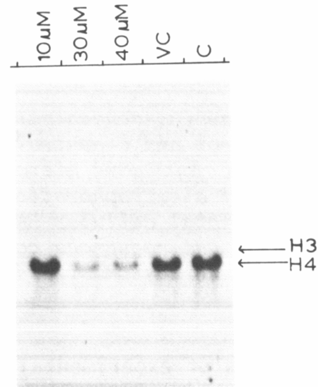


FIGURE 3F

Effects of varying concentrations (10 µM, 30 µM, 40 µM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of mRNAs for the four core histones. The Signals shown were obtained when 50 µg of electrophoretically fractionated nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. A) WI38 and B) SV40-WI38 total cellular RNA hybridized to a DNA probe (pFF435) encoding H2A, H2B and H3 histones; C) WI38 and D) SV40-WI38 total cellular RNA hybridized to a DNA probe (pFO422) encoding H3 histone; E) WI38 and F) SV40-WI38 total cellular RNA hybridized to a DNA probe (pF0108A) encoding H4 histone.

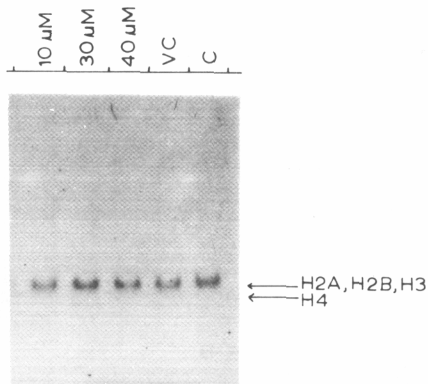


FIGURE 4A

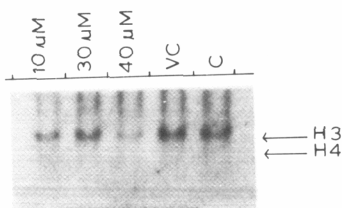


FIGURE 4B

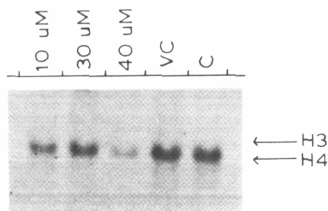


FIGURE 4C

Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ9-THC on the representation of mRNAs for the four core histones. The signals shown were obtained when 50 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs from A549 cells were hybridized to cloned human DNA sequences coding for: A) H2A, H2B, and H3 histones (pFF435); B) H3 histone (pFF422); C) H4 histone (pF0108A).

Several lines of experimental evidence suggest that the Δ9-THC-induced reductions in histone mRNA levels we have observed in normal and transformed human cells are not merely a reflection of a general, nonspecific cannabinoid-induced inhibition in RNA synthesis. As reported previously, the cannabinoid-induced inhibition of ^{3}H-uridine incorporation into total cellular RNAs largely reflects a drug-induced influence on the intracellular nucleotide precursor pool rather than an effect on cellular RNA metabolism (Mon et al. 1981a,b). The absence of a significant quantitative effect of psychoactive and nonpsychoactive cannabinoids on levels of nuclear (Mon et al. 1981a,b) or chromatin (Mon et al. 1981a,b) transcription in vitro further suggests that these drugs do not interfere with the general levels or rates of cellular RNA synthesis.

The inability of Δ^9-THC, at concentrations between 10 and 40 μM, to modify the levels of ribosomal RNAs provides more direct evidence for some extent of specificity to the cannabinoid-mediated decrease in histone mRNA levels. In all experiments reported in this paper, the representation of 18S and 28S ribosomal RNAs was monitored in control, and in Δ^9-THC-treated, cells by staining gels with ethidium bromide and by ultraviolet shadowing. A typical example of a gel showing the levels of the major ribosomal RNAs in control and in drug-treated cells is shown in figures 1B and 1C. Additionally, when electrophoretically fractionated cellular RNAs from control and Δ^9-THC-treated cells were hybridized with ^{32}P-labeled cloned human 18S (LS-2) and 28s (LS-6) ribosomal RNA coding sequences, a dose-dependent decrease in the representation of these RNAs was not observed. Figures 5A and 5B show no change in the levels of 28S ribosomal RNAs from Δ^9-THC-treated HeLa and SV40-transformed WI38 cells in the same RNA samples where greater than 80% reduction was observed for the representation of core histone mRNAs in treated cells. Figure 5C shows unchanged levels of 18S ribosomal RNA in these same cells following hybridization with ^{32}P-labeled human 18s ribosomal DNA.

A long-standing question has been whether cannabinoids influence the expression of specific genetic sequences. While cannabinoid-induced effects on cell structure and function, coupled with cannabinoid-mediated modification in macromolecular biosynthesis, are consistent with such a contention, direct experimental evidence for an effect of cannabinoids on expression of specific genes has to date not been reported. In this paper we present data which indicate that treatment of exponentially growing normal diploid and transformed human cells with Ag-THC results in a dose-dependent decrease in the representation of histone mRNAs, with a decreased sensitivity of cells with highly developed drug metabolizing systems. This cannabinoid-mediated reduction of cellular histone mRNA levels does not simply reflect a general decrease in cellular mRNA levels or in cellular RNA metabolism. We also present data indicating that the levels of ribosomal RNAs are not altered by the concentrations of Δ^9-THC used in our studies, and we have reported previously that general levels of <u>in vitro</u> and <u>in vivo</u> RNA synthesis are not quantitatively affected by either psychoactive or nonpsychoactive cannabinoids.

While our results clearly indicate that Δ^9-THC preferentially inhibits expression of histone genes, the levels at which regulation is perturbed and the biological implications of this cannabinoid-mediated effect remain to be resolved. The reduction in cellular levels of histone mRNAs after cannabinoid treatment may be attributable to alterations in mRNA stability, transcription, or processing of histone transcripts. Additionally, drug-induced structural modifications in the histone genes and in their flanking regulatory sequences should also be considered within this context. By analogy with other moderately reiterated eukaryotic sequences which have been shown to unaergo structural modifications in conjunction with phenotypic changes, cannabinoid-induced effects on the structural features of human histone genes

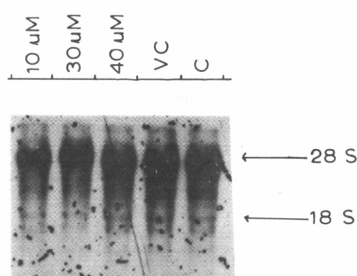


FIGURE 5A

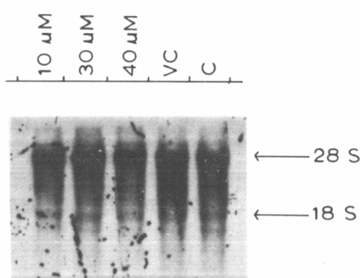


FIGURE 5B

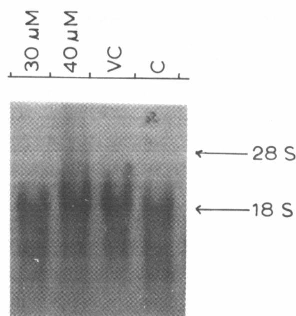


FIGURE 5C

Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of 28S and 18S ribosomal RNAs. The signals shown were obtained when 10 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. A) HeLa and B) SV40-WI38 total cellular RNA hybridized to a DNA probe (LS-6) encoding 28S RNA; C) HeLa total cellular RNA hybridized to a DNA probe (LS-2) encoding 18S RNA.

could be a possibility. The extent to which the expression of specific genetic sequences other than histone sequences is affected by cannabinoids is also an open-ended question--one which is particularly important because the organization and regulation of the moderately reiterated human histone genes differ considerably from those of the more complex spliced single copy genes.

From a biological standpoint the selective effect of Δ^9-THC on expression of histone genes may be understandable. Expression of histone genes has been shown to be temporally and functionally coupled with DNA replication (Stein et al. 1979; Stein and Borun 1972; Wu and Bonner 1981), and cannabinoids have been shown to bring about a dose-dependent inhibition in cell proliferation (Blevins and Regan 1976; Carchman et al. 1976a,b; Desoize et al. 1979; End et al. 1977; Green et al. 1983; Nahas et al. 1974a,b, 1977; Lemberger 1973; McClean and Zimmerman 1976; Mon et al. 1978, 1981a,b; Nahas and Desoize 1974; Nahas and Paton 1979; White et

al. 1976; Zimmerman and McClean 1973; Zimmerman and Zimmerman 1976; Zimmerman et al. 1979). In fact, in the normal and transformed cell lines we have examined, the extent to which histone mRNA levels are affected by Δ^9-THC is paralleled by the extent to which proliferative activity is affected by cannabinoids. It remains to be determined whether expression of other genetic sequences, whose expression is prerequisite for DNA replication or mitotic division, are preferentially inhibited by cannabinoids. Equally important is whether the cannabinoid-mediated modifications in cellular histone mRNA levels are attributable to a direct effect on the histone genes or the transcripts, or alternatively, whether the effects of cannabinoids on histone gene expression are indirect, e.g., acting initially on other genetic sequences or cellular macromolecules.

III. Approaches to Defining Effects of Cannabinoids on Specific Genes

Cannabinoid-induced modifications in cell structure and function have been well-documented as have a series of physiological effects resulting from such drug-induced cellular changes. Two pivotal biological processes which have been shown to be dramatically influenced by cannabinoids are endocrine function and cell proliferation--both of which have been reviewed in this monograph. Moreover, these are not unrelated processes since in many cases proliferation is responsive to hormonal control. Understanding the manner in which drug-induced alterations in gene expression are brought about should provide insight into the molecular basis of cannabinoid-related modifications in cellular function.

Since cannabinoid-induced alterations in gene expression can result from changes in the organization of genetic sequences and/or in the manner in which genetic information is transcribed and processed, a critical and systematic evaluation of the influence of cannabinoids on the structure and expression of specific genetic sequences, particularly in human cells, should be a high priority. Understanding cannabinoid-induced effects on human gene organization and expression is prerequisite to evaluating possible short-term, long-term, and hereditable disorders that may arise from the use of these drugs either therapeutically or as abused substances. Of equal importance, despite the history of fragmentary and often controversial reports of cannabinoid-induced modification in genome-related phenomena (e.g., chromosomal changes, alterations in RNA synthesis, etc.), we are now in a position to address these issues directly and definitively. Availability of a series of cloned human genes permits evaluation of drug-related effects on specific genes, on defined regions of genes, and on transcription and processing of genetic information. Examples of ways in which cloned genetic sequences can be utilized as high resolution probes for the identification and quantitation of several specific human gene transcripts were presented in the previous section of this chapter.

It will be instructive to focus efforts where possible on human studies; for example, drug-induced effects on the organization of specific genetic sequences or regions thereof can be performed using DNA from only 20 ml of blood. Thereby the opportunity is available to determine the effects of cannabinoias on the genomes of subjects participating in endocrine function and behavior studies. A number of normal and tumor-derived human cell lines are available and should be utilized to complement such an approach. By combining both intact organism and cell culture approaches, it is possible to draw on the physiological reality of the organism and the biochemical simplicity of isolated cells. It would also be appropriate to concentrate efforts on evaluating drug-induced effects on a limited series of genetic sequences, those related to proliferation and endocrine function, where phenotypic effects are well understood and the information obtained can be integrated with other ongoing investigations.

In summary, we have attempted to discuss a series of high resolution approaches and procedures which can provide important information regarding the influence of cannabinoids on genome structure and function. Examples of the applications of several of these approaches and procedures have been presented in an attempt to document the feasibility of their implementation. We are highly optimistic that in the next few years our understanding of the genetic effects of cannabinoids at the cellular and molecular level will be significantly enhanced. These same approaches can be implemented for assaying the influence of unfractionated marijuana extracts, psychoactive and nonpsychoactive components of marijuana, natural and synthetic cannabinoids, and cannabinoid metabolites as well as other abused substances, individually or in conjunction with cannabinoids.

REFERENCES

Blevins, R.D., and Regan, J.D. Δ^9-tetrahydrocannabinol: Effect of macromolecular synthesis in human and other mammalian cells. In: Nahas, G.G., ed. Marihuana: Chemistry, Biochemistry and Cellular Effects. New York: Springer, 1976 p. 213.

Carchman, R.A.; Harris, L.S.; and Munson, A.E. The inhibition of DNA synthesis by cannabinoids. Cancer Research 36:95, 1976a.

Carchman, R.A.; Warner, W.; White, A.C.; and Harris, L.S. Cannabinoids and neoplastic growth. In: Nahas, G.G., ea. Marihuana: Chemistry, Biochemistry and Cellular Effects. New York: Springer, 1976b, p. 329.

Desoize, B.; Leger, C.; and Nahas, G. Plasma membrane inhioition of macromolecular precursor transport by THC. Biochem Pharmac 28:1113, 1979.

End, D.W.; Thoursen, K.; Dewey, W.L.; and Carchman, R.A. A comparative study of the disposition of (-)-Δ^9-tetrahydrocannabinol in neuroblastoma and glioma cells in tissue culture: Relation to cellular impairment. Molec Pharmacol 13:864, 1977.

Green, L.; Marashi, F.; Stein, J.L.; and Stein, G.S. A decreased influence of cannabinoids on macromolecular biosynthesis and cell proliferation in human cells which metabolize polycyclic hydrocarbon carcinogens. Anticancer Research, in press, 1983.
Lemberger, L. Tetrahydrocannabinol metabolism in man. Drug Metabolism and Disposition 1:461, 1973.
Maniatis, T.; Jeffrey, A.; and Kleid, D.G. Nucleotide sequence of the rightward operator of phage. Proc Natl Acad Sci USA 72:1184-1188, 1975.
McClean, D.K., and Zimmerman, A.M. Action of Δ^9-tetrahydro-cannabinol on cell division and macromolecular synthesis in division-synchronized protozoa. Pharmacology 14:307-321, 1976.
Mon, M.J.; Haas, A.E.; Stein, J.L.; and Stein, G.S. Influence of psychoactive and nonpsychoactive cannabinoids on cell proliferation and macromolecular biosynthesis in human cells. Biochem Pharmacol 30:31, 1981a.
Mon, M.J.; Haas, A.E.; Stein, J.L.; and Stein, G.S. Influence of psychoactive and nonpsychoactive-cannabinoids on cnromatin structure and function in human cells. Biochem Pharmacol 30-45 1981b.
Mon, M.J.; Jansing, R.L.; Doggett, S.; Stein, J.L.; and Stein, G.S. Influence of Δ^9-tetrahydrocannabinol on cell proliferation and macromolecular biosynthesis in human cells. Biochem Pharmacol 27:1759-1765, 1978.
Nahas, G.G., and Desoize, B. Effect inhibiteur du 5 n-amyl-resorcinol sur la transformation lymphoblastique. C R Acad Sci Paris Series D 279:1607-1608, 1974.
Nahas, G.C., and Paton, W.D.M. Marihuana: Biological Effects. Oxford: Pergamon Press, 1979.
Nahas, G.G.; Armand, J.P.; and Hsu, J. Inhibition in vitro de la blastogenese des lymphocytes T par le Δ^9-tetrahydrocannabinol. C R Acad Sci Paris Series D 278:679, 1974a.
Nahas, G.G.; Desoize, B.; Armand, J.P.; Hsu. J.; and Morishima, A. Inhibition in vitro de-la transformation iymphocytaire par divers cannaibinoids naturels. C R Acad Sci Paris Series D 279:785-787, 1974b.
Nahas, G.G.; Morishima, A.; and Desoize, B. Effects of cannabi-noids of macromolecular synthesis and replication of cultured lymphocytes. Fed Proc 36:1748-1752, 1977.
Rave, N.; Crkvenjakov, R.; and Boedtker, H. Identification of procollagen mRNAs transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. Nucleic Acids Res 6:3559-3567, 1979.
Sierra, F.; Lichtler, A.; Marashi, F.; Rickles, R.; Van Dyke, T.; Clark, S.; Wells, J.; Stein, G.; and Stein, J. Organization of human histone genes. Proc Natl Acad Sci USA 79:1795, 1982.
Southern, E.M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Jour Mol Biol 98:503, 1975.
Stein, G.S., and Borun, T.W. The synthesis of acidic chromosomal proteins during the cell cycle of HeLa S3 cells. I. The accelerated accumulation of acidic residual nuclear protein before the initiation of DNA replication. Jour Cell Biol 52:292, 1972.

Stein, G.S.; Hochhauser, S.; and Stein, J.L. Histone genes: Their structure and control. In: Busch, H.; ed. The Cell Nucleus. Chromatin, Part D. New York: Academic Press 1979, p. 259
Stein, G.S.; Spelsberg, T.C.; and Kleinsmith, L.J. Nonhistone chromosomal proteins and gene regulation. Science 183:817, 1974.
Stein, G.S.; Stein, J.L.; and Kleinsmith, L.J. Chromosomal proteins and gene regulation. Scientific Amer 232.46, 1575.
White, A.C.; Munson, J.A.; Munson, A.E.; and Carchman, R.A. Effects of Δ^9-tetrahydrocannabinol in lewis lung adenocarcinoma cells in tissue culture. Jour Natl Cancer Inst 56:6556, 1976.
Wu, R.S., and Bonner, W.M. Separation of salistone syntnesis from S-phase histone synthesis in dividing cells. Cell 27:321-331, 1981.
Zimmerman, A.M., and McClean, D.K. Action of narcotic and hallucinogenic agents on the cell cycle. In: Zimmerman. A.M.: Padilla, G.M.; and Cameron, I.L., eds. Drugs and the Cell Cycle New York: Academic press, 1973, pp. 67-94.
Zimmerman, A.M., and Zimmerman, S.B. The influence of marihuana on eukaryote cell growth and development. In: Nahas, G.G.. ed. Marihuana: Chemistry, Biochemistry and Cellular Effects. New York: Springer, 1976. pp. 195-205.
Zimmerman, A.M.; Bruce, W.R.; and Zimmerman, S. Effects of cannabinoids on sperm morphology. Pharmacology 18(3):143, 1979.

ACKNOWLEDGMENTS

These studies were supported by research grants DA-01188 and DA-02033 from the National Institute on Drug Abuse. We are indebted to Drs. Roy Schmickel and Golder Wilson for providing us with cloned human ribosomal genes.

AUTHORS

Gary S. Stein, Ph.D.
Department of Biochemistry and Molecular Biology
University of Florida College of Medicine
Gainesville, Florida 32610

Janet L. Stein, Ph.D.
Department of Immunology and Medical Microbiology
University of Florida College of Medicine
Gainesville, Florida 32610

Effects of Cannabis and Natural Cannabinoids on Chromosomes and Ova

Akira Morishima, M.D., Ph.D.

EFFECTS OF CANNABIS AND NATURAL CANNABINOIDS ON CHROMOSOMES

Introduction

During the past dozen years, a number of cytogenetic investigations on the effects of cannabis and natural cannabinoids have been reported in the literature. These studies have yielded contradictory results, leading to a controversy as to whether or not these agents are capable of inducing chromosonal abnormalities and, therefore, whether marijuana abuse carries any risk of producing chromosome damage. As recently as 1980, Zimmerman and Yesoda Raj (1980, p. 258) stated, in discussing their cytogenetic observations, "Although the current investigation further supports the concept of a detrimental action of cannabinoids on somatic cells, there remains some doubt concerning a direct action of THC on chromosomes." The purpose of this paper is to review these published studies in an attempt to understand better the basis from which this controversy arose.

An environmental agent, including chemical substances, capable of modifying the structure of chromosomes or the numbers of chromosomes of a normal nuclear complement falls into the broad definition of a chromosomal mutagen. However, the majority of investigations on chromosomal mutagens during the past decade, have focused on induction of alterations in the chromsomal structure, mainly chromosome breaks. This was largely due to the initial finding on lysergic acid diethylamide (LSD-25) which was reported to induce breaks in chromosomes of human lymphocytes (Cohen et al. 1967). A number of chemical agents which can induce chromosome breakages have been identified. These clastogens, e.g., mitomycin C (Cohen and Shaw 1964, Adler 1976), generally induce a marked increase in the incidence of chromatic and isochromatid breaks.

The standard technique for examining chromosome breaks utilizes a rapidly proliferating tissue, such as culture of peripheral lymphocytes or fibroblasts, or bone marrow aspirates. The specimens are usually, but not always, exposed to a mitotic arrestant to increase the number of observable metaphases. The cells are then exposed to

446-384 O - 84 - 3

a hypotonic solution in order to disperse the chromosomes so that the overlaps are minimizd when chromosomes are examined microscopically. Although these preparations yield suitable specimens for morphological observation of individual chromosanes, the use of both the mitotic arrestant and hypotonic solution disrupts the natural spacial relationship of chromosomes. Thus, these preparations cannot be used to examine the segregational process of chromosomes. Further, because the technique tends to induce metaphase plates from which some chromosomes are lost due to an excessive dispersion, metaphases are usually scanned under a low-power mangnification in order to choose only those plates which appear to contain the full complement of well spread chromosomes before they are subjected to a detailed analysis under a high-power microscopic observation. A trained observer, using the standard procedure, is usually capable of discriminating those metaphase plates containing several chromosomes less than the modal number, under a low-power magnification. Thus, the usual method for observation of chromosome breakage precludes detection of hypoploid metaphases with a greatly reduced number of chromosomes.

Other methods used to examine clastcgenic substances include the dominant lethal method (Rohrborn 1970), examination of sister chromatid exchanges (Kato 1974) and of unscheduled DNA repair synthesis (Stich et al. 1971). Although these tests may detect events related to chromosome breakage, there is no proof that the results can be correlated directly with the clastogenic effect of an agent. Further, few studies on marijuana have utilized these methods of investigation (Legator et al. 1976, Zimmerman et al. 1978).

Clastogens may affect chranosanes at various stages of a cell cycle (Röhrborn 1970). However, the vast majority of induced chromosome breaks should be repaired at the original breaking point leaving no detectable abnormality at metaphase of the succeeding cell cycle. Chromatid and isochromatid breaks probably represent breakage which occurred during the cell cycle in which the metaphase is observed. Only occasional breaks may result in non-repair or simultaneous cooperative repair between two or more breaking points, involving one or more chromosomes, resulting in structural rearrangements (Evans et al. 1967). These events would be detectable at a subsequent metaphase, provided that the cell bearing such an abnormality is capable of surviving and undergoing the mitotic process. Thus, detection of even a relatively low incidence of chromosomal rearrangements would imply a much greater clastogenic effect of an agent. Morphologically observable structural rearrangements of chromosomes include fragments, chromatid exchanges between two chromosomes, chain and ring formations, translocations, deletions, and inversions.

Some chromosomes mutagens are known to have little or no clastogenic effect, but principally act by disrupting the normal mitotic process. Thus, there are at least two classes of chemical mutagens. When HeLa cells were exposed to nitrous oxide, formation of microtubules became anomalous and the mitoses were arrested at metaphase (Brinkley and Rao 1973). Olivetol, 5-n-amylresorcinol, which has a molecular structure of the C-ring common to all cannabinoids, was

found to induce marked hypoploidy and abnormal chromosomal segregation in human lymphocytes when added to the culture medium (Morishirra et al. 1976a, 1976b). Halogenated inhalation anesthetics (Sturrock and Nunn 1976, Kusyk and Hsu 1976) and other volatile liquids such as benzene, toluene and chloroform (Liang and Hsu 1983) have been found to act in a similar fashion. These mitotic disrupters seem to have little effect on interphase chromo somes, but affect the mitotic apparatus through their effect on spindle and microtubular formation during a cell division (Morishima et al. 1976b, Grant et al. 1977). Their mutagenic effects are not likely to be detected by use of prokaryotes which are commonly employed in screening tests for environmental mutagens, since these organisms do not utilize a mitotic apparatus for cell division (Kusyk and Hsu 1976).

The effects of a mitotic disrupter mainfest themselves as abnormal movements of chromosomes errors of chromosome segregation (FCS), during mitosis, and may give rise to aneuploidy or gross imbalance of genetic material of the cell (Kusyk and Hsu 1976, Henrich et al. 1980). Rapidly proliferating tissue specimens can be fixed for microscopic examination without the use of a mitotic arrestant or a hypotonic solution. In these preparations, spindles are not artificially disrupted and chromosomes are not dispersed. Therefore, these specimens are suitable for observation of metaphase, anaphase, and telophase chromosomes in more or less natural spacial relationships, and are referred to as anaphase preparations. Although the morphology of individual chromosomes is not readily discernible due to overlaps, ECS can be examined directly in these preparations (Fig. 1). These include bridge formation, anaphase lag, misalignment of chromosomes (chromosomes out of phase with others), unequal segregation in bipolar division and multipolar division (Henrich et al. 1980). ECS commonly leads to formation of aneuploid cells.

The effects of a mitotic disrupter may also be examined indirectly by observation of unselected metaphase plates prepared in the usual fashion with the use of a mitotic arrestant and hypotonic solution (Morishima et al. 1976b). This method allows detection of hypoploidy, including those cells which are missing a large number of chromosomes from the complement. However, because of the artefactual loss of chromosomes attendant to the hypotonic treatment, an extremely large number of metaphases must be examined in both the control and the experimental groups of cells before a valid conclusion can be drawn (Morishima et al. 1976a).

Induction of aneuploidy by a mitotic disrupter also may be detected by measuring the DNA content of cells using microspectrography (Leuchtenberger et al. 1973a). An euploid metaphase should contain 4DNA (tetraploid amounts of DNA), and an euploid telophase cell should contain 2DNA (diploid amounts of DNA). A normal cell in S-phase (synthetic period of DNA in the interphase) would contain DNA in amounts between 2DNA and 4DNA.

An acentric chromosome fragment, produced by a break in the chromosome or as a sequela of anaphase bridge formation, will fail to

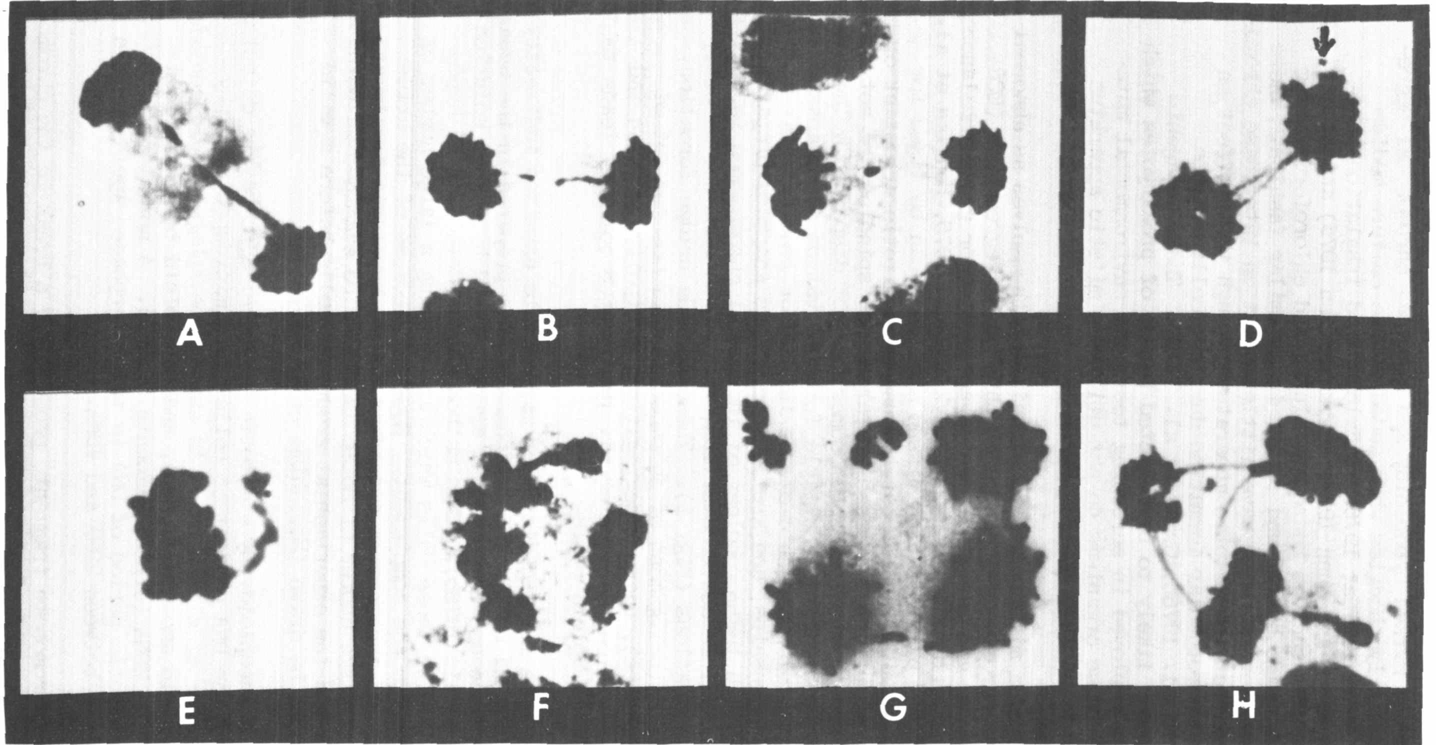


Figure 1. Errors of chromosome segregation (ECS). From Henrich et al. (1980). (A) Anaphase bridge. (B) Severed bridge. (C) Anaphase Zag. (D) Micronucleus (arrow) and multiple bridges. (E) Chromosome out of phase with others in metaphase. (F) Unequal segregation in bipolar diversion. (G) Multipolar division. (H) Multipolar division and bridges.

move toward a pole during the anaphase movement. The resultant anaphase lag leads to the exclusion of the fragment from either daughter nucleus, although it is retained within the cytoplasm. This cytoplasmic chromosomal material is referred to as a micronucleus. Since polychromatic erythrocytes of bone marrow lack the nucleus, presence of a micronucleus can be detected readily in these cells. The micronucleus test in polychromatic erythrocytes has been developed as a useful *in vivo* test system for detecting chromosomal fragments induced by chemical mutagens (Schmid 1975). That a positive result may arise from either chromosomal breakage or from ECS should be kept in mind.

The disparate results of cytogenetic studies on the effects of cannabis and cannabinoids are reviewed, below, by considering their ability to act as clastogens separately from their effects as mitotic disrupters. Some published data were retabulated in order to make them compatible with the format of presentation and for easier comparison with the data of others. In a number of publications, different types of observations were made, or multiple types of studies were performed. These data were considered separately.

Cannabis and Cannabinoids as Clastogens

Chromosome breakage has been examined extensively in human lymphocytes because of their ready accessibility. For some *in vivo* studies, lymphocytes of marijuana abusers were cultured, and the incidence of chromosome breaks in metaphase preparations was compared with that found in lymphocytes of non-marijuana users who served as the controls (Dorrance et al. 1970, Gilmour et al. 1971), Herha and Obe 1974, Martin et al. 1974, Stenchever et al. 1974). In other studies, marijuana users were asked to abstain from its use for a period of time, and then were given cannabis or delta-9-tetrahydrocannabinol (THC), the principal psychoactive component of cannabis, by mouth, or were given marijuana cigarettes to smoke under a controlled environment (Nichols et al. 1974, Matsuyama et al. 1976, 1977). In these experiments, the users served as their own control during the period of abstinence, and the incidence of chromosome breakage during the exposure to cannabis or THC was compared with that observed during the control period. The results of these studies are summarized in table 1. Of the 9 studies, 7 yielded no significant increase in the incidence of chomosome breaks. When the results were positive (Gilmour et al. 1971, Stenchever et al. 1974), the increased incidence was a few percent over the controls.

For *in vitro* studies, human lymphocytes from non-marijuana smokers were cultured in the presence of THC, cannabis resin or marijuana extracts (Table 1). These studies uniformly yielded negative results (Neu et al. 1970, Stenchever and Allen 1972, Martin et al. 1974, Stenchever et al. 1976).

From these studies, it may be concluded that the human lymphocyte system is relatively insensitive to the clastogenic effects of

TABLE 1. The effects of cannabis and natural cannabinoids as clastogens* in human lymphocytes

Experimental system and design	Results Magnitude of effect (%) Control/Experiment	Reference
In vivo studies		
Marijuana smokers	Neg	Dorrance et al. 1970
Marijuana smokers, light	Neg	Gilmour et al. 1971
Marijuana smokers, heavy + LSD	0.52/2.18	Gilmour et al. 1971
Cannabis users + LSD	Neg	Herha and Obe 1974
Marijuana smokers	Neg	Martin et al. 1974
Cannabis or THC given by mouth	Neg	Nichols et al. 1974
Marijuana smokers	1.2/3.4	Stenchever et al. 1974
Marijuana smokers; controlled smoking	Neg	Matsuyama et al. 1976
Marijuana smokers; controlled smoking	Neg	Matsuyama et al. 1977
In vitro studies		
Δ^8-THC added to cultures	Neg	Neu et al. 1970
THC added to cultures	Neg	Stenchever and Allen 1972
Cannabis resin added to cultures	Neg	Martin et al. 1974
Marijuana extract or THC added to cultures	Neg	Stenchever et al. 1976

* Includes observations on single chromatid and isochromal breaks, fragments, chromatid exchanges, chain and ring formations, translocations, deletions, and inversions, but excludes chromosome gaps.

± LSD--With or without history of abuse of Lysergic acid diethylamide.

cannabis and cannabinoids, or that these substances have little or no clastogenic effects.

Studies on induction of chromosome breakage by cannabis or cannabinoids in tissues other than human lymphocytes are summarized in table 2. When rats were exposed to THC, and the lymphocytes of exposed mothers and their offspring were examined, no increase in chromosome breaks was observed (Pace et al. 1971). From the cultured fibroblasts of embryos whose mothers were injected with cannabis resin, a similar result was obtained (Martin et al. 1974). However, when mice were exposed to THC, cannabinol (CBN), cannabidol (CBD), or marijuana extracts, and their primary spermatocytes were examined, a significant increase in the chromosomal rearrangements was observed (Zimmerman et al. 1979, Dalterio et al. 1982). A similar increase in structural rearrangements of chromosomes was found in bone marrow cells of the treated mice (Zimmerman et al. 1980). The magnitude of the clastogenic effect ranged from several percent to 15% over the controls. The effects of THC, CBN, CBD, and marijuana extracts were essentially the same. A study on dominant lethal test, using THC-treated male mice mated with untreated females, yielded negative results (Legator et al. 1976).

When various types of somatic cells in tissue culture were exposed to marijuana smoke (Leuchtenberger et al. 1973a), cannabis resin (Martin et al. 1974), or THC (Zimnerman et al. 1978), disparate results were obtained (Table 2). In one study, chromosome breaks in metaphase preparations, the sister chromatid exchanges, and the unscheduled DNA repair synthesis were examined without detecting mutagenic effect of THC (Zimmerman et al. 1978). Thus, the *in vitro* studies on the clastogenic effects were mostly negative-

Reviewing all of the above studies as a group, it appears that cannabis and cannabinoids are extremly weak clastogens, and that their clastogenic effects become apparent only in appropriately sensitive test systems such as primary sperm-atocytes and bone marrow cells, whereas the human lymphocyte system is relatively insensitive to their clastogenic effects (Table 1 and 2). Varying sensitivity of different tissues to a single chemical mutagen has been noted previously (Röborn 1970).

Cannabis and cannabinoids as mitotic disrupters

In vivo studies on induction of ECS by cannabis and cannabinoids are summarized in table 3. The parameters studied included observations on hypoploidy in metaphase preparations (Morishima et al. 1976a, 1979), ECS in anaphase preparations (Morishima et al. 1979, Zimmerman and Yesoda Raj 1980), aneuploidy and univalent sex chromosomes in primary spermatocytes (Zimmerman et al. 1979, Dalterio et al. 1982) and micronuclei in the polychromatic erythrocytes (Legator et al. 1976, Zimmerman and Yesoda Raj 1980). The systems utilized in these studies varied from human lymphocytes to bone marrow cells and primary spermatocytes of the mouse. Nevertheless, all but 2 out of 9 studies yielded statistically significant increases in the ECS. The effect was apparent for marijuana smoking,

TABLE 2. The effects of cannabis and natural cannabinoids as clastogens* in other than human lymphocyte system

Experimental system and design	Results Magnitude of effect (%) Control/Experiment	Reference
In vivo studies		
THC injections into pregnant rats; dams and offspring examined	Neg	Pace et al. 1971
Cannabis resin injections into pregnant rats; embryos examined	Neg	Martin et al. 1974
Cannabinoids injected into mice; primary spermatocytes examined	6.30/11.45-THC 6.30/10.72-CBN 6.30/11.72-RD	Zimmerman et al. 1979
Cannabinoids injected into mice; bone marrow cells examined	2.0/12.3-THC 2.0/17.0-CBN 2.0/14.3-CBD	Zimmerman and Yesoda Raj 1980
Marijuana extract or cannabinoids fed to mice; primary spermatocytes examined	0.81/5.74-Extract 0.81/4.54-THC 0.81/6.49-CBN	Dalterio et al. 1982
In vitro studies		
Cultured human lung cells exposed to marijuana smoke	Pos (Quantitative data not available)	Leuchtenberger et al. 1973a
Cannabis resin added to rat embryonic fibroblast cultures	Neg	Martin et al. 1974
THC added to fibroblast and XP cell cultures; metaphase and sister chromatid exchange examined	Neg	Zimmerman et al. 1978

* Includes observations on single chromatid and isochromatid breaks, fragments, chromatid exchanges, chair and ring formations, translations, deletions, inversions, and sister chromatid exchanges, but excludes chromasome gaps.

TABLE 3. The effects of cannabis and natural cannabinoids as mitotic disrupters*: *in vivo* studies

Experimental system and design	Results Magnitude of effect (%) Control/Experiment	Reference
THC fed to mice; micronuclei in bone marrow cells examined	Neg	Legator et al. 1976
Hypoploid metaphases in lymphocytes of marijuana smokers, heavy	15.52/36.23	Morishima et al. 1976a
Hypoploid metaphases in lymphocytes of marijuana smokers, light	31.19/37.09	Morishima et al. 1976a
Hypoploid metaphases in lympho cytes of marijuana smokers, controlled smoking	12.83/35.66	Morishima et al. 1979
ECS in lymphocytes of marijuana smokers; controlled smoking	1.20/2.01	Morishima et al. 1979
Cannabinoids injected into mice; aneuploidy in primary sperma tocytes examined	Neg	Zimmerman et al. 1979
Cannabinoids injected into mice; micronuclei in bone marrow cells examined	0.9/3.7-THC 0.9/5.3-CBN 0.9/5.0-CBD	Zimmerman and Yesoda Raj 1980
Cannabinoids injected into mice; ECS in bone marrow cells examined	8.0/17.3-THC 8.0/12.0-CBN 8.0/12.0-CBD	Zimmerman and Yesoda Raj 1980
Cannabinoids given by mouth to mice; aneuploidy and univalent sex chromosomes in primary spermatocytes examined	8.22/13.87-Extract 8.22/34.38-THC 8.22/17.32-CBN	Delterio et al. 1982

* Includes observations on hypoploid cells, aneuploidy, polyploidy, micronuclei in polychromatic erythrocytes of the bone marrow, univalent chromosomes during meiosis and errors of chromosome segregation (ECS) as seen in metaphase or anaphase preparations.

THC, CBN, CBD, and marijuana extract. The magnitude of effects ranged from a few percent to as high as 26% over the controls.

In vitro studies on induction of ECS are summarized in table 4. Again, methods used to examine the property of mitotic disrup tion varied from one study to another. These included observations on abnormal DNA contents of somatic and germ cells (Ieuchtenberger and Leuchtenberger 1971, Ieuchtenberger et al. 1973a, 1976), ECS in anaphase preparations (Ieuchentenberger and Ieuchentenberger 1971, Ieuchtenberger et al. 1973a, 1979, Henrich et al. 1980), and aneuploid and hypoploid metaphases (Ieuchtenberger et al. 1973b, Morishim et al. 1976a, Stenchever et al. 1976). The systems utilized for the experiments were also variable, including lung cells of man and mouse in culture, explants of testis of the mouse, and human lymphocytes or human breast cancer cells in tissue culture. Nevertheless, all 8 studies resulted in statistically significant increases of abnormal cells. Marijuana smoke, THC, and marijuana extracts were all effective in includiug the ECS. The magnitude of the effects ranged from a few percent to about 30% over the controls.

Conclusions

The contrast between the two groups of studies, one in which mainly the clastogenic effects were examined (Table 1 and 2) and another in which principally the mitotic disruptive effects were observed (Table 3 and 4), is striking. Taking all the studies together in which mitotic or meiotic movements of chromosomes were examined, it is apparent that cannabis and cannabinoids are mitotic disrupters capable of preventing the normal segregation of chromosomes (Table 3 and 4). The apparent contradictory results on the effects of cannabis and cannabinoids on induction of chromosomal abnormalities were explained previously as possibly being due to variations in the criteria for microscopic observations from one laboratory to another, effects of drugs other than marijuana being used by marijuana abusers, or variations in amounts of cannabinoids used. However, by separating those studies which examined mainly the clastogenic effects from those which examined the property of mitotic disruption, the controversy can be reconciled. It may be concluded that cannabis and cannabinoids possess the property of a mitotic disrupter, and that they may also have extremely weak clastogenic activity.

Whether or not cannabis and cannabinoids can produce stable chromosome alterations which can be transmitted through many cell generations is a major concern to all cytogenetic investigators. Although in all probability the majority of cells bearing observed chromosome abnormalities are incapable of surving more than a few cell cycles, it seems reasonable and prudent to assume that occasional cells affected by these chromosomal mutagens will bear stable chromosomal aberrations which will not be incompatible with cellular proliferation. Induction of such cells could lay a foundation for oncogenesis, teratogenesis, and mutagenesis. The observation of induced chromosomal abnormalities in primary spermatocytes (Zimmerman et al.

TABLE 4. The effects of cannabis and natural cannabinoids as mitotic disrupters*: *in vitro* studies

Experimental system and design	Results Magnitude of effect (%) control/Experiment	Reference
Cultured lung cells of mice exposed to marijuana and cigarette smoke; DNA contents and ECS examined	5 fold increase in tetraploid DNA contents. Increased ECS	Leuchtenberger and Leuchtenberger 1971
Cultured human lung cells exposed to marijuana smoke; DNA content and ECS examined	5-7 fold increase in aneuploid amounts of DNA or ECS	Leuchtenterger et al. 1973a
Cultured human lung cells exposed to marijuana smoke; aneuploidy examined	44/69	Leuchtenterger et al. 1973b
Explants of testes of mice exposed to marijuana smoke; DNA contents in spematids examined	20/50	Leuchtenberger et al. 1976
THC added to human lymphocyte cultures; hypoploidy examined	22.56/33.13	Morishima et al. 1976
Marijuana extract added to human lymphocyte culture; aneuploidy examined	2.0-4.0/9.0-12.0	Stenchever et al. 1976
Cultured human breast cancer cells exposed to marijuana make; abnormal mitosis examined	8.0/21.5	Leuchtenberger et al. 1979
THC added to human lymphocyte cultures; ECS examined	1.11/3.54	Henrich et al. 1980

* Includes observations on aneuploid DNA contents per cell, aneuploidy, abnormal mitoses, and errors of chromosome segregation (FCS) as see in metaphase or anaphase preparations.

1979, Dalterio et al. 1982) suggests that induction of chromosome abnormalities transmissible to offspring is still a possibility.

EFFECTS OF THC ON OVA

Although there is a growing body of evidence indicating that cannabis and cannabinoids can adversely affect the germ cells of males, little information is available on their effects upon female germ cells. Chronic administration of cannabis and cannabinoids has resulted in decreased sperm counts and motility (Dixit at al. 1974, Hembree et al. 1976, 1979, Huang et al. 1979, Dalterio et al. 1982). Although these findings may be mediated through the suppressive effects of cannabis and cannabinoids on the secretion of gonadotropins (Kolodny et al. 1974, Cohen 1976, Smith et al. 1976, 1980, Halclerode et al. 1979), there is evidence to indicate that these substances can directly affect germ cells. Chromosomal abnormalities have been induced in primary spermatocytes in *in vivo* experiments (Zimmerman et al. 1979, Dalterio et al. 1982), as well as in an *in vitro* study (Leuchtengerger et al. 1976).

Studies on female gem cells are, as yet, meager. *In vitro* steroidogenesis by isolated follicles was inhibited by THC, and resumption of meiosis occurred without the normal accompaniment of detachment of cumulus-granulosa cells from the ova, as assessed by the germinal vesicle breakdown (Reich et al. 1982).

The Effect of THC on Early Embryogenesis

In our recent studies the effects of chronic administration of THC on early embryogenesis were investigated in mice treated with THC prior to sexual maturation (Nogawa et al. 1983, Shinohara et al. 1983). The study was designed to imitate prolonged abuse of marijuana by sexually immature and adolescent girls. Female mice were injected, intraperitoneally, with THC 5 m/kg/d. or with the vehicle for 21 consecutive days, starting on the 30th day of life. Vaginal opening generally occurred in these mice on the 35th day of life. The dose of THC was approximately equivalent to that absorbed by man smoking 1 to 2 marijuana cigarettes per day (Rosenkrantz 1976). The mice were injected intraperitoneally with 5 I.U. of pregnant mare serum gonadotropin on the last day of THC injection, and 48 hours later with 5 I.U. of human chorionic gonadotropin (hCG) in order to induce superovulation. The time of hCG injection was taken as time zero. Females were placed with untreated young adult males for 16 hours immediately after hCG injection and allowed to mate. One group of females was sacrificed 22 to 26 hours after hCG injection. Another group of female mice was sacrificed at 49 to 52 hours. The ova were recovered from the oviducts and immediately examined microscopically. After the morphological examination either metaphase or anaphase preparations of these ova were made.

The rate of mating, ovulation, and fertilization did not differ between the control and the experimental group.

From 85 control and 95 THC-treated mice sacrificed between 22 and 26 hours, 2,495 and 3,238 ova were recovered, respectively. Only 1.0% and 1.4% of these from the control and the THC-treated mice, respectively, were at the 2-cell or later stages of development and therefore had completed the first cleavage division. Thus, these samples represented ova just prior to the first cleavage division. The incidence of morphologically abnormal ova, 12.3% for the control and 11.2% for the THC-treated animals, did not differ between the two groups.

From 261 control and 201 THC-treated mice sacrificed between 49 and 52 hours, 8,001 and 6,345 ova were obtained, respectively. The majority of ova were in the 2-cell stage, but a significant proportion of the ova were in the process of or had completed the second cleavage division (Table 5). Thus, these samples represented ova at the time of the second cleavage division. The incidence of morphologically abnormal ova (Fig. 2) was 13.6% in the control and 19.1% in the THC-treated group (Table 5). This difference was significant at $p<0.001$. Therefore, it was concluded that the incidence of abnormal ova increased from the period just before the first cleavage division to the time of the second cleavage division in the THC-treated animals. It appeared that morphological degeneration of ova occurs progressively between these two periods, resulting in an accumulation of abnormal ova at the time of the second cleavage division. The incidence of ova that had successfully completed the first cleavage division, those in the 2-cell or later stages of development, was 77.2% for the control and 71.7% for the THC-treated group (Table 5). This difference was significant at $p<0.005$. Therefore, it was concluded that THC induced an increased rate of failure in completing the first cleavage division. In order to assess the success rate of the second cleavage division, the ova in the 1-cell stage and the morphologically abnormal ova were eliminated from tabulation (Table 6). There were a total of 6,183 ova in the control and 4,552 in the THC-treated group, representing apparently healthy ova that had successfully completed the first cleavage division. Of these ova, 15.4% in the control and 14.3% in the THC-treated group, were at either the 3-cell or 4-cell stage of developmnt, thus having successfully undergone the process of the second cleavage division. The difference between the two groups was not significant. Therefore, THC treatment did not appear to interfere with the second cleavage division for those ova that had completed the first cleavage division.

Among 2,215 ova from the control and 2,078 from the THC-treated group examined in the anaphase preparations, 365 and 301, respectively, were at metaphase, anaphase, or telophase of the second cleavage division. The only type of ECS found in these cells was anaphase bridge formation found in 5 cells from the controls and in 2 cells from the THC-treated group. Thus, THC did not induce ECS at the time of the second cleavage division.

When 2,426 ova obtained from the control animals and 1,018 ova from the THC-treated group were examined in the metaphase preparations, the mitotic index was 6.7% for the control and 7.5% for

TABLE 5. The incidence of morphologically normal and abnormal ova of the muse, 49 to 52 hours after the hCG injection (at the time of the second cleavage division)

	Normal (%) Cell Stage				Abnormal (%)	Total No. of ova
	1	2	3	4		
Control	9.1	65.3	4.2	7.7	13.6	8,001
THC-treated	9.2	61.4	3.2	7.1	19.1	6,345

TABLE 6. The success rate of the second cleavage division in apparently health ova that had completed the first cleavage division, 49 to 52 hours after the hCG injection

	Stages of development of ova (%)		Total No. of ova
	3- and 4-cell	2-cell	
Control	15.4	84.6	6,183
THC-treated	14.3	85.7	4,552

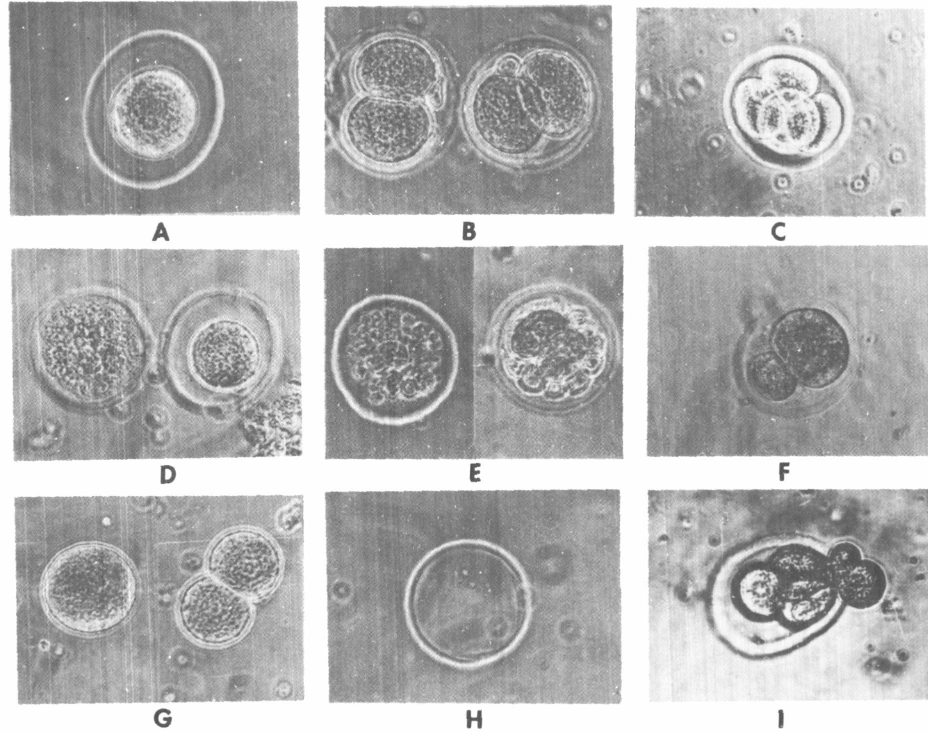


Figure 2. Normal and Abnormal Ova. From Nogawa et al. (1983). (A), (B), (C) Normal 1-cell, 2-cell, and 4-cell stage ova. (D) Liquefaction degeneration of the left ovum, and a normal ovum on the right. (E) Fragmentation of ooplasm in 2 ova. (F) Unequal cleavage. (G) Premature loss of zona pellucida in a 1-cell and 2-cell stage ova. (H) Zona only. (I) Premature hatching.

the THC-treated group. No obvious aneuploidy was observed in 114 metaphases from the controls or in 69 metaphases from the THC treated animals, although dispersion of chromosomes a number of metaphases was less than ideal.

Discussion

Chronic administration of THC, at a modest dose, to presumably sexually developing mice and continuing through their early adulthood induced a significant increase in morphologically abnormal ova. Vaginal opening, which takes place under the influence of estrogen, occurred at about the 35th day of life in the mice used in this study. Therefore, it is reasonable to assume that the protocol for THC administration covered the prepubertal stages of sexual maturation.

Although the absolute percentage of increase in abnormal ova was relatively small at 5.5%, the order of magnitude was similar to the increased cytogenetic abnormalities induced by THC in the primry spermatocytes of the mouse (Zimmerman et al. 1979), and to the 2.4% increase in ECS induced in human lymphocytes by THC *in vitro* (Henrich et al. 1980).

Although there is no clear evidence of increased fertility or fetal wastage among chronic marijuana abusers, the effect of the drug may be difficult to detect by epidemiological studies, even if the observed effect of THC in the mouse were to occur in man. Natural loss of concepti in humans has been estimated to be as high as 78% (Roberts and Lowe 1975), but recognizable spontaneous abortion accounts for only 11.5% of known pregnancies (Shapiro et al. 1962). Therefore, if marijuana abuse increases the wastage of early concepti by the same order of magnitude as that observed in the mouse, the extremely high incidence of natural loss would readily mask the increase and prevent clinical detection.

Despite the relatively small percentage of increase in degenerating ova induced by THC, the biological significance of this observation may differ greatly from the adverse effect of THC on other tissues. In contrast to most somatic tissues and sperm, *de novo* formation of oocytes does not occur after birth (Zuckerman 1956, Tsafriri 1978). Thus, any damage inflicted upon oocytes is likely to be irreversible and permanent.

The observed increase in degenerating ova was caused by their inability to undergo successfully the first cleavage division. The pathogenesis, therefore, is likely to lie in the process of meiosis, which takes place during the time-frame just prior to ovulation and shortly after fertilization. It is reasonable to assume that some 24 to 48 hours would elapse before morphologic changes of degeneration can be detected in an ovum that undergoes aberrant meiosis. Fragmentation of ooplasm, for example, occurs 24 hours after an oocyte reaches full maturity (Zenzes and Engel 1976), when not fertilized. That the THC prevented the ova from completing the first cleavage division was further supported by our failure to detect an increase in ECS or aneuploidy during the second cleavage

division. Presumably only those ova that were not significantly affected during meiosis would have completed the first cleavage division successfully and would have survived to undergo the second cleavage division.

The pathogenic process leading to the observed degeneration of ova is likely to lie in meiosis. The evidence implicating THC as a mitotic disrupter, which interfers with the normal segregation of chromosomes, has been reviewed above. Currently, studies are in progress to examine directly the effects of THC on the meiotic process in ova of the mouse.

REFERENCES

Adler, I.D. Aberration induction by mitomycin C in early primary spermatocytes of mice. Mutat Res, 35:247-256, 1976.

Brinkley, B.R., and Rao, N.J. Nitrous oxide: Effects on the mitotic apparatus and chromosome movement in HeLa cells. J Cell Biol 58:96-106, 1973.

Cohen, M.M., and Shaw, M.W. Effects of mitomycin C on human chromosomes. J Cell Biol, 23:386-395, 1964.

Cohen, M.M., Marinello, M.J., and Back, N. Chromosomal damage in human leukocytes induced by lysergic acid diethylamide. Science, 155:1417-1419, 1967.

Cohen, S. the 94-day cannabis study. Ann NY Acad Sci, 282:211-220, 1976.

Dalterio, S., Badr, F., Bartke, A., and Mayfield, D. Cannabinoids in male mice: Effects on fertility and spermatogenesis. Science, 216:315-316, 1982.

Dixit, V.P., Arya, M., and Lohiya, N.K. The effect of chronically administered cannabis extract on the female genital tract of mice and rats. Endokrinologie, 66:365-368, 1974.

Dorrance, D., Janiger, and Teplitz, R.L. In vivo effects of illicit hallucinogens on human lymphocyte chromosomes. JAMA, 212:1488-1491, 1970.

Evans, H.J., Court Brown, W.M., and McLean, A.S. Human Radiation Cytogenetics. Amsterdam, North-Holland Publishing., 1967.

Gilmour, D.G., Blood, A.D., Lele, K.P., Robbins, E.S., and Maximilian, C.Chromosomal aberrations in users of psychoactive drugs. Arch Gen Psychiat, 24:268-272, 1971.

Grant, C.J., Powell, J.N., and Radford, S.G. The induction of chrmosomal abnormalities by inhalational anesthetics. Mutat Res, 46:177-184, 1977.

Harcelrode, J., Nyquist, S.E., Nazar, B., and Lowe, D. Effects of cannabis on sex hormones and testicular enzymes of the rodent. In: Nahas, G.G., and Paton, W.D.M., ed. Advances in the Biosciences. Vol. 22 & 23. Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979. pp. 395-405.

Hembree, W.C., III, Zeidenberg, P., and Nahas, G.G. Marihuana's effects on human gonadal function. In: Nahas, G.G., Paton, W.D.M., and Idänpään-Heikkilä, J.E., ed. Marihuana: Chemistry, Biochemistry, Cellular Effects. New York, Heidelberg, Berlin: Springer-Verlag, 1976. pp. 521-532.

446-384 O - 84 - 4

Hembree, W.C., III, Nahas, G.G., Zeidenberg, P., and Huang, H.F.S. Changes in human spermatozoa associated with high dose of marihuana smoking. In: Nahas, G.G., Paton, W.D.M., ed. Advances in the Biosciences. Vol. 22 & 23. Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979. pp. 429439.

Henrich, R.T., Nogawa, T., and Morishima, A. In vitro induction of segregational errors of chromosomes by natural cannabinoids in normal human lymphocytes. Environment Mutagenesis, 2:139-147, 1980.

Herha, J., and Obe, G. Chromsomal damage in chronical users of cannabis: In vivo investigation with two-day lymphocyte cultures. Pharmakopsychiat, 7:328-337, 1974.

Huang, H.F.S., Nahas, G.G., and Hembree, W.C., III. Effects of marihuana inhalation on spermatogenesis of the rat. In: Nahas, G.G., and Paton, W.D.M., ed. Advances in the Biosciences. Vol. 22 & 23. Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979. pp. 419-427.

Kate, H. Induction of sister chromatid exchanges by chemical mutagens and its possible relevance to DNA repair. Cell Res, 85:239-247. 1974.

Kolodny, R.C:, Masters, W.H., Kilodner, R.M., and Toro, G. Depression of plasma testosterone levels after chronic intensive marijuana use. N Engl J Med, 290:872-874, 1974.

Kusyk, C., and Hsu, T.C. Mitotic anomalies induced by three inhalation halogenated anesthetics. Environment Res, 12:366-370, 1976.

Legator, M.S., Weber, E., Connor, T., and Stoeckel, M. Failure to detect mutagenic effects of Δ^9-tetrahydrocannabinol in the dominant lethal test, host-mediated assay, blood-urine studies, and cytogenetic evaluation with mice. In: Braude. M.C. and Szara, S., ed. Pharmacology of Marihuana. Vol. 2: New York: Raven Press. 1976. pp. 699-709.

Leuchtenberger, C., and Leuchtenberger, R. Morphological and cytochemical effects of marijuana cigarette smoke on epitheloid cells of lung explants from mice. Nature, 234:227-229, 1971.

Leuchtenberger, C., Leuchtenberger. R., and Schneider. A. Effects of marihuana and tobacco smoke on human lung physiology. Nature, 241:137-139, 1973a.

Leuchtenberger, C., Leuchtenberger, R., and Ritter, U. Effects of marihuana and tobacco smoke on DNA and chromosomal complement in human lung explants. Nature, 242:403-404, 1973b.

Leuchtenberger, C., Leuchtenberger, R., Zbinden, J., and Schleh, E. Cytological and cytochemical effects of whole smoke and of the gas vapor phase of marihuana cigarettes on growth and DNA metabolism of cultured mammalian cells. In: Nahas, G.G., Patton, W.D.M., and Idänpään-Heikkilä, J.E., ed. Marihuana: Chemistry Biochemistry, and Cellular Effects. New York Heidelberg, Berlin: Springer-Verlag, 1976. pp. 243-256.

Leuchtenberger, C., Leuchtenberger, R., and Chapus, L. Difference in response to vitamin C between marihuana and tobacco smoke exposed human cell cultures. In Nahas, G.G., and Paton, W.D.M., ed. Advances in the Biosciences. Vol. 22 & 23. Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979. pp. 209-218.

Liang, J.C., and Hsu, T.C. A simple cytogenetic assay for detecting mitotic-arresting effects of volatile liquids. Proc 14th Annual Meeting of Environmental Mutagen Society, San Antonio, Texas March 1983. pp. 69-70 (Abstract).

Martin, P.A., Throburn, M.J., and Bryant, S.A. In vivo and in vitro studies on the cytogenetic effects of Cannabis sativa in rats and men. Teratol 9:81-85, 1974.

Matsuyama: Jarvik, L.F., Ful, T.K., and Yen, F.S. Chromosane studies before and after supervised marijuana smoking. In: Braude, M.D., and Szara, S., ed. Pharmacology of Marihuana. Vol. II. New York: Raven Press, 1976. pp. 723-731.

Matsuyama, S.S., Yen, F.S., Jarvik, L.F., Sparkes, R.S., Fu, T.K., Fisher, H., Reccius, N., and Frank, I.M. Marihuana exposure in vivo and human lymphocyte chromosomes. Mutat Res, 48:255-266, 1977.

Morishima, A., Milstein, M., Henrich, R.T., and Nahas, G.G. Effects of marihuana smoking, cannabinoids, and olivetol on replication of lymphocytes: Formation of micronuclei. In: Braude, M.C., and Szara, S., ed. Pharmacology of Marihuana. Vol. II. New York: Raven Press, 1976a. pp. 711-722.

Morishima, A., Henrich, R.T., Jou, S., and Nahas, G.G. Errors of chromosome segregation induced by olivetol, a compound with the structure of C-ring common to cannabinoids: Formation of bridges and multipolar divisions. In Nahas, G.G., Patton, W.D.M., and Iänpään-Heikkilä, J.E., ed. Marihuana: Chemistry, Biochemistry, and Cellular Effects. New York, Neidelberg, Berlin: Spinger-Verlag, 1976b. pp. 265-271.

Morishima, A., Henrich, R.T., Jayaraman, J., and Nahas, G.G. Hypoploid metaphases in cultured lymphocytes of marihuana smokers. In: Nahas, G.G., and Paton, W.D.M., ed. Advances in the Biosciences. Vol. 22 & 23. Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction Brain. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Permagon Press, 1979. pp. 371-376.

Neu, R.L., Powers, H.O., King, S., and Gardner, L.I. Δ8-and Δ9-tetrahydrocannabinol: Effects on cultured human leucocytes. J Clin Pharmacol, 10:228-230, 1970.

Nicholas, W.W., Miller, R.C., Heneen, W., Bradt, C., Hollister, L., and Kanter, S. Cytogenetic studies on human subjects receiving marihuana and Δ-9-tetrahydrocannabinol. Mutation Res, 26:413-417, 1974.

Nogawa, T., Shinohara, O., Henrich, R.T., and Morishima, A. The Effects of chronic administration of delta-9-tetrahydrocannabinol on the early embryonic development of the mice. In: Dewey, W.L., Agurell, S., and Willette, B., ed. Proceedings of Cannabinoids 1982 Symposium. New York, London: Academic Press, 1983 (in press).

Pace, H.B., Davis, W.M., and Borgen, L.A. Teratogenesis and marijuana. Ann NY Acad Sci, 191:123-131, 1971.
Reich, R., Laufer, N., Lewysohn, O., Cordova, T., Ayalon, D., and Tsafriri, A. In vitro effects of cannabinoids on follicular function in the rat. Biol Reprod, 27:223-231, 1982.
Roberts, C.J., and Lowe, C.R. Where have all the conceptions gone? Lancet, i:498-499, 1975.
Röhborn, G. The dominant lethals: Method and cytogenetic examination of early cleavage stages. In: Vogel, F., and Rohrborn, G., ed. Chemical Mutagenesis in Mammals and Man. New York, Heidelberg: Springer-Verlag, 1970. pp. 148-155.
Rosenkrantz, H. The immune responses and marihuana. In: Nahas, G.G., Patton, W.D.M., and Idänään-Heikkilä, J.E., ed. Marihuana: Chemistry, Biochemistry, and Cellular Effects. New York, Heidelberg, Berlin: Springer-Verlag, 1976. pp. 441-456.
Schmid, W. The micronucleus test. Mutation Res, 31:9-15, 1975.
Shapiro, S., Jones, E., and Densen, P. A life table of pregnancy terminations and correlates of fetal loss. Milbank Men Fund Quart, 40:1, 1962.
Shinohara, O., Henrich, R.T., and Morishima, A. The effects of chronic administration of delta-9-tetrahydrocannabinol on early embryogenesis of the mouse. Biol Reprod, 1983 (submitted).
Smith, C.G., Moore, C.E., Besch, N.F., and Besch. P.K. Effect of delta-9-tetrahydrocannibinol (THC) on the secretion of male sex hormone in the rhesus monkey. Pharmacologist, 18:248, 1976.
Smith, C.G., Besch, N.F., and Asch, R.H. Effects of marihuana on the reproductive system. In: Thomas, J.A., and Singhal, R.L., ed. Advances in Sex Hormone Research. Baltimore, Munich: Urban and Schwarzenberg, 1980. pp. 273-294.
Stenchever, M.A., and Allen, M. The effects of delta-9-tetrahydrocannabinol on the chromosomes of human lymphocytes in vitro. Am J Obstet Gynecol, 114:819-821, 1972.
Stenchever, M.A., Kunysz, T.J., and Allen, M.A. Chromosome breakage in users of marihuana. Am J Obstet Gynecol, 118:106-113, 1974.
Stenchever, M.A., Parks, K.J., and Stenchever, M.R. Effects of Δ^8-tetrahydrocannabinol, Δ^9-tetrahydrocannabinol, and crude marihuana on human cells in tissue culture. In: Nahas, G.G., Paton, W.D.M., and Idänpään-Heikkilä, J.E., ed. Marihuana: Chemistry, Biochemistry, and Cellular Effects. New York, Heidelberg, Berlin: Springer-Verlag, 1976. pp. 257-263.
Stich, H.F., San, R.H.C., and Kawazoe, Y. DNA repair synthesis in mamnalian cells exposed to a series of oncogenic and non-oncogenic derivatives of 4-nitroquinoline-1-oxide. Nature, 229:416-419, 1971.
Sturrock, J.E., and Nunn, J.F. Synergism between halothane and nitrous oxide in the production of nuclear abnormalities in the dividing fibroblast. Anesthesiol, 44:461-471, 1976.
Tsafriri, A. Oocyte maturation in mammals. In: Jones, R.E., ed. The Vertebrate Ovary: Comparative Biology and Evolution. New York and London: Plenum Press, 1978. pp. 409-422.
Zenzes, M.T., and Engel, W. Aging of the female gamate. I. Oocyte fragmentation during postnatal development of the rat. Cytobios, 16:53-61, 1976.

Zimmerman, A.M., Stich, H., and San, R. Nonmutagenic action of cannabinoids *in vitro*. *Phamacol*, 16:333-343, 1978.

Zimmerman, A.M., Zimmerman, S., and Yesoda Raj, A. Effects of cannabinoids on spermatogenesis in mice. In: Nahas, G.G., and Patton, W.D.M., ed. *Advances in the Biosciences*. Vol. 22 & 23. *Marihuana: Biological Effects; Analysis, Metabolism, Cellular Responses, Reproduction and Brain*. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press, 1979. pp. 407-418.

Zimmerman, A.M., and Yesoda Raj, A. Influence of cannabinoids on somatic cells *in vivo*. *Pharmacol*, 21:277-287, 1980.

Zuckerman, S. The regenerative capacity of ovarian tissue. *Ciba Found Coll Ageing*, 2:31, 1956.

ACKNOWLEDGEMENT

This work was supported by a grant from NIDA, lR0 DA02833.

AUTHOR

Akira Morishima, M.D., Ph.D.
Department of Pediatrics
College of Physicians & Surgeons
Columbia University
630 West 168th Street, New York, NY 10032

Endocrine Effects of Marijuana in the Male: Preclinical Studies

Jack Harclerode, Ph.D.

INTRODUCTION

The research efforts of many investigators in the recent past have made it abundantly clear that exposure to marijuana has significant effects upon the reproductive system and the effects of cannabinoid treatment are equally significant on both male and female reproductive systems. Among the effects of cannabinoid treatment on the male reproductive system that have been reported arc altered testicular function, in the form of depressed male hormone secretion, and changes in both the quantity and quality of the sperm produced by the seminiferous tubules. There have been changes reported in the weight and in certain of the enzymes associated with the reproductive organs. Much research effort focused on the ability of THC to depress the secretion of the gonadotropins from the pituitary that are responsible for stimulating testosterone production by the Leydig cells of the testis and the action on the hypothalamus to depress gonadotropic releasing hormone (GnRH).

Maintenance and regulation of normal reproductive capacity in the male is a complex and highly integrated phenomenon. It requires proper nutritional and hormonal support, not only by the hormones directly involved in reproduction, hut also by the synergistic action of hormones produced by other endocrine organs throughout the body. The regulation of many endocrine organs in the body is though the hypothalamus, an area which is sensitive to chemical, hormonal, and sensory input from all parts of the body. Thus, it is easy to understand why psychoactive drugs which alter neural function in various parts of the brain have so much of an effect on the output of trophic hormones from the hypothalamus. This review will attempt to summarize the effect that marijuana and its various constituents have on the reproductive system of laborator animals. Although this review emphasizes effects on the male reproductive system, some female data are included for comparative purposes.

THE EFFECTS OF THC ON GONADOTROPINS

In 1973 Marks reported that luteinizing hormone levels in ovariectomized rats were reduced sharply by Δ^9-tetrahydrocannibinol (THC)

(1, 3, and 10 mg/kg I.V.). Marks concluded that the action of THC was not related to activation of muscarinic receptors but that it might be due to the observed ability to increase the uptake and retention of catecholamines by brain tissue, which would in effect produce an inhibition of GnRH release from the hypothalamus. Symons et al. (1976) showed that both acute and chronic treatment of rats with THC (5 mg/kg 2 times a week for 6 weeks i.m.) produced a decrease in both plasma luteinizing hormone (LH) and testosterone levels. They also observed that the effect of an acute dose was more dramatic than the chronic treatment and that THC somehow decreased the pituitary's response to GnRH.

Collu et al. (1975) reported that injection of prepubertal male rats with 10 mg/kg THC three times a week for 30 days resulted in reduced levels of LH in the plasma while pituitary levels of luteinizing hormone were unmodified. They similarly reported that plasma levels of follicle stimulating hormone (FSH) were not affected by the drug treatment. Dalterio et al. (1978) showed that oral administration of THC (50 mg and 100 mg/kg) to male mice resulted in a reduction of plasma testosterone, LH, and FSH levels, but that single doses of cannabinol (CBN) had no effect on any of these three hormones. They concluded that the reduction in plasma hormone levels was due to inhibition of pituitary LH release, as well as to a direct effect on the testis to alter the responsiveness to LH stimulation.

Smith et al. (1976) injected male Rhesus monkeys with THC (5 mg/kg I.M. acute) and produced a 65% reduction in serum testosterone levels that returned to normal over the next 3-day period. They found a depression in LH levels that was comparable in magnitude and duration to the depression in testosterone levels. Fran these data they postulated that the THC inhibition of male sex hormones was due to its interaction with the central nervous system. Thus it appears that THC affects the hypothalamus and pituitary of males in a similar manner to the way it affects females.

In female ovariectomized monkeys, Snith et al. (1979a) compared the effects of various doses of THC on the levels of LH and on the levels of follicle stimulating hormone. Five mg/kg produced a 68% reduction in LH levels and a 56% reduction in FSH levels. The maximum decrease occurred at the same time for both gonadotropins. Smith et al. (1979a) have also reported that the decrease in both LH and FSH were equivalent in response to equal doses of THC whether the THC was administered as a pure substance or as the same percent in crude marijuana extract (CME). These data suggest that the inhibitory action of marijuana on gonadotropin levels is produced by THC and that the other cannabis derivatives contained in marijuana do not contribute to the effect, since neither cannabidol (CBD) nor CBN had any significant effect on gonadotropin levels, even up to dosages of 10 mg/kg. Thus they have concluded that the inhibitory effect of cannabis derivatives on gonadotropins is related to their psychoactivity. It should be pointed out, however, that certain non-psychoactive cannabinoids which may not be involved in altering hormonal changes in the hypothalamus, the pituitary, or the testis, may contribute to a direct effect on other reproductive

functions such as sperm production by the seminiferous tubules, augmentation of hormonal effects on growth and secretory activity of accessory sex organs (such as prostate and seminal vesicles), interaction with hormones at the target tissue level, or altering receptors for hormones on target tissues.

THE EFFECT OF MARIJUANA ON GONADOTROPIN RELEASING HORMONE

The hypothalamus contains neurosecretory neurons which are responsible for the synthesis and secretion of factors that regulate the release of the hormones elaborated by the anterior lobe of the pituitary gland. A single hypothalamic factor seems to be responsible for the release of both LH and FSH and this factor is gonadotropin releasing hormone. The secretion of GnRH is affected by a variety of factors including neural, chemical, sensory, hormonal, and various drug treatments of the animal. Normally, release of GnRH is regulated by the neural transmitters of the hypothalamus. Thus, factors which alter dopamine and norepinephrine concentrations also alter GnRH release. As a general rule, things which enhance the release of adrenergic or dopaminergic substances in the hypothalamus should stimulate the release of GnRH, and those substances, such as drugs, which antagonize adrenergic and dopaminergic release should inhibit the release of GnRH.

Several studies have indicated that the pharmacological effect of THC on gonadotropin levels involves the alteration of gonadotropin releasing hormone. Smith et al. (1979a) using the overiectomized monkey, showed that administration of GnRH to monkeys that had received THC 6 hours before (2.5 mg/kg I.M.), was able to reverse THC-induced reduction in LH and FSH within 30 minutes after GnRH administration. Tyrey (1978) similarly showed that administration of GnRH to ovariectomized rats previously treated with THC reversed the effects of THC induced depression of LH and FSH. Nir et al. (1973) had shown earlier that GnRH might be involved, in a study that examined the effect; of THC-induced suppression of ovulation in rats by the administration of GnRH; and Asch et al. (1979a) also reported that GnRH could reverse the THC-induced suppression of ovulation in rabbits.

Thus it appears that THC blocks GnRH release by the hypothalamus. Since the pituitary remains capable of responding to exogenous GnRH in the presence of THC, it seems likely that the observed THC-induced suppression of gonadotropin output arises indirectly from an action of THC upon the hypothalamus and not through a direct effect upon the pituitary.

In contrast to the acute studies reported above, Rosenkranz and Esber (1980) reported that prolonged oral administration of THC to young rats (2, 10, or 50 mg/kg for 14 to 180 days) tended to increase gonadotropins, indicating that tolerance to the effects of THC may develop in males. They also reported that cannabidiol treated male monkeys had increased follicle stimulating hormones (CBD, 20, 100, and 300 mg/kg oral), but that the steroid hormones were essentially unchanged.

It is not surprising that the psychoactive ingredient in marijuana, THC, has as a major effect the depressing of gonadal hormone levels by acting through the hypothalamus to suppress GnRH release. In view of the psychoactive nature of THC, it is possible that it alters neural transmitter substances throughout the brain, but especially in that region of the central nervous system that is important in regulating GnRH release from the hypothalmus. Very few studies have examined whether hypothalamic neural transmitters are in fact affected by marijuana smoke or THC treatment. There are, however, some studies which have shown that THC affects neural transmitter substances in other areas of the brain. Kramer and Ben-David (1974) have shown that both serotonin and dopamine are probably responsible for the inhibitory effect of THC on prolactin, and Marks (1973) indicated that hypothalamic cholinergic mechanisms are probably not involved in the THC depression of LH release. Several early studies (Truitt and Anderson 1972; Fuxe and Janssen 1972; and Welch et al. 1971), reported that biogenic amines in the CNS are altered by THC treatment. Others (Howes and Osgood 1974; and Hershkowitz et al. 1977) showed that THC prevents reuptake of dopamine, norepinephrine, and serotonin into the respective nerve endings throughout the brain. Other authors (Revuelta et al. 1979) have shown that THC depressed cholinergic activity in the rat hippocampus.

These reports indicate that serotonin levels in certain areas of the brain may increase with concomittant decrease in catecholamines in other areas of the brain. Thus it appears that future studies, which address themselves to clarifying the effect of marijuana or THC on neurotransmitters in the hypothalamic area and their sub sequent effect on GnRH and other factors involved in regulating pituitary function, would be quite important.

There are other possible mechanisms to partially explain the observed effects of THC on pituitary horn-one output other than through altering hypothalamic regulation of GnRH. Jakubovic and McGeer (1972) showed that THC (but not nonpsychoactive cannabinoids) decreased the synthesis of protein and nucleic acid in the infant rat brain. Cannabinoids also may affect the cell membranes of nerves, for Greenberg et al. (1978) have shown an inhibition of LPC acyltransferase, a plasma membrane bound enzyme, which may be involved in neurotransmitter uptake mechanisms in the synpatosomes of mouse brain. Also, marijuana may produce morphological changes, at the ultrastructural level in the neural tissue, such as synaptic cleft widening and clumping of synaptic vesicles in the synaptic cleft (Harper et al. 1977).

EFFECT OF MARIJUANA ON THE TESTIS AND ACCESSORY REPRODUCTIVE ORGANS

Roth crude marijuana extract (CME) and THC produced a decrease in the weight of the reproductive organs of rats when given over a prolonged period of time and over several dosage ranges. The greatest effects were reported for high dosages (15 and 75 mg/kg orally daily for 77 days). Fujimoto et al. (1978) showed significant reduction in ventral prostate, seminal vesicles, and epididymal weight, which was correlated with a decrease in plasma

testosterone levels and was accompanied by a reduced number of sperm in the fluids of the epididymus. Treatment of the rats for only 5 days produced none of these changes. The effects of the cannabinoids appeared to be reversible, because there was a return to control levels of organ weights 30 days after cessation of drug treatment.

Similarly, Dixit and Lohiya (1975) report that cannabis extract produced a marked reduction in the weights of seminal vesicles, ventral prostate, epididymus, and preputial glands of castrated adult male mice. Further, if cannabis extract was administered in combination with testosterone propionate, the growth stimulation produced by testosterone propionate alone was inhibited, indicating that cannabis extract may be antiandrogenic. However, there was no indication as to which compounds in the extract might produce the antiandrogenic effect. Vyas and Singh (1976) report that cannabis administered to pigeons for 30, 60, or 90 days produced a significant decrease in the gross weight of the testis, decrease in the diameter of seminiferous tubules, degenerative changes in the seminiferous tubules, and a complete cessation of sperm production. Dixit et al. (1977) also showed that CME (10 mg/kg I.P. for 10 days) produced degenerative changes in the testis of a toad including decreased Leydig cell nuclei, a reduction of RNA and protein contents in the testis, and an indication of reduced androgen production.

Purohit et al. (1979) report that THC administered to hypophysectomized rats was able to block the stimulatory effect of human chorionic gonadotropin on ventral prostate and seminal vesicle weights but that serum testosterone and dihydroxytestosterone levels were unaffected. They concluded that THC must have both a direct tissue effect and an indirect hypothalamic-hypophyseal effect to reproduce the changes observed in the testis. Ghosh et al. (1981) report that THC (10 mg/kg for 7 days) prevented the testosterone induced changes of DNA, RNA and protein content, acid phosphatase, and an isoenzyme variant of acid phosphatase in the ventral prostate tissues of adult castrated rats. They theorized that THC acted antiandrogenically directly at the level of the male accessory sex organs.

The reports in the literature of reduction in reproductive organ weights are accompanied by reports that show that the quality and quantity of sperm produced by the testis is affected by cannabinoids. Fosenkrantz and Hayden (1979) report that rats exposed to single daily doses of Turkish marijuana smoke, containing .25% THC, exhibited seminiferous tubule degeneration with an interference with sperm maturation. Zimmerman et al. (1979a, 1979b) have shown that treatment of mice for as few as 5 days with THC (5 mg/kg I.P.) or cannabidiol (CBD) (10 and 25 mg/kg I.P.) produced an increase in the number of abnormal sperm, including sperm which had an increased number of ring and chain translocations when the cells were evaluated 16 days after the last dose of cannabinoids. When calls were evaluated 35 days after the last of 5 consecutive days of cannabinoid treatment, among the abnormal sperm reported where those which had heads without hooks, banana shaped heads, amorphous

heads, and folded heads. Huang et al. (1979) reported that rats receiving .4 or 3 mg THC/kg as marijuana smoke for 75 days exhibited a decreased number of epididymal sperm, and an increased number of sperm with head to tail dissociations. These sperm changes were accanpanied by a decrease in testicle weight, a decrease in the weight of the seminal vesicles, and an increase in adrenal gland weight.

Dalterio et al. (1982) treated male mice with THC, CBD, or CME (25 mg/kg) daily for 5 days. The mice were killed 50 to 60 days after cessation of drug treatment and meiotic chromosomes were prepared for microscopic examination from the testes. They reported that THC treated animals had a higher frequency of unpaired sex chromosomes at metaphase than did controls. Other chromosomal aberrations reported for cannabinoids include: ring and chain translocations, an increased incidence of aneupolidy, and a higher frequency of polyploidy. They also report that the cannabinoid treated males impregnated significantly fewer females, that the females successfully impregnated had a higher pre- and post-natal death rate and an increased percentage of pregnancies with fetal loss. The F^1 male offspring, of these cannabinoid exposed males, who successfully impregnated females produced litters that had a higher incidence of developmental anomalies or that exhibited chromsomal rearrangements.

Several studies have been reported that may help to explain the reduced sperm production as well as the abnormalities that are seen in the semen after treatment of animals with various cannabinoids. Jakubovic and McGeer (1977) studied the effects of THC and various water insoluble cannabinoids on metabolism in testicular slices and testicular cell suspensions. Cannabinoids (.1 mM) caused significant diminution in the amount of radioactivity that was incorporated into nucleic acids, lipids, and proteins in the testis slices. The cannabinoids inhibited the synthesis of labeled nucleotides and the incorporation of radioactive amino acids into proteins. They concluded that cannabinoid treatment produces a direct inhibitory effect of the cannabinoids on the kinases and/or polymerases involved in the RNA or DNA synthesis and that the inhibition of protein synthesis may be, in turn, a result of decreased chromosomal DNA, which could ultimately lead to alteration in spermatogenesis.

Jakubovic et al. (1979) also examined the effects of cannabinoids on testosterone and protein synthesis in rat testis Leydig cells _in vitro_. Various cannabinoids were added to the Leydig cell preparation at .15 mM concentration. It was found that HOG stimulated preparations incurred a 50% reduction in testosterone synthesis. However, the nonstimulated cultures had no effect on basal steroidogenesis even at cannabinoid concentrations of 15 mM. They concluded that cannabinoids at this high concentration may interfere directly with the Leydig cells to inhibit both protein synthesis and testosterone synthesis, and that the consequence of this inhibition might be affected spermatogenesis.

Several investigators have examined the effect that cannabinoids have on the enzymes of the testis in both _in vivo_ and _in vitro_

systems. There is evidence that cannabinoids affect the enzymes found in Sertoli cells in the seminiferous tubules which are important in sperm maturation. Schwartz et al. (1977) have shown that treatment of rats with THC (2 mg/kg I.P. for 9 days) or with cannabidiol (2 mg/kg I.P. for 9 days), produced a decrease in both microsomal cytochrome P-450 Leydig cells of the testis (an enzyme which is involved in the biosynthesis of testosterone) and gamma-glutanlyl transpeptidase (a marker protein for Sertoli cells in the seminiferous tubules of the testis). Normal levels of cytochrome P-450 and gamma-glutamyl transpeptidase were restored when exogenous LH and FSH were supplied to the THC treated animals. These results indicate that some enzymatic defects caused by cannabinoid exposure may be prevented if normal levels of the gonadotropins are maintained and that cannabinoid effects on the testis might be due to defects of pituitary hormones rather than a direct action of the drugs on the testis. Goldstein et al. (1977) reported that CBD (2 mg/kg/day, I.F.) and THC (2 mg/kg/day I.P.) depressed an esterase isozyrne of the rat Ieydig cells after 10 days of treatment with the cannabinoids. Also, Hubbard et al. (1979) reported a reduced cholesterol esterase activity in cultured rat Leydig cells at a concentration of 16 mM. This enzyme hydrolyzes the cholesteryl esters of oleic, arachadonic, and palmitic acids.

A group of investigators (Burstein et al. 1978a, 1978b, 1979 and Shoupe et al. 1980) has examined the effect of various cannabinoids on the enzymes, involved in the synthesis of testosterone, that are located in the Leydig cells. An early study (Burstein 1978a) indicated that neither THC nor CBN (1 and 5 micrograms/ml) altered the transformation of either pregnenalone or progesterone to testosterone, a dose of cannabinoid that caused a 60% decrease in overall testosterone synthesis *in vitro*. They postulated that the cannabinoids either blocked the conversion of cholesterol to pregnenalone or somehow affected the availability of precursor-cholesterol. Burstein et al. (1979) used isolated mouse Leydig cells as a model to examine the stepwise steroidogenic pathway for the site of action of THC and concluded that THC inhibition was effected at the release of precursor cholesterol from its ester storage. Shoupe et al. (1980) showed that THC inhibited crude Leydig cell cholesterol esterase preparations with a mixed type of inhibition.

Cannabinoids are relatively insoluble in the aqueous medium used to culture testicular cells which raises the question of what the concentration of cannabinoids is in the culture media and how cannabinoids may affect other constituents of the culture media. A further complication is the fact that the actual tissue concentration *in vivo* may never approach the levels that were added to cultures to cause the results reported *in vitro*. At least one study (Nahas et al. 1981) indicated that there was no selective concentration of radioactive Δ^8-THC in the cells of the testis of mice administered the compound. Thus, some of the observations reported for alteration in protein synthesis and testosterone synthesis may be an artiface of an *in vitro* system and may not be representative of what was actually occurring *in vivo*.

THE EFFECTS OF THC ON PROLACTIN SECRETION

The reports of research which examined the effect of THC on prolactin secretion in the male are somewhat controversial and contain conflicting reports. Some of the apparent conflict can be attributed to the method of administration of the THC, and perhaps the dosage of THC as well. Collu (1976) administered THC (20µg) intraventricularly for a week to prepubertal and to adult rats. He reported that pituitary levels of prolactin were increased in both prepubertal and adult animals, and noted that there were no changes in brain levels of noradrenaline, dopamine, or serotonin following drug treatment. Kramer and Ben-David (1974) had reported a suppression of prolactin release by acute THC treatment. The administration of the serotonin antagonist, cyprohepatidine, or the dopamine antagonists, perphenazine and chemozine, was able to abolish the THC induced suppression of prolactin. They also questioned whether the ability of THC to decrease TRH, a hormone known to cause prolactin release, might be responsible for the THC-induced suppression of prolactin release.

Bromley et al. (1977) administered a rather high dose of THC (30 mg/kg I.P.) to male rats and showed a marked suppression of prolactin as quickly as 15 minutes after THC injection. They reported that THC blocked the release of prolactin in ether-stressed rats, and also found that THC impaired, but did not prevent, a significant pituitary response to perphenazine.

Dalterio et al. (1981) reported a reduction of plasma prolactin levels in stressed and non-stressed male mice after a single dose of THC (50 mg/kg P.O.). However, chronic treatment had no effect on prolactin under these conditions (3 times/week/3 weeks). Chronic exposure to CBN resulted in reduced plasm prolactin levels in stresssed mice, indicating that perhaps non-psychoactive ingredients in marijuana, at least in the dosages used in this study, can exert effects on endocrine function. Daley et al. (1974) reported that male rats injected with THC (4 and 16 mg/kg I.P.) had heavier pituitary gland weights than controls at both dosage levels and that, although serum prolactin was significantly increased, there was no increase in the total piuitary concentration of prolactin.

Smith et al. (1979b) and Asch et al. (1979b) have shown that administration of THC (2.5 or 2 mg/kg I.M.) produced a short-lived inhibition of prolactin levels in the serum of male Rhesus monkeys. Administration of thyrotropin releasing hormone (TRH) was able to increase prolactin levels in THC treated monkeys, indicating that the hypothalamus was the site of action in the THC-induced depression of prolactin release fran the pituitary.

Although there are conflicting reports on the ability of THC to either increase or decrease prolactin levels in the pituitary and in the serum, it appears that the effect of THC and perhaps other non-psychoactive ingredients in marijuana is to produce a prompt depression of prolactin secretion from the pituitary gland in the male. The exact mechanism of this inhibition is not known at this

time; however, marijuana probably has some action on the hypothalamus to alter secretion of the several neural transmitters which are probably involved in eliciting prolactin release fran the pituitary gland. The ability of THC to inhibit the release of prolactin, LH, and FSH fran the pituitary would appear to be unique, and in apparent contrast to many other psychoactive drugs which stimlate prolactin release while inhibiting the secretion of both LH and FSH. It is also apparent that the male pattern of response to THC with repressed prolactin levels is the same as has been shown to occur in female animals and it is probable that the reduction is caused by the same mechanism that cause the reduction in females.

EFFECT OF MARIJUANA THE ADRENAL GLAND

Cortical Hormones

Exposure to stressful situations elicits a prompt secretion of adrenocortical steriods which help the organisms to counteract the stress. The adrenal cortex responds to acute cannabinoid treatment with a prompt rise in corticosterone levels in the plasma. Exposure to a wide range of dosages of THC ranging fran 2 to 50 mg/kg body weight produced increased corticosterone levels in the plasm of both the rat and the mouse. Dewey et al. (1970) showed that ascorbic acid, which is inversely correlated to adrenal cortical hormne secretion, was depleted fran the adrenal cortex of laboratory rats. Maier and Maitre (1975) demonstrated that. the increased corticosterone in plasma of rats pretreated with THC was accompanied by a decrease in adrenal cortical cholesterol, a precursor to adrenal cortical hormones, and an increase in unesterified fatty acids; however, the rabbit did not respond to THC with a similar increase in wrtisol.

Birmingham and Bartova (1976) showed that the response of elevated plasma corticosterone to THC disappeared after 8 days of treatment with a dose of 3 mg/kg body weight. Pertwee (1974) also showed that tolerance developed to the effect of THC on corticosterone levels in muse plasm and did so without impairing the effect of stress in mobilizing wrticosterone release. Mitra et al. (1977) found no evidence of tolerance after 21 days of THC (10 mg/kg/day) administration.

Bromley and Zimmerman (1976) examined the possibility that THC acts as a general systemic stressor. Generally, stress causes an increase in both prolactin and corticusterone levels in male rats. However, their results indicated that, unlike most stressful stimuli, acute administration of THC caused a depression in prolactin while stimulating corticosterone release.

Several studies have indicated that the wrticosterone response to THC was mediated through the pituitary by the action of THC on central nervous structures to modify the secretion or release of ACTH from the hypothalamus. Barry et al. (1973) ruled out the possibility that THC acted directly on the adrenal cortex to elicit corticosterone release. Their study employed hypophysectomized rats which had been administered THC at 2 mg/kg body weight, a

dosage which significantly increases plasma corticosterone levels. After hypophysectcmy, THC was unable to increase plasma corticosterone levels. Puder et al. (1982) further showed that adult male rats, bearing complete hypothalamic deafferentations, who were injected with THC (5 mg/kg) had no significantly altered serum concentration of either ACTH or corticosteroids. These results demonstrate that extrahypothalamic sites and/or neural pathways mediate the effect of THC to decrease corticosterone secretion.

Further evidence for a central action of THC comes fran the study of Malor et al. (1978). Mice treated with THC had a dosedependent rise in plasma nonesterified fatty acids (NEFA) due to THC's action in elevating corticosterone. The THC induced rise in plasma NEFA was blocked by prior administration of the dopamine receptor antagonists, perphenazine or pimozide. Thus the elevation of plasma NEFA produced in mice, by THC, is probably centrally mediated and requires the presence of functional dopaminergic receptors which presumably cause ACTH to be released to effect the corticosterone induced rise in plasma NEFA. Moreover, pentobarbital (75 mg/kg) (Mitra et al. 1977) and dexamethasone (Kokka and Garcia 1974), two agents which block ACTH secretion, also prevent the adrenocortical response to THC (10 mg/kg).

Several investigators have examined the accmulation of radioactive THC by various brain structures. Erdmann et al. (1976) showed that the amount of radioactive THC taken up by the preoptic areas, hypothalamus and pituitary, structures which are important for the regulation and release of hormones, did not concentrate more radioactive compound than other brain structures. Also there was a reduction in the amount of radioactive wrticosterone that was accumulated by the hypothalamus and thalamus if rats were treated with THC at high doses (9 mg/kg I.P.). Interestingly, and perhaps in apparent contrast, smaller doses (3 mg/kg I.P.) actually increased corticosterone uptake (Drew and Slagle 1973). However, Johnson et al. (1978) showed that high doses of cannabinoids (30 and 100 mg/kg) increased uptake of radioactive wrticosterone by the whole brain. The cannabinoids examined were 11-hydroxy-delta-9-THC, THC, and cannabinol (CBN). Pretreatment with THC at 3, 10, or 100 mg/kg SC increased the affinity of the hippocampus for tritiated corticosterone, but its concentration was decreased in the hypothalamus, mid-brain, pons, and medulla.

Collu (1976) injected THC (20 µg) directly into the ventricles of the rat brain and produced an increase in the general activity of cells in the adrenal and pituitary glands which was correlated with an increased corticoceterone production. Chronic treatment of rats and mice with THC produced an increased adrenal weight, which returned to control levels upon cessation of drug treatment (Dixit et al. 1974). Pats pretreated with THC (5 mg/kg I.P.) were still able to release corticosterone in response to stress (Jacobs et al. 1979).

Apparently not all of the effects of cannabinoids on adrenocortical function are due to the effect that they have to stimlate ACTH and corticosterone production by a direct endocrine route. Several

investigators have examined the effect of THC, CNB, and CBD on muse and rat adrenal cortical cells grown in tissue culture. Addition of 10^{-6} to 10^{-4}M cannabinoid to the incubation medium in which the cultured adrenal cortical cells were being grown prevented the cells from responding to added ACTH (Carchman et al. 1976, Warner et al. 1977). The cannabinoids appear to depress the synthesis of adrenal cortical steroids at a site between synthesis of cAMP and of pregnenalone (Warner et al. 1977). Delta-9-tetrahydrocannibinol (3.2 and 16 µM) added to an incubation medium that contained homogenized rat adrenal cells produced an inhibition of cholesterol esterase activity similar to that found in Leydig cells (Burstein et al. 1978a).

Adrenal Medulla

Several studies have examined the effect of THC on other cannabinoids on catecholamines and 5-hydroxytryptamine turnover in brain and adrenals. Johnson et al. (1981) showed that 30 mg/kg of THC increased the ammount of tritiated tryptophane found in the brains of mice and increased the amount of tritiated 5-hydroxytryptamine that was synthesized during the lo-minute period before decapitation. Previous studies have shown that this effect of THC was mediated by increased levels of plasma corticosterone in the mice produced by treatment with THC, since adrenalectomy inhibited it. Thus, there is a possibility that wrticosterone may mediate the effect of THC on tryptophane disposition and metabolism.

Mazurkiewics-Kwilecki and Filczewski (1973) showed that administration of THC (2 mg/kg daily) for 1 week caused an increase in the synthesis of tritiated catecholamines in the brain and adrenals. However, the endogenous norepinephrine and dopamine concentration in the brain, and epinephrine and norepinephrine levels in the adrenals, were apparently unaltered. The ability of THC to increase serotonin in the brain of mice has been confirmed by Welch et al. (197l), who showed that intraperitoneal injection of 10 mg/kg elevated serotonin concentration in the telencephalon by 30% within 10 minutes after injection but had no effect on the endogenous levels of brain norepinephrine or dopmine. Delta-9-tetrahydrocannabinol, on the other hand, depleted adrenal epinephrine by 25% within 10 minutes. Mitra et al. (1976) showed that administration of a single dose of THC (10 or 50 mg/kg) produced a dose-dependent increase in the content of norepinephrine and epinephrine in the rat adrenal gland and that THC given acutely both at low and high doses decreased the rate of synthesis and increased the rate of depletion of dopamine, decreased the rate of depletion of norepinephrine, and increase the rate of depletion of epinephrine. Biswas et al. (1975) also showed that THC affected the rat adrenal medulla; acute treatment (10 mg/kg) caused a decrease in catecho lamines, and chronic treatment (10 mg/kg for 30 days) produced a similar decrease in total catecholamine content of the adrenal medulla, as well as an hypertrophy of chromaffin substance.

THE EFFECT OF MARIJUANA ON THYROID GLAND

The function of thyrotropin releasing hormone (TRH) is, to elicit thyrotropin (TSH) secretion from the pituitary which in turn stimulates the thyroid gland to concentrate iodide from the blood, and to synthesize and secrete the thyroid hormones. TRH is secreted from the hypothalamus in response to dopamine and norepinephrine stimulation. Grimm and Reichlin (1973) showed that incubation of the hypothalamic tissue from mouse brain with either neurotransmitter, dopamine, or norepinephrine, was effective in causing a release of TRH. However, agents which blocked conversion of dopamine to norepinephrine were unable to elicit this response. They also showed that whereas acetylcholine had no effect on TRH release, serotonin tended to inhibit TRH release

The first indication that thyroid gland function might be affected by marijuana was reported in 1965 by Miras, who showed that cannabis resin administered to rats produced a depression of radioactive iodine accumulation in the thyroid gland. He was, however, unable to show whether the cannabinoids in the extract affected thyroid gland function directly at the site of the thyroid gland or whether the action of the cannabinoids was by alteration of hypothalamic output of TSH. Lomax (1970) injected marijuana distillate extract into rats and found a decreased release of radioactive iodine from the thyroid glands of rats. He showed that the hypothalamus was probably the site of action by injecting TSH during the period when marijuana was maximally depressing the radioactive iodine release and reversed the inhibition of marijuana on radioactive iodine release.

Nazar et al. (1977) reported that acute administration of THC at 2.5 mg/kg, and chronic treatment for 3 days, depressed serum thyroxine concentration in the rat. Single injections depressed thyroxine in the serum for 6 hours following drug treatment. Administration of TSH elevated serum thyroxine, indicating that THC probably had its action on thyroid function at the level of the hypothalamus. Under chronic THC treatment, however, twice daily for 14 days, an apparent tolerance to the thyroid depressant action of THC appeared to develop. They further showed that the presence of the adrenal hormone was not required for the depressant action of THC on thyroid gland function. Esber et al. (1976) administered 2 and 4 mg of THC per day by inhalation to rats and produced a significant lowering of triicdothyronine (T_3) in the serum; serum thyroxine was not affected. Oral administration of THC to rats (10 mg/kg/day for 14 days) had a more dramatic effect on serum hormonal levels than administration by inhalation of marijuana smoke. Oral administration significantly depressed both triiodothyronine and thyroxine.

Administering THC or smoking marijuana produced a decrease in body temperature, with a peak response observed between 1 and 2 hours after drug administration, which lasted from 5 to 6 hours. Bhargava (1980) was able to antagonize the hypothermic response to THC in mice by either intracerebral or intraperitoneal administration of TRH prior to the THC injection. Similar effects were produced by

446-384 O - 84 - 5

histidyl proline diketopiperazine (HPD), postulated as a metabolite of TRH, if given intracerebrally; however, it was completely ineffective if given intraperitoneally. Thus, the antagonism of THC-induced hypothermia by TRH may be mediated by its conversion to HPD in the central nervous sytem.

EFFECT OF MARIJUANA ON GROWTH HORMONE

There is a body of evidence to indicate that the exposure of animals to marijuana, a marijuana extract, cannabinoids, or the psychoactive ingredient, THC, produced depressed body weights and altered organ sizes. There are a number of reasons to believe that marijuana affects the normal growth processes. Growth of the organism and its organ systems is a complex and highly integrated system affected by many external and internal factors such as nutrition, heredity, and hormonal regulation. Although many hormones interact to affect growth processes, one of the more important hormones is growth hormone; for this reason several research workers have examined the effect that THC has on growth hormone. Collu et al. (1975) injected prepubertal male rats intraperitoneally three times a week for a month with either 1 or 10 mg/kg of THC. Treatment with 10 mg/kg produced rats with smaller tails and prostates than non-treated animals and, in addition, plasma levels of growth hormone and LH were decreased at all drug dosages. While pituitary quantities of GH and LH remain unchanged, they reported no effect on FSH and brain levels of norepinephrine, epinephrine, serotonin, and 5-hydroxyindole acetic acid which were comparable to those of the control group.

Collu (1976) administered THC (20 µg) each day for a week to prepubertal and adult rats intraventricularly. Prepubertal rats had lower prostate weight; however, they had increased plasma and pituitary levels of growth hormone. Kokka and Garcia (1974) showed that administration of 5 to 20 µm/kg of THC to adult rats caused an inhibition of growth hormone secretion and a stimulation of ACTH secretion. Dexamethasone, a potent supressor of ACTH secretion, blocked the stimulatory affect of an acute injection of THC on ACTH secretion but did not affect its inhibitory action of GH secretion. Treatment of rats with pentobarbital usually induces a rise of plasma growth hormone; however, THC was able to suppress the pentobarbital induced rise of plasma GH. Dalterio et al. (1981) report that growth hormone levels in the plasma of male mice that received a single dose of THC were reduced only in non-stressed animals, whereas chronic treatment with THC did not affect prolactin or growth hormone levels under either stressed or non-stressed conditions. In apparent contrast to the effects of THC, acute administration of CBD, a non-psychoactive ingredient in marijuana, produced increased plasma GH levels in non-stressed mice, while repeated CBN treatments reduced GH levels in stressed animals.

SUMMARY

Marijuana affects a variety of hormones that are regulated by hypothalamic function and it appears that the psychoactive ingredient, THC, is the major compound responsible for this action. It is

probable that THC affects these hormones through its ability to alter various neural transmitters in the hypothalamus or neural transmitters in the CNS which impinge on the hypothalarms. The dopaminergic and serotonergic fibers seem to be particularly important. The two gonadotropins, LH and FSH, secreted by the pituitary gland are of major importance to reproduction in the male. Both gonadotropins appear to respond to a single releasing factor fran the hypothalmus, GnRH, which is sensitive to catecholamine neurotransmitters. The THC-induced block of GnRH release results in lowered LH and FSH which is responsible for reduced testosterone production by the Leydig cells of the testis. Other hormones that might have a synergistic or antagonistic effect upon reproduction in the male are the adrenal cortical hormones, prolactin, thyroid hormones, and growth hormones. THC appears to depress prolactin, thyroid gland function, and growth hormone while elevating adrenal cortical steriods.

Chronic exposure of laboratory animals, such as rats, mice, and monkeys to marijuana and to the various cannabinoids in marijuana has altered the function of several of the accessory reproductive organs. Reports of reduced prostate and seminal vesicle weights, as well as altered testicular function, have been partially explained by the effect of marijuana in lowering serum testosterone needed for proper function and support. Although some of the change in organ weight may be due to lowered testosterone production by the Leydig cells of the testis, sane of the weight changes may be due to a direct action of THC, and perhaps some of the other nonpsychoactive cannabinoids in marijuana, on the tissue themselves. Also, of concern are the reports that acute cannabinoid treatments affects the quality and quantity of spermatozoa produced by the testis. The question is still unanswered as to whether or not the effects observed on spermatozoa are due to a direct action of the cannabinoids on spermatogenesis, or whether some of the observed effects may be due to altered hormone levels which are necessary for the support of spermatogenesis. Reduced testosterone and FSH may be important in producing the observed changes in sperm production by the seminiferous tubules.

Many of the effects on the endocrine system caused by chronic treatment of animals with THC are completely reversible with time and there is reason to believe that tolerance develops to these effects with acute exposure to THC. Still, many unanswered questions remain regarding the long-term consequence of marijuana use on such important functions of male reproduction as sperm formation and maturation, and the long-term effects on the function of the sexual organs. Until the time that answers to these and other questions are forthcoming, it is questionable whether marijuana should be consumed by adolescent males or those males with marginal fertility problems.

REFERENCES

Asch, R.H., Fernandez, E.O., Smith, C.C., and Pauerstein, C.J. Precoital single doses of delta-9-tetrahydrocannabinol block ovulation in the rabbit. Fertil Steril, 31:331, 1979a.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., and Pauerstein, C.J. Acute decrease in serum prolactin concentrations caused by delta-9-tetrahydrocannabinol in nonhuman primates. Fertil Steril, 32(5):571-575, 1979b.

Barry, J., III, Kubena, R.K., Perhach, J-L., Jr. Pituitary-adrenal activation and related responses to delta-1-tetrahydrocannabinol. Prog Brain Res, 39:323-330, 1973.

Bhargava, H.N. The effect of thyrotropin releasing hormone and histidylproline diketopiperazone on delta-9-tetrahydrocannabinol-induced hypothermia. Life Sci, 26(11):845-850, 1980.

Birmingham, M.K., and Bartova, A. Effects of cannabinol derivatives on blood pressure, body weight, pituitary-adrenal function, and mitochondrial respiration in the rat. In: Nahas, G.G. et al., eds. Springer, New York, 1976. pp. 425438.

Biswas, N., Deb, G., and Ghosh, J.J. Changes in rat adrenal medulla following delta-9-tetrahydrocannabinol treatment. A histochemical study. Acta Endocrinol, 80(2):329-338, 1975.

Bromley, B., Gordon, H., and Zimmerman, E. Delta-9-tetrahydrocannabinol inhibition of ether and perphenazine induced release of prolactin in male rats. Fed Proc, 36(3):1026, 1977.

Bromley, B., and Zimmerman, E. Divergent release of prolactin and corticosterone following delta-9-tetrahydrocannabinol injection in male rats. Fed Proc Am Soc Exp Biol, 35:220, 1976.

Burstein, S., Hunter, S.A., Shoupe, T.S., and Taylor, P. Cannabinoid inhibition of testosterone synthesis by mouse Leydig cells. Res Com Chem Pathol Pharmacol, 19(3):557-560, 1978a.

Burstein, S., Hunter, S.A., and Shoupe, T.S. Inhibition of cholesterol esterases by delta-1-tetrahydrocannabinol. Life Sci, 23: 979-982, 1978b.

Burstein, S., Hunter, S.A., and Shoupe, T.S. Site of inhibition of Leydig cell testosterone synthesis by delta-l-tetrahydrocannabinol. Mol Pharmacol, 15(3):633-640, 1979.

Carchman, R.A., Warner, W., White, A.C., and Harris, L.S. Cannabinoids and neoplastic growth. In Nahas, G.G., ed. Marihuana: Chemistry, Biochemistry and Cellular Effects. New York: Springer-Verlag, 1976. pp, 329-345.

Collu, R. Endocrine effects of chronic intraventricular administration of delta-9-tetrahydrocannabinol to prepuberal and adult female rats. Life Sci, 18:223-230, 1976.

Collu, R., Letarte, J., Leboeuf, G., and Ducharme, J.R. Endocrine effects of chronic administration of psychoactive drugs to prepuberal male rats. I: delta-9-tetrahydrocannabinol. Life Sci, 16:533-542, 1975.

Daley, J.D., Branda, L.A., Rosenfeld, J., and Younglai, E.V. Increase of serum prolactin in male rats by (-)-trans-delta-9-tetrahydrocannabinol. J Endocrinol, 63:415-416, 1974.

Dalterio, S., Badr, F., Bartke, A., and Mayfield, D. Cannabinoids in male mice: effects on fertility and spermatogenesis. Science, 216(4543):315-316, 1982.

Dalterio, S., Bartke, A., Roberson, C., Watson, D., and Burstein, S. Direct and pituitary-mediated effects of delta-9-tetrahvdrocannabinol and cannabinol on the testis. Pharmacol Biochem Behav 8:673-687, 1978.

Dalterio, S., Michael, S.D., MacMillan, B.T., and Bartke, A. Differential effects of cannabinoid exposure and stress on plasma prolactin growth hormone and corticosterone levels in male mice. Life Sci, 28(7):761-766, 1981.

Dewey, W.L., Peng, T.C., and Harris, L.S. The effects of 1-trans-delta-9-tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of rats. Eur J Pharmacol, 12(3):382-384, 1970.

Dixit, V.P., Jain, H.C., Verma, O.P., and Sharma, A.N. Effects of cannabis extract on the testicular function of the toad Bufo andersonii Boulenger. Indian J Exp Biol, 15(7):555-557, 1977.

Dixit, V.P., and Lohiya, N.K. Effects of cannabis extract on the response of accessory sex organs of adult male mice to testosterone. Indian J Physol Pharmacol, 19(2):98-100, 1975.

Dixit, V.P., Sharma, V.N., and Lohiya, N.K. The effect of chronically administered cannabis extract on the testicular function of mice. Eur J Pharmacol, 26:111-114, 1974.

Drew, W.G., and Slagel, D.E. Delta-9-tetrahydrocannabinolinol selective impairment of corticosterone uptake by lymbic structures of the rat. Neuropharmacol, 12(9):909-914, 1973.

Erdmann, G., Just, W.W., Thel, S., Werner, G., and Wiechmann, M. Comparative autoradiographic and metabolic study of Δ-8- and Δ-9-tetrahydrocannabinol in the brain of the marmoset Callithrix jacchus. Psychopharmacol, 47:53-58, 1976.

Esber, H.J., Rosenkrantz, and Bogden, A.E. Assessment of the effect of delta-9-tetrahydrocannabinol on testicular and thyroid hormone levels in rats. Fed Proc, 35(3);727, 1976.

Fujimoto, G.I., Rosenbaum, R.M., Ziegler, D., Rettura, G., and Morrill, G.A. Effect of marijuana extract given orally on male rat reproduction and gonads. Proc 60th Ann Meet Endocr Soc, 1978. pp. 373.

Fuxe, K., and Jansson, G. Effect of tetrahydrocannabinol on central monoamine neurons. Acta Pharm Suec, 8:695, 1972.

Ghosh, S.P., Chatterjee, T.K., and Ghosh, J.J. Antiandrogenic effect of delta-9-tetrahydrocannabinol in adult castrated rats. J Reprod Fert, 162(2):513-517, 1981.

Goldstein, H., Harcelrode, J., and Nyquist, S.E. Effects of chronic administration of delta-9-tetrahydrocannabinol and cannabidiol on rat testicular esterase isozymes. Life Sci, 20(6):951-954, 1977.

Greenberg, J.H., Mellors, A., and McGowan, J.C. Molar volumes relationships and the specific inhibition of a svnaptosomal enzyme by psychoactive cannabinoids. J Med Chem 21:1208-1212, 1978;

Grimm, Y., and Reichlin, S. Thyrotropin-releasing hormone (TRH): Neurotransmitter regulation of secretion by mouse hypothalamic tissue in vitro. Endocrinology, 93:626-631, 1973.

Harper, J.W., Heath, R.G., and Myers, W.A. Effects of Cannabis saliva on ultrastructure of the synapse in monkey brain. J Neuroscience, 3:87-93, 1977.

Hershkowitz, M., Goldman, R., and Raz, A. Effect of cannabinoids on neurotransmitter uptake. ATPase activity and morphology of mouse brain synaptosanes. Biochem Pharmacol, 26:1327-1331, 1977.

Howes, J., and Osgood, P. The effect of delta-9-tetrahydrocannabinol on the uptake and release of ^{14}C-dopamine from crude striatal synaptosomal preparations. Neuropharmacology, 13:1109-1114, 1974.

Huang, H.F.S., Nahas, G.G., and Hembree, W.C. Effects of marihuana inhalation on spermatogenesis of the rat. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana: Biological Effects. New York: Pergamon Press, 1979. pp. 419-427.

Hubbard, C.D., Burstein, S., Hunter, S.A., and Shoupe, T.S. Inhibition of cholesterol esterase by cannabinoids. Fed Proc Am Soc Exp Biol, 38(3):510, 1979.

Jacobs, J.A., Dellarco, A.J., Manfredi, R.A., and Harclerode, J. The effects of delta-9-tetrahydrocannabinol, cannabidiol, and shock on plasma corticosterone concentrations in rats. J Pharm Pharmacol, 31:341-342, 1979.

Jakubovic, A., and McGeer, P.L. Inhibition of rat brain protein and nucleic-acid synthesis by cannabinoids in vitro. Canad J Biochem, 50:654-662, 1972.

Jakubovic, A., and McGeer, P.L. Biochemical changes in rat testicular cells in vitro produced by cannabinoids and alcohol: metabolism and incorporation of labeled glucose, amino acids, and nucleic acid precursors. Toxicol Appl Pharmacol, 41(3):473-486, 1977.

Jakubovic, A., McGeer, E.G., and McGeer, P.L. Biochemical alterations induced by cannabinoids in the Leydig cells of the rat testis in vitro: effects of testosterone and protein synthesis. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana: Biological Effects. New York: Pergamon Press, 1979. pp. 251-264.

Johnson, K.M., Dewey, W.L., and Bloom, A.S. Adrenalectomy reverses the effects of delta-9-tetrahydrocannabinol on mouse brain 5-hydroxytryptamine turnover. Pharmacology, 23:223-229, 1981.

Johnson, K.M., Dewey, W.L., Ritter, K.S., and Beckner, J.S. Cannabinoid effects on plasma corticosterone and uptake of ^{3}H-corticosterone by mouse brain. Eur J Pharmacol, 47:303-310, 1978.

Kokka, N., and Garcia, J.F. Effects of delta-9-tetrahydrocannabinol on growth hormone and ACTH secretion in rats. Life Sci, 15: 329-338, 1974.

Kramer, J., and Ben-David, M. Suppression of prolactin secretion by acute administration of delta-9-tetrahydrocannabinol in rats. Proc Soc Exp Biol Med, 147:482-484, 1974.

Lomax, P. The effect of marijuana on pituitary-thyroid activity in the rat. Agents and Actions, 1:252-257, 1970.

Maier, R., and Maitre, L. Steroidogenic and lipolytic effects of cannabinoids in the-rat and the rabbit. Biochem Pharmacol, 24 (8):1695-1699, 1975.

Malor, R., Jackson, D.M., and Chesher, G.B. Possible central dopaminergic modulation of the rise in plasma concentration of non-esterified fatty-acids produced in the mouse by levo trans-delta-9-tetrahydrocannabinol. Biochem Pharmacol, 27(4):407-414, 1978.

Marks, B.H. Δ^1-tetrahydrocannabinol and luteinizing hormone secretion. Prog Brain Res, 39:331-338, 1973.

Mazurkiewics-Kwilecki, I.M., and Filczewski, M. The effects of chronic treatment with delta-9-tetrahydrocannabinol on catecholamine synthesis in the rat. Psychopharmacologia (Germany-West), 33(1):71-79, 1973.

Miras, C.J. Some aspects of cannabis action. In: Wolstenholme, G.E.W., and Knight, J., eds. Hashish: Its Chemistry and Pharmacology, CIBA Found. Study group 21. Boston: Little, Brown, 1965. pp. 37-52.

Mitra, G., Poddar, M.K., and Ghosh, J.J. 1976. Delta-9-tetrahydrocannabinol: effect on adrenal catecholamines. Toxicol Appl Pharmacol, 37:517-524, 1976.

Mitra, G., Poddar, M.K., Ghosh, J.J. Interaction of detal-9-tetrahydrocannabinol with resperine. phenobarbital, and LSD-25 on plasma and adrenal corticosterone. Toxicol Appl Pharmcol, 42(3):505-512, 1977.

Nahas, G., Leger, C., Tocque, B., and Hoellinger, H. The kinetics of cannabinoid distribution and storage with special reference to the brain and testis. J Clin Pharmcol, 21:208-214, 1981.

Nazar, B., Kairys, D.J., Fowler, R., and Harclerode, J. Effects of delta-9-tetrahydrocannabinol on serm thyroxine concentrations in the rat. J Pharm Pharmcol, 29(12):778-779, 1977.

Nir, I., Ayalon, D., Tsafriri, A., Cordova, T., and Lindner, N.R. Suppression of the cyclic surge of luteinizing hormone secretion and of ovulation in the rat by delta-1-tetrahydrocannabinol. Nature, 243(100):470-471, 1973.

Pertwee, R.G. Tolerance to the effect of delta-l-tetrahydrocannabinol on corticosterone levels in mouse plasma produced by repeated administration of cannabis extract or delta-l-tetrahydrocannabinol. Br Jour Pharmacol, 51(3):391-397, 1974.

Puder, M., Weidenfeld. J., Chowers, I., Nir, I., Conforti, N., and Siegel, R.A. Corticosterophin and Corticosterone secretion following delta-1-tetrahydrocannabinol, in intact and in hypothalamic deafferentiated male rats. Exp Brain Res (Gemany, West), 46(1):85-88, 1982.

Purchit, V., Singh, H.H., and Ahluwalia, B.S. Evidence that the effects of methadone and marihuana on male reproductive organs are mediated at different sites in rats. Biol Reprod, 20:1039-1044, 1979.

Revuelta, A.V., Cheney, D.L., Wood, P.L., and Costa, E. GABAergic mediation in the inhibition of hippocampal acetylcholine turn--over rate elicited by Δ^9-tetrahydrocannabinol. Neuropharmcology, 1:525-530, 1979.

Rosenkrantz, H., and Esber, H.J. Cannabinoid-induced hormone changes in monkeys and rats. T Toxicol Environ Health, 6(2):297-313, 1980.

Rosenkrantz, H., and Hayden, D.W. Acute and subacute inhalation toxicity of Turkish marihuana, cannabichromene, and cannabidiol in rats. Toxicol Appl Pharmacol, 48(3):357-386, 1979.

Schwarz, S., Harcelrode, J., and Nyquist, S.E. Effects of delta-9-tetrahydrocannabinol administration on marker proteins of rat testicular cells. Life Sci, 22:7-14, 1977.

Shoupe, T.S., Hunter, S.A., Burstein, S.H., and Hubbard, C.D. The nature of the inhibition of cholesterol esterase by delta-l-tetrahydrocannabinol. Enzyme, 25(2):87-91, 1980.

Smith, C.G., Moore, C.E., Besch, N.F., and Besch, P.K. The effect of marihuana delta-9-tetrahydrocannabinol on the secretion of sex hormones in the mature male Rhesus monkey. Clin Chem, 22(7): 1184, 1976.

Smith, C.G., Smith, M.T., Besch, N.F., Smith, R.G., and Asch, R.H. Effect of Δ^9-tetrahydrocannabinol (THC) on female reproductive function. In: Nahas, G.G., and Paton, W.D.M., eds. Marihuana: Biological Effects. New York: Pergamon Press, 1979a. pp. 449-467.

Smith, C.G., Ruppert, M.J., Asch, R.H., and Siler-Khodr, T. The effects of tetrahydrocannabinol on prolactin in the Rhesus monkey. Paper presented at the NIDA Conference on Genetic, Perinatal, and Developmental Effects of Abused Substances, Airlie, Virginia, 1979b.

Symons, A.M., Teale, J.D., and Marks, V. Effect of delta-9-tetrahydrocannabinol on the hypothalamic-pituitary-gonadal system in the maturing male rat. J Endocrinol, 68(3):43-44, 1976.

Truitt, E.B., Jr., and Anderson, S.H. Biogenic amine alterations produced in the brain by tetrahydrocannabinol and their metabolites. Ann NY Acad Sci, 191:68-73, 1972.

Tyrey, L. Δ^9-tetrahydrocannabinol suppression of episodic luteinizing hormone secretion in the ovariectomized rat. Endocrinology, 102(6):1808-1814, 1978.

Vyas, D.K., and Singh, R. Effect of cannabis and opium on the testis of the pigeon Columbia livia Gmelin. Ind J Exp Biol, 14: 22-25, 1976.

Warner, W., Harris, L.S., and Carchman, R.A. Inhibition of corticosteroidogenesis by delta-9-tetrahydrocannabinol. Endocrinology, 101(6):1815-1820, 1977.

Welch, B.L., Welch, A.S., Messiha, F.S., and Berger, H.G. Rapid depletion of adrenal epinephrine and elevation of telenephalic serotonin by (-)-trans-9-tetrahydrocannabinol in mice. Res Common Chem Pathol Pharmacol, 2(3):382-391, 1971.

Zimmerman., Bruce, W.R., and Zimmerman, S. Effects of cannabinoids on sperm morphology. Pharmacology, 18(3):143-148, 1979a.

Zimmerman, A.M., Zimmerman, S., and Raj, A.Y. Effects of cannabinoids on spermatogenesis in mice. In Nahas, G.G., and Paton, W.D.M., eds. Marihuana: Biological Effects. New York: Pergamon Press, 1979b. pp. 407-418.

AUTHOR

Jack Harclerode, Ph.D.
Department of Biology
Bucknell University
Lewisburg, Pennsylvania 17837

Endocrine Aspects of Cannabinoid Action in Female Subprimates: Search for Sites of Action

Lee Tyrey, Ph.D.

INTRODUCTION

There is no longer any doubt that marijuana and the cannabinoids which it contains have pronounced effects on endocrine function in humans and a variety of experimental animals. The increased availability of delta-9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana (Mechoulam 1970), and other cannabinoids has greatly stimulated research into the physiological effects of these unique agents. The results of this research clearly indicate that THC administration alters the plasma levels of several pituitary hormones, presumably by altering their secretion. Among these hormones are the gonadotropins and prolactin, the pituitary hormones that regulate reproductive function. The effects of THC on these reproductive hormones have been most pronounced in the female at least in part because the rather wide fluctuations in their secretion and the consequent pronounced cyclic changes in target tissues make almost any perturbation of this system easily discernible. At the same time, the fact that changes in a target tissue such as the uterus depend upon ovarian hormones, which in turn depend upon appropriate stimulation from the pituitary whose activity is regulated by the brain makes this system particularly complex. The functional dependencies make it difficult to determine dhere cannabinoids may exert their influence in the chain of interrelated events from the brain to the target tissue. It is not surprising, therefore, that questions remain regarding the extent of cannabinoid action on the uterus, ovary, pituitary, and brain. What follows is an attempt to review the evidence for the impact of cannabinoids, primarily THC, on these potential sites of action.

THE UTERUS

The possibility that THC may have a direct “estrogenic” effect on the uterus was raised by Solomon et al. (1976) who treated young adult ovariectomized rats with daily intraperitoneal injections of THC in dosages of 1, 2.5, or 10 mg/kg body weight (BW). Inject ions started on the day of ovariectomy and continued for 14 days. The reproductive tracts were removed for examination. All three doses

of THC had significant uterotropic effects as indicated by the occurrence of uterine weights greater than those in vehicle-treated ovariectomized rats. Nonetheless, the uterine stimulation was substantially below that produced by 2 ug/kg doses of estradiol benzoate. Of the three THC doses, the 2.5 mg/kg dose appeared to have a somewhat greater stimulatory effect than the lower or higher doses. Subsequently, Solomon et al. (1977) reported that cytologic examination of the reproductive tracts removed from the THC-treated ovariectomized animals indicated hyperplasia and hypertrophy of the uterus which was most pronounced in those animals treated with the intermediate 2.5 mg/kg dose. The uteri were reported to show growth of the surface epithelium, endometrial stroma, and myometrium, as welI as proliferation of the endometrial glands. The vaginal epithelium showed increased stratification and cornification, frequently with a superficial layer of mucoid cells. These findings contrasted to those with control animals in which the uteri were small and poorly vascularized as typical for the ovariectomized rat. While these interesting findings suggested that THC may have direct estrogenic activity, a possibility subsequently supported by a report that THC competitively inhibited the binding of 3H-estradiol to estrogen receptors (Rawitch et al. 1977), the bulk of the evidence detracts from that hypothesis.

Several studies in which intact or ovariectomized rats and mice were treated chronically with cannabis extract or THC (Dixit et al. 1975; Rosenkrantz et al. 1975; Okey and Truant 1975; Virgo 1979) indicated that uterine weights were either reduced or unaltered, even when treatment was continued as long as 180 days (Rosenkrantz et al. 1975). Okey and Truant (1975) found no evidence of uterine stimulation in immature or ovariectomized female rats after treatment with cannabis resin administered either in the diet or by intraperitoneal injection. Moreover, cannabis resin added to rat uterine cytosol preparations failed to compete with the binding of ^{3}H-estradiol to estrogen receptors. In later studies, Okey and Bondy (1977; 1978) conducted a detailed assessment of the potential 3H-THC binding to rodent mammary and uterine cytosol receptors, as well as of the ability of unlabeled cannabinoids to compete with estradiol for binding to receptor sites. They found that THC added to cytosol preparations even at the limit of its solubility did not compete with ^{3}H-estradiol for receptor binding. Similar results were obtained with the THC metabolite, 11-OH-THC. Studies utilizing sucrose density gradients indicated that while the ^{3}H-estradiol binding peak was typically in the 8s region, ^{3}H-THC binding was restricted to the 4-5s region and was not inhibited by excess amounts of either unlabeled THC or estradiol. These data provide compelling evidence that THC does not exert a direct estrogen effect on target tissues, since binding to the specific high-affinity estradiol receptor is a necessary precondition for such action.

Because THC does not interact with the uterine cytoplasmic estrogen receptor, the earlier findings of Solomon et al. (1976; 1977) await satisfactory explanation. Since THC activates the pituitary-adrenal axis and increases plasma corticosterone concentration (Kubena et al. 1971; Jacobs et al. 1979), Solomon et al. (1977) suggested that a presumptive increase in the secretion of adrenal steroids induced by THC treatment in their study may have altered the response of the castrate's reproductive tract to THC. It seems possible, however, that insofar as the adrenal cortex is capable of producing sex steroids which could act on the uterus, including considerable amounts of progesterone (Holzbauer et al. 1969), the adrenal activation by THC might in fact explain its "estrogenic" activity. Perhaps an examination of the uterine effects of THC in adrenalectomized-ovariectomized rats would settle the issue

THE OVARY

The possibility that cannabinoids exert direct effects on the ovary was raised by the observation that THC or its metabolites concentrate in the corpora lutea of mice (Freudenthal et al. 1972). Although this accumulation could be related merely to the lipophilic character of cannabinoids, the possibility of direct action was given physiological support by the observation of Nir et al. (1973). These investigators found that exogenous LH administration in a dose which was adequate to cause full ovulation in rats whose normal ovulation had been prevented by pentobarbital treatment was able to induce ovulation in only 40% of the animals in which ovulation was blocked by THC. Subsequently, the same group (Ayalon et al. 1977) found that two- to four-fold greater doses of ovine LH were required to restore ovulation in THC-treated animals as compared with those blocked with pentobarbital. Since the ovulatory surge of gonadotropins in the rat is followed by prostaglandin accumulation in the ovarian follicles (Bauminger et al. 1975), and since prostaglandins have been implicated in the mechanism of follicle rupture at ovulation (Tsafrifi et al. 1972), Ayalon and his colleagues (1977) measured the accumulation of ovarian prostaglandins following the injection of LH into proestrous rats whose spontaneous LH surges were blocked with either pentobarbital or THC. Consistent with the reduced ovulatory response to LH, the ovaries of the THC-treated animals had substantially reduced levels of prostaglandin E in comparison with the ovaries of pentobarbital-treated rats. The fact that higher doses of LH brought about a graded increase in prostaglandin accumulation and ovulation suggested that THC or its metabolites may interfere with the ovulatory process through an inhibition of ovarian prostaglandin synthesis.

Ovarian steroid synthesis and secretion also seem to be subject to direct inhibition by cannabinoids. Consistent with the inhibitory effects of cannabinoids on steroidogenesis in testicular Leydig cells (Burstein et al. 1978b) and adrenal tumor cells (Warner et al. 1977), hCG-stimulated progesterone secretion from dispersed rat luteal cells was reported to be inhibited by the addition of

THC or cannabinol to the incubation medium in micromolar concentrations (Burstein et al. 1979). Later work extended the observation of these THC inhibitory effects to isolated granulosa cells (Moon et al. 1982). FSH-stimulated progesterone secretion from incubated porcine granulosa cells was inhibited in a dose-related fashion by THC, 11-OH-THC, and SP-111A, a water soluble derivative of THC. The latter also was shown to inhibit basal as well as FSH-stimulated progesterone secretion from incubated rat granulosa cells. When entire rat follicles, which include the thecal compartment in addition to the granulosa cells, were cultured (Reich et al. 1982). THC and cannabidiol inhibited the release not only of progesterone but also of testosterone and estradiol. The inhibition was observed for both basal steroid secretion and that stimulated by the addition of LH to the explant incubation medium. It is interesting to note that the nonpsychoactive cannabinoids are somewhat more potent than THC in inhibiting steroidogenesis in ovarian cells (Burstein et al. 1979; Reich et al. 1982).

A unifying hypothesis to explain the similar actions of cannabinoids on steroidogenesis in the testis, ovary and adrenal was suggested by Burstein et al. (1978a) based on their observation that THC inhibits cholesterol esterase activity over the same range of concentrations effective in inhibiting steroid secretion. Since steroidogenesis is to a major extent dependent upon the availability of precursor free cholesterol, the inhibition of esterase activity can be viewed as the likely explanation of reduced steroid secretion from cells and tissues incubated with cannabinoids. Consistent with this hypothesis are observations that cannabinoids do not reduce LH-stimulated cyclic AMP production in rat follicle explants and that the addition of cholesterol to explant culture medium at least partially restores progesterone production in the presence of cannabinoids (Reich et al. 1982).

Thus there is clear evidence that both psychoactive and nonpsychoactive cannabinoids exert a direct inhibitory action on steroidogenesis. Whether the cellular site of action is cholesterol esterase or is ultimately shown to be elsewhere, it seems that the anti-steroidogenic activity of cannabinoids is unrelated to the unique psychoactive properties possessed by THC. The physiological consequences of these direct ovarian effects of cannabinoids, of course, remain to be demonstrated. Attempts to do this in estrous rabbits whose ovarian steroid secretion was stimulated by hCG injection failed to find any effect of THC on the secretory patterns of progesterone, testosterone, or dihydrotestosterone (Asch et al. 1979b). Of course, it is possible that the large dose of hCG (100 IU) overcame direct steroidogenic inhibitory effects of THC. In any case, one may speculate that the direct ovarian effects of THC identified *in vitro* are unlikely to be of major consequence in the functional sense view of the profound effects of THC on the ovarian regulatory hormones emanating from the pituitary.

THE HYPOTHALAMIC-PITUITARY AXIS

In the intact animal, the effects of cannabinoid treatment on ovarian function, as well as those on the adrenal (Kubena et al. 1971; Jacobs et al. 1979) and thyroid (Nazar et al. 1977), can be accounted for by the action of THC at the level of the brain or pituitary. The suppression of plasma thyroxine subsequent to THC treatment is readily overcome by the administration of thyroid stimulating hormone (Nazar et al. 1977), and the corticosterone elevation is accompanied by a marked elevation in ACTH levels (Puder et al. 1982). In the case of those pituitary hormones which act on the ovary, especially LH and prolactin, there is now abundant evidence that THC exposure can alter their secretion to an extraordinarv extent.

Luteinizing Hormone

The acute intravenous administration of THC to ovariectomized rats in doses of 1 to 10 mg/kg BW was found to significantly reduce the serum concentration of LH in samples taken 1 hr after treatment (Marks 1973). Since the secretion of LH from the pituitary occurs in an episodic fashion, consisting of semi-regular periodic pulsatile releases, a lowering of serum LH concentrations by THC could result either from an attenuation or reduced frequency of the secretory pulses or from a reduction in the basal secretion upon which such pulses occur. Subsequent work has shown that the decline in serum LH following THC treatment results not merely from an attenuation, but from a complete suppression of the episodic LH secretory pulses (Tyrey 1978). Serial blood sampling of individual animals after intravenous injections of THC in doses of 0.5 to 8 mg/kg BW revealed a complete, but transient, inhibition of LH pulses. The consequent rapid decline in serum LH occurred at a rate consistent with estimates for the serum half-life of endogenous LH in the ovariectomized rat (Weick 1977). It was thus not possible to discriminate between serum LH levels following THC treatment and those observed 20 and 45 min after hypophysectomy (Tyrey 1978) an observation implying that for a time following THC treatment little new LH was secreted into the circulation.

In later experiments with ovariectomized rats, the episodic secretion of LH was inhibited by acute intravenous THC treatment with doses as low as 62.5 ug/kg BW (Tyrey 1980). In both these and the earlier experiments with higher THC dosages no relationship was apparent between the THC dosage and the degree of LH inhibition produced; however, the duration of the inhibitory effect clearly depended upon the amount of THC administered. Although THC doses of 62.5 ug/kg suppressed LH secretion for less than 30 min, the duration of suppression increased with larger doses in a dose-related manner, continuing for 1 to 2 hrs when the dose reached 4 mg/kg BW (Tyrey 1978; 1980). The finding that the duration, but not the degree, of THC-induced LH suppression in the ovariectomized rat is dose-dependent is consistent with observations made in the ovariectomized monkey (Besch et al. 1977; Smith et al. 1979). The

rat and monkey data differ, however., in that THC administration to monkeys by intramuscular injection in doses equivalent to those utilized in the rat studies produced a considerably longer period of LH suppression, continuing for as long as 24 hrs in some cases. Whether this difference results solely from the different routes of drug administration used in these studies remains to be determined.

While the elevated gonadotropin secretion in the ovariectomized animal makes this model system particularly useful for investigating the inhibitory actions of THC, serum LH in intact cycling female rats also is suppressed following acute (Chakravarty et al. 1975) or chronic (Chakravarty et al. 1979) THC treatment. Moreover, experiments with intact cyclic female rats have shown that, in addition to suppressing circulating LH levels, THC also inhibits the spontaneous increase in LH secretion which brings about ovulation and conversion of the ruptured follicle to a corpus luteum. Nir et al. (1973) found that intraperitoneal administration of THC in doses which approximated 10 mg/kg BW would prevent the proestrous surge of LH and thereby block ovulation. Similar effects have been noted for the rabbit, in which the ovulatory surge of gonadotropins is not released spontaneously, but rather is induced by mating. In this species, the acute intramuscular injection of THC in doses of 2.5 or 5 mg/kg BW at 2 hrs before mating completely blocked the reflex LH surge and ovulation (Asch et al. 1979a).

In spontaneous ovulation, the ovulatory surge of gonadotropin is believed to be initiated by the positive feedback action of ovarian estrogen on brain centers regulating LH secretion. It is possible to demonstrate this positive feedback action of estrogen in ovariectomized rats which have been estrogen primed. In this model, a second estrogen injection 3 days after the priming injection induces a surge of LH not unlike that occurring on the afternoon of proestrus. Thus it is noteworthy that the estrogen-induced LH surge in the estrogen-primed ovariectomized rat is also blocked by THC administration (Steger et al. 1980). Treatment with THC therefore appears to prevent expression of the cyclic gonadotropin release mechanism responsive to estrogen or, in the case of reflex ovulators such as the rabbit, neural activation. Whether cannabinoid exposure actually prevents activation of this cyclic release mechanism or, alternatively, simply prevents its expression by inhibiting LH secretion through an action which may be equivalent to that operating to suppress the tonically elevated LH secretion in the ovariectomized animal remains a matter for speculation. Of course, in either case, the actual mechanism of THC action is unknown.

Although cannabinoids may have direct effects on steroid secretion from the ovary or other steroid secreting glands, it is not possible to explain the inhibition of LH secretion on the basis of a direct effect on the pituitary. Several studies have established that the administration of exogenous GnRH readily overcomes the suppression of LH secretion induced by THC exposure (Ayalon et al. 1977; Tyrey 1978; Smith et al. 1979; Asch et al. 1979a). Moreover, the

magnitude of the LH response to an injection of exogenous GnRH in ovariectomized rats during THC-induced LH suppression was equivalent to that induced by GnRH in ovariectomized rats in which LH secretion was suppressed by estrogen and progesterone pretreatment rather than by THC (Tyrey 1978). In contrast to these studies, there is a single report of reduced LH responsiveness to exogenous GnRH in male rats after acute or chronic THC treatment (Symons et al. 1976).

Follicle Stimulating Hormone

The effect of cannabinoid treatment on FSH secretion is neither as clear nor as extensively studied as that on LH. While serum FSH levels in ovariectomized monkeys were suppressed in a fashion parallel to that of LH following THC treatment (Smith et al. 1979), THC treatment of ovariectomized rats in doses that suppressed LH concentrations had no effect on serum FSH levels (Steger et al. 1981). However, the proestrous surge of FSH in the cyclic rat, like that of LH, can be prevented by appropriately timed THC injections (Ayalon et al. 1977). Additional studies are required to clarify this apparent difference in the FSH responses to THC treatment in rats and monkeys.

Prolactin

Prolactin, which has lactogenic and, in the rat, luteotropic actions, also is suppressed by cannabinoid treatment. While one group (Daley et al. 1974) has reported a modest increase in serum prolactin in male rats at 24 hrs after the last of 4 daily THC injections, most reports indicate reduced serum prolactin levels after THC treatment. Studies with intact male (Kramer and Ben-David 1974; 1978) and female (Chakravarty et al. 1975) rats, ovariectomized rats (Hughes et al. 1981), and male and ovariectomized female monkeys (Asch et al. 1979) all revealed a clear suppression of basal prolactin secretion by THC. This is of particular interest since, unlike LH whose release requires active stimulation from the hypothalamus, prolactin secretion is under tonic inhibitory control. Dopamine plays a central role in this inhibitory control by acting directly on the prolactin secreting cells of the pituitary as a prolactin inhibiting factor (PIF), and, perhaps, by acting in the hypothalamus to bring about the release of some other PIF. Thus basal serum prolactin concentrations, normally maintained at relatively low levels as a result of tonic inhibition, can be further decreased by THC treatment. This raises the question as to whether active increases in prolactin secretion, which would require withdrawal of the tonic inhibition or stimulation by a releasing factor, perhaps TRH, also might be suppressed by THC. In the rodent there are three recognized physiological stimuli for prolactin secretion: rising estrogen titers, stimulation of the uterine cervix, and suckling of the lactating female. As indicated below, the secretion of prolactin induced by any of these stimuli can be blocked by THC treatment.

The estrogen provoked rise in serum prolactin occurring coincident with the proestrous gonadotropin surge in the rat is blocked, as is

the gonadotropin surge itself, by THC administration (Ayalon et al. 1977). Thus THC inhibits the surge secretion of LH, FSH and prolactin during proestrus.

The secretion of prolactin induced by cervical stimulation occurs in a pattern quite distinct from that of proestrus and without the coincident surges in LH and FSH. Stimulation of the uterine cervix, whether resulting from mating or some artificial means, initiates a series of twice daily prolactin surges, one nocturnal and one diurnal, which continue for several days. The effect of THC treatment on these surges was studied in rats in which pseudopregnancies were induced by electrical stimulation of the cervix (Hughes and Tyrey 1982). When such animals were given a single intravenous injection of THC in a dose of 4 mg/kg BW at a time just before the beginning of a nocturnal surge was expected, the initiation of that surge was delayed for approximately 1 hr; but not otherwise affected. On the other hand, when the THC was administered in hourly doses of 1 mg/kg BW throughout the interval during which the prolactin rise normally occurs, the surge was prevented altogether. It should be noted that even though the hourly THC injections continued into the next day, there was no apparent inhibition of the presumably basal prolactin secretion occurring at that time. The reason for the failure of these continued injections to suppress prolactin below control levels is not clear, but conceivably could be related to the repetitive treatment at rather close intervals leading to the development of tolerance to continued drug action. Additional investigation is required to clarify this issue.

An early indication of the potential inhibitory influence of THC on the increase in prolactin secretion induced by the suckling stimulus was provided by the report (Borgen et al. 1971) of marked postnatal mortality among rat pups born to mothers chronically treated with THC during pregnancy. These investigators suggested that this effect resulted from inadequate maternal lactation, rather than from some defect in the newborn pups. Cross-fostering of the offspring of THC-treated mothers to control mothers and of the control offspring to THC-treated mothers allowed the drug-exposed offspring to survive, while the cross-fostered control pups did poorly. Starvation was apparently the cause of the increased pup mortality, for milk was not always visible in the stomachs of pups suckling treated mothers. Similar effects associated with perinatal THC treatment have since been reported for mice (Szepsenwol et al. 1979; Hatoum et al. 1981).

The reports of lactational problems in THC-treated rodents suggested that THC treatment may interfere with the increased prolactin secretion required for normal lactation. Indeed a delay in the post-partum prolactin rise in mice chronically treated with THC during pregnancy and the early post-partum period (Raine et al. 1978) indicated that prolactin secretion is altered in treated mothers. Of particular importance was the question of whether the prolactin surge induced by each suckling episode, necessary for the continuation of adequate lactation, might be sensitive to the

inhibitory actions of JHC. An early attempt to answer this question (Bromley et al. 1978) was compromised by the immediate disruption of maternal behavior resulting from THC administration. Consequently it was necessary to remove pups from the mother at the time of THC administration and to judge the effects of THC treatment on suckling-induced prolactin secretion by comparisons of the relative rates of decline in serum prolactin in the THC-treated and control mothers. A somewhat more rapid decline of prolactin concentrations in THC-treated animals was interpreted as indicating an inhibition of suckling-induced prolactin secretion, since it was assumed that prolactin secretion in the controls would continue for a time even after pup removal. Later studies utilized urethane anesthesia to circumvent the disruption of nursing behavior by THC administration and, since this procedure allows the neuroendocrine reflex response to suckling to remain intact, a clear demonstration of the inhibitory effect of THC on suckling-induced prolactin secretion was possible (Tyrey and Hughes 1983). The intravenous injection of THC into lactating rats just prior to suckling prevented the reflex prolactin surge in most animals. When THC administration was delayed until after the surge was under way, prolactin secretion was nonetheless curtailed and the serum prolactin concentration declined even though suckling continued.

As in the case of LH, there is no evidence that cannabinoids inhibit prolactin secretion through direct action on the pituitary. THC added to hemi-pituitary incubations in concentrations of 10^{-6} or 10^{-4} M had no detectable effect on the release of prolactin into the medium (Hughes et al. 1981). That these incubated hemi-pituitaries could respond to agents which have a direct suppressive action was demonstrated by the clear inhibition of prolactin secretion following the addition of the dopamine agonist bromocryptine (CB-154) to the incubation medium. Since the *in vitro* studies did not exclude the possibility that prolactin suppression *in vivo* resulted from the direct pituitary action of a THC metabolite rather than from THC itself, Hughes et al. (1981) also investigated the potential effect of THC on prolactin secretion from ectopic pituitary autografts. The elevated prolactin secret ion from autografts placed beneath the kidney capsule in hypophysectomized rats was unaffected by the intravenous injection of THC in a dose of 1 mg/kg BW, even though this dose was maximally effective in suppressing serum prolactin from the pituitary remaining under CNS control. The failure of THC to inhibit prolactin secretion from these pituitary autografts or from pituitary tissue incubated *in vitro* provides strong evidence against the possibility that THC inhibits prolactin secretion through action at the pituitary level. A similar conclusion has been drawn from the observation in the primate that TRH administration stimulates prolactin release during THC suppression (Asch et al. 1979c). Thus, the effect of THC on both gonadotropin and prolactin secretion appears to involve some alteration of the central neuroendocrine control of the pituitary.

446-384 O - 84 - 6

CENTRAL NERVOUS SYSTEM

If THC does not act directly on the pituitary to alter hormone secret ion, the next logical potential site of action would be the medial basal hypothalamus (MBH). This region of the hypothalamus contains the greatest abundance of nerve endings containing the hypothalamic releasing and inhibiting factors which form the functional link between the brain and anterior pituitary. While it is reasonable to suggest that THC may disrupt anterior pituitary function through a direct effect on these neurosecretory elements, the most recent evidence indicates that this is probably not the case.

Hughes et al. (1983) recently utilized microsurgical procedures in female rats to produce complete deafferentation of the MBH and then tested the ability of THC to inhibit prolactin secretion in these animals. The deafferentation procedure disconnects the MBH from other parts of the brain, but leaves it anatomically and functionally connected to the pituitary. Low serum prolactin concentrations are maintained in these animals as a result of continued tonic inhibitory control by the remaining hypothalamic “island.” When such animals were treated with intravenous THC in a dose which caused profound inhibition of prolactin secretion in the intact animal, there was no detectable decrease in serum prolactin concentrations. The hypothalamic region included within the surviving “islands”, determined histologically, included, in addition to the median eminence, most of the arcuate nucleus and the ventral portions of the ventromedial nuclei. Since this region of the hypothalamus is generally acknowledged to be directly involved in the regulation of prolactin secretion, the lack of THC effectiveness in suppressing serum prolactin levels in these rats suggests that THC does not act at a site within the MBH, and thus presumably not on those elements (most directly involved in prolactin regulation.

Since isolation of the MBH from the remainder of the brain prevented expression of the THC inhibitory effect on prolactin, other animals were subjected to partial deafferentation of the MBH by the placement of cuts in the frontal plane at the level of the suprachiasmatic nuclei or just rostral to these nuclei. These cuts were intended to interrupt rostral afferent pathways into the MBH believed to be important in the regulation of prolactin secretion. Both the frontal cuts at the suprachiasmatic level and those placed slightly more rostrally prevented the suppression of prolactin secretion by THC. Moreover, in some animals with the more rostral presuprachiasmatic cuts, a brief rise in serum prolactin was noted, the significance of which remains to be explained. Nonetheless, the failure of THC to bring about the expected suppression of prolactin secretion in animals with these frontal cuts implies that the inhibitory action of THC on prolactin secretion depends either upon the anterior hypothalamic region destroyed by these cuts, or upon the rostral fiber pathways which pass through this region to reach the MBH.

Additional experiments utilizing more selective lesioning techniques were conducted in an attempt to identify an afferent system which might be involved in conveying the THC inhibitory effect. Septal lesions interrupting the lateral corticohypothalamic tract which includes a suspected prolactin regulatory pathway from the orbital frontal neocortex to the preoptic area (Tindal and Knaggs 1977) failed to prevent suppression of serum prolactin after THC administration. Similarly, although lesions of the amygdala influence prolactin secretion in the rat (Peters and Gala 1980), destruction of amygdalar projections into the preoptic and hypothalamic areas by way of the diagonal band of Broca and the stria terminalis failed to prevent expression of the THC inhibitory effect on prolactin secretion. Finally, destruction of the periventricular region at the rostral midbrain level, intended to interrupt a presumptive ascending prolactin regulatory pathway (Tindal and Knaggs 1977), also failed to alter the suppressive effect of THC.

The deafferentation studies thus provide evidence that the THC-induced suppression of prolactin secretion in the female rat is not attributable to site of action within the MBH. Furthermore, prevention of the THC suppressive effect by frontal cuts at the suprachiasmatic level suggests that rostral pathways into the MBH convey the THC inhibitory effect. If this is the case, those pathways carrying hypothalamic projections from the orbitofrontal neocortex, amygdalar, and midbrain periventricular areas thought to be involved in prolactin regulation do not appear critical.

In addition to the evidence that THC acts at a site outside of the MBH in inhibiting prolactin secretion, other evidence indicates that this is also the case for THC stimulation of ACTH secretion (Puder et al. 1982). The ACTH surge normally induced in the rat by THC administration does not occur in rats previously subjected to complete hypothalamic deafferentation. Thus extra-hypothalamic sites appear to be involved in the mediation of the stimulatory effects of THC on ACTH secretion as well as its inhibitory effects on prolactin.

SUMMARY

The search for a site of cannabinoid action in non-primate experimental animals has raised the possibility of drug action at each level of the female reproductive system. The early suggestion that THC may have a direct “estrogen-like” action on the uterus has not been substantiated by subsequent investigations indicating that THC does not interact with the estrogen cytoplasmic receptor. Since receptor recognition is a fundamental requirement for hormone action, it is unlikely that THC acts as an estrogen. The experiments suggesting such action should be repeated under conditions where the potential confounding effects of steroids secreted from non-gonadal sources are controlled,

Direct ovarian effects of THC on the ovulatory process in the rodent as well as on steroid secretion from the cells of both the

corpus luteum and the preovulatory follicle have been demonstrated. Whether these effects have significance with respect to physiological function remains a question, however, in view of the rather pronounced effects of cannabinoids on the secretion of those pituitary hormones regulating these ovarian events. Only additional investigation *in vivo* of the more subtle gonadal effects of THC treatment can clarify this issue.

With respect to the pituitary hormones, there is clear evidence for the profound effects of cannabinoid exposure, of which the most pronounced may be those on the secretion of LH and prolactin. Effects on these reproductive hormones carry the threat of potential disturbance of the reproductive process, especially in the female where there is great dependence upon the appropriate cyclic changes in hormone levels. The full biologic impact of the pituitary effects of cannabinoids requires careful and thorough assessment.

It can be concluded with reasonable confidence that THC alters the secretion of the pituitary reproductive hormones, and that of ACTH as well, through actions in the brain. While it would be reasonable to suggest that this site of action may reside in the MBH, the region of the hypothalamus most intimately associated with pituitary function, that does not seem to be the case for effects on prolactin and ACTH. Prevention of the expression of THC effects on these hormones by MBH deafferentation points to a more distant site for THC action. Some of the more likely possible sites for THC inhibition of prolactin secretion have been investigated, but direct evidence for their involvement was not forthcoming. Similarly, the pineal apparently is not involved in the suppression of LH secretion by THC (Johnson et al. 1980). Thus the search for a site of THC action which had narrowed to a focus upon the hypothalamic-pituitary axis must again be expanded into a probing of higher brain regions. This means, of course, that the search for a site of drug action, and ultimately a mechanism, has been enormously complicated. Nevertheless, the need for a more complete understanding of cannabinoid actions on central neuro-endocrine regulation clearly justifies the effort.

REFERENCES

Asch, R.H., Fernandez, E.O., Smith, C.G., and Pauerstein, C.J. Precoital single doses of delta-9-tetrahydrocannabinol block ovulation in the rabbit. *Fertil Steril* 31:331-334. 1979a.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., and Pauerstein, C.J. Effects of delta-9-tetrahydrocannabinol administration on gonadal steroidogenic activity *in vivo*. *Fertil Steril* 32:576-582, 1979b.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., and Pauerstein, C.J. Acute decreases in serum prolactin concentrations caused by delta-9-tetrahydrocannabinol in nonhuman primates. *Fertil Steril* 32:571-575, 1979c.

Ayalon, D., Nir, I., Cordova, S., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., and Lindner, H.R. Acute effect of delta-1-tetrahydrocannabinol on the hypothalamic-pituitary-ovarian axis in the rat. Neuroendocrinology 23:31-42, 1977.

Bauminger, S., Lieberman, M.E., and Lindner, H.R. Steroid-independent effect of gonadotropins on prostaglandin synthesis in rat Graafian follicles in vitro. Prostaglandins 9:753-764, 1975.

Besch, N.F., Smith, C.G., Besch, P.K., and Kaufmann, R.H. The effect of marihuana (delta-9-tetrahydrocannabinol) on the secretion of luteinizing hormone in the ovariectomized rhesus monkey. Am J Obstet Gynecol 128:635-640, 1977.

Borgen, L.A., Davis, W.M., and Pace, H.B. Effects of synthetic Δ^9-tetrahydrocannabinol on pregnancy and offspring in the rat. Toxicol Appl Pharmacol 20:480-486, 1971.

Bromley, B.L., Rabii, J., Gordon, J.H., and Zimmerman, E. Delta-9-tetrahydrocannabinol inhibition of suckling-induced prolactin release in the lactating rat. Endocr Res Commun 5:271-278. 1978.

Burstein, S., Hunter, S.A., and Shoupe, T.S. Inhibition of cholesterol esterases by Δ^1-tetrahydrocannabinol. Life Sci 23: 979-982, 1978a.

Burstein, S., Hunter, S.A., Shoupe, T.S., Taylor, P., Bartke, A., and Dalterio, S. Cannabinoid inhibition of testosterone synthesis by mouse Leydig cells. Res Commun Chem Pathol Pharmacol 19:557-560, 1978b.

Burstein, S., Hunter, S.A., and Shoupe, T.S. Cannabinoid inhibition of rat luteal cell progesterone synthesis. Res Commun Chem Pathol Pharmacol 24:413-416, 1979.

Chakravarty, I., Shah, P.G., Sheth, A.R., and Ghosh, J.J. Mode of action of delta-9-tetrahydrocannabinol on hypothalamo-pituitary function in adult female rats. J Reprod Fertil 57:113-115, 1979.

Chakravarty, I., Sheth, A.R., and Ghosh, J.J. Effect of acute Δ^9-tetrahydrocannabinol treatment on serum luteinizing hormone and prolactin levels in adult female rats. Fertil Steril 26: 947-948, 1975.

Daley, J.D. Branda, L.A., Rosenfeld, J., and Younglai, E.V. Increase of serum prolactin in male rats by (minus)-trans-delta-9-tetrahydrocannabinol. J. Endocrinol 63:415-416, 1974.

Dixit, V.P., Arya, M., and Lohiya, N.K. The effect of chronically administered cannabis extract on the female genital tract of mice and rats. Endokrinologie 66:365-368, 1975.

Freudenthal, R.I., Martin, J., and Wall, M.E. Distribution of Δ^9-tetrahydrocannabinol in the mouse. Brit J Pharmacol 44:244-249, 1972.

Hatoum, N.S., Davis, W.M., Elsohly, M.A., and Turner, C.E. Perinatal exposure to cannabichromene and delta-9-tetrahydrocannabinol: separate and combined effects on viability of pups and on male reproductive system at maturity. Toxicol Lett 8:141-146, 1981.

Holzbauer, M., Newport, H.M., Birmingham, M.K., and Traikov, H. Secretion of pregn-4-ene-3,20-dione(Progesterone) in vivo by the adrenal gland of the rat. Nature 221:572-573, 1969.

Hughes, C.L., Jr., Everett, J.W., and Tyrey, L. Delta-9-tetrahydrocannabinol suppression of prolactin secretion in the rat: lack of direct pituitary effect. Endocrinology 109:876-880, 1981.

Hughes, C.L., Jr., Everett, J.W., and Tyrey, L. Effects of Δ^9-tetrahydrocannabinol on serum prolactin in female rats bearing CNS lesions: implications for site of drug action. In: Agurell, S., Dewey, W.L., and Willette, R., eds. Cannabinoids 82 New York: Academic Press, 1983, in press.

Hughes, C.L., Jr., and Tyrey, L. Effects of (-)-trans-delta-9-tetrahydrocannabinol on serum prolactin in the pseudopregnant rat. Endocr Res Commun 9:25-36, 1982.

Jacobs, J.A., Dellarco, A.J., Manfredi, R.A., and Harclerode, J. The effects of Δ^9-tetrahydrocannabinol, cannabidiol, and shock on plasma corticosterone concentrations in rats. J Pharm Pharmacol 31:341-342, 1979.

Johnson, L.Y., Asch, R.H., and Reiter, R.J. Failure of pinealectomy to affect acute changes in plasma levels of luteinizing hormone and prolactin in ovariectomized rats following delta-9-tetrahydrocannabinol administration. Subst Alcohol Actions Misuse 1:355-359, 1980.

Kramer, J., and Ben-David, M. Suppression of prolactin secretion by acute administration of delta-9-THC in rats. Proc Soc Exp Biol Med 147:482-484, 1974.

Kramer, J., and Ben-David, M. Prolactin suppression by (-) delta-9-tetrahydrocannabinol (THC): involvement of serotonergic and dopaminergic pathways. Endocrinology 103:452-457, 1978.

Kubena, R.K., Perhach, J.L., Jr., and Barry, H., III. Corticosterone elevation mediated centrally by Δ^1-tetrahydrocannabinol in rats. Eur J Pharmacol 14:89-92, 1971.

Marks, B.H. Δ^1-Tetrahydrocannabinol and luteinizing hormone secretion. Prog Brain Res 39:331-337, 1973.

Mechoulam, R. Marihuana chemistry. Science 168:1159-1166, 1970.

Moon, Y.S., Duleba, A.J., and Jakubovic, A. Effect of cannabinoids on progesterone production by ovarian granulosa cells of pig and rat. Life Sci 31:315-318, 1982.

Nazar, B., Kairys, D. J., Fowler, R., and Harclerode, J. Effects of Δ^9-tetrahydrocannabinol on serum throxine concentrations in the rat. J Pharm Pharmac 29:778-779, 1977.

Nir, I., Ayalon, D., Tsafriri, A., Cordova, T., and Lindner, H.R. Suppression of the cyclic surge of luteinizing hormone secretion and of ovulation in the rat by Δ^1-tetrahydrocannabinol. Nature 243:470-471, 1973.

Okey, A.B., and Bondy, G.P. Is delta-9-tetrahydrocannabinol estrogenic? Science 195:904-905, 1977.

Okey, A.B., and Bondy, G.P. Delta-9-tetrahydrocannabinol and 17-beta-estradiol bind to different macromolecules in estrogen target tissues. Science 200:312-314, 1978.

Okey, A.B., and Truant, G.S. Cannabis demasculinizes rats but is not estrogenic. Life Sci 17:1113-1118, 1975.

Peters, J.A., and Gala, R.R. The effect of corticomedial amygdaloid lesions on prolactin secretion in male and female rats. Endocrinology 106:1665-1669, 1980.

Puder, M., Weidenfeld, J., Chowers, I., Nir, I., Conforti, N., and Siegel, R.A. Corticotropin and corticosterone secretion following delta-1-tetrahydrocannabinol, in intact and in hypothalamic deafferentated male rats. Exp Brain Res 46:85-88, 1982.

Raine, J.M., Wing, D.R., and Paton, W.D. The effects of delta-1-tetrahydrocannabinol on mammary gland growth, enzyme activity and plasma prolactin levels in the mouse. Eur J Pharmacol 51:11-17, 1978.

Rawitch, A.B., Schultz, G.S., Ebner, K.E., and Vardaris, R.M. Competition of Δ^9-tetrahydrocannabinol with estrogen in rat uterine estrogen receptor binding. Science 197:1189-1191, 1977.

Reich, R., Laufer, N., Lewysohn, O., Cordova, T., Ayalon, D., and Tsafriri, A. In vitro effects of cannabinoids on follicular function in the rat. Biol Reprod 27:223-231, 1982.

Rosenkrantz, H., Sprague, R.A., Fleischman, R.W., and Braude, M.C. Oral Δ^9-tetrahydrocannabinol toxicity in rats treated for periods up to six months. Toxicol Appl Pharmacol 32:399-417, 1975.

Smith, C.G., Besch, N.F., Smith, R.G., and Besch, P.K. Effect of tetrahydrocannabinol on the hypothalamic-pituitary axis in the ovariectomized rhesus monkey. Fertil Steril 31:335-339, 1979.

Solomon, J., Cocchia, M.A., and DiMartino, R. Effect of delta-9-tetrahydrocannabinol on uterine and vaginal cytology of ovariectomized rats. Science 195:875-877, 1977.

Solomon, J., Cocchia, M.A., Gray, R., Shattuck, D., and Vossmer, A. Uterotrophic effect of delta-9-tetrahydrocannabinol in ovariectomized rats. Science 192:559-561, 1976.

Steger, R.W., Silverman, A.Y., Johns, A., and Asch, R.H. Interactions of cocaine and delta-9-tetrahydrocannabinol with the hypothalamic-hypophysial axis of the female rat. Fertil Steril 35:567-572, 1981.

Steger, R.W., Silverman, A.Y., Siler-Khodr, T.M., and Asch, R.H. The effects of delta-9-tetrahydrocannabinol on the positive and negative feedback control of luteinizing hormone release. Life Sci 27:1911-1916, 1980.

Symons, A.M., Teale, J.D., and Marks, V. Effect of Δ^9-tetrahydrocannabinol on the hypothalamic-pituitary-gonadal system in the maturing male rat. J Endocrinol 68:43p-44p, 1976.

Szepsenwol, J., Fletcher, J., Murison, G.L., and Toro-Goyco, E. Long term effects of delta-9-tetrahydrocannabinol in mice. In: Nahas, G.G. and Paton, W.D.M., eds. Marihuana: Biological Effects. Adv. Biosci, Vol. 22-23, Pergamon Press, New York, pp.359-370, 1979.

Tindal, J.S., and Knaggs, G.S. Pathways in the forebrain of the rat concerned with the release of prolactin. Brain Res 119:211-221, 1977.

Tsafriri, A., Lindner, H.R., Zor, U., and Lamprecht, S.A. Physiological role of prostaglandins in the induction of ovulation. Prostaglandins 2:1-10, 1972.

Tyrey, L. Δ-9-tetrahydrocannabinol suppression of episodic luteinizing hormone secretion in the ovariectomized rat. Endocrinology 102:1808-1814, 1978.

Tyrey, L. Δ^9-tetrahydrocannabinol: a potent inhibitor of episodic luteinizing hormone secretion. J Pharmacol Exp Ther 213:306-308, 1980.

Tyrey, L., and Hughes, C.L., Jr. Inhibition of suckling-induced prolactin secretion by Δ^9-tetrahydrocannabinol. In: Agurell, S., Dewey, W.L., and Willette, R., eds. Cannabinoids 82. New York: Academic Press, 1983, in press.

Virgo, B.B. The estrogenicity of delta-9-tetrahydrocannabinol (THC): THC neither blocks nor induces ovum implantation, nor does it effect uterine growth. Res Commun Chem Pathol Pharmacol 25: 65-77, 1979.

Warner, W., Harris, L.S., and Carchman, R.A. Inhibition of corticosteroidogenesis by delta-9-tetrahydrocannabinol. Endocrinology 101:1815-1820, 1977.

Weick, R.F. A comparison of the disappearance rates of luteinizing hormone from intact and ovariectomized rats. Endocrinology 101: 157-161, 1977.

ACKNOWLEDGMENT

The preparation of this review was supported by grant DA 02006 from the National Institute on Drug Abuse.

AUTHOR

Lee Tyrey, Ph.D.

Department of Obstetrics & Gynecology
Duke University Medical Center
Durham, North Carolina 27710

Acute, Short-Term, and Chronic Effects of Marijuana on the Female Primate Reproductive Function

Carol Grace Smith, Ph.D., and Ricardo H. Asch, M.D.

INTRODUCTION

The reproductive effects of marijuana and delta-9-tetrahydrocannabinol (THC) have received much attention fran the scientific community for the past several years. Early clinical reports indicated that chronic marijuana use might be associated with decreased hormone levels and infertility. later studies failed to confirm these findings. Studies in laboratory animals clearly demonstrate that THC has pronounced effects on reproductive hormones and on ovulation and spermatogenesis, and have provided much informatiom on how these effects are produced. These studies have not, however, provided much insight into the apparent discrepancy in the pronounced drug effects reported in laboratory animal studies and the less impressive effects or absence of disruptive effects reported in clinical studies. Since much of our knowledge of human reproductive physiology has been obtained from studies in laboratory animals, it is difficult to ascribe these differences to species variations. It is more likely that the discrepancies in laboratory animal studies and the clinical studies are based on differences in experimental designs and failure to consider the role of the development of drug tolerance in the conclusions of these studies.

The sexually mature rhesus monkey is one of the best experimental animal models for studying the human reproductive system. The female commonly has a 28-day menstrual cycle that is controlled by negative and positive feedback mechanisms between gonadal steroids and pituitary gonadotropins identical to those found in the human menstrual cycle. Specific ammounts of marijuana derivatives can be administered to these animals and reproductive paramters can be examined directly. The purpose of this review is to sumarize the effects of acute, short-term (less than 30 days), and chronic treatment with THC on the primate menstrual cycle.

ACUTE EFFECTS OF MARIJUANA (THC) OF FEMALE REPRODUCTIVE HORMONES

Acute Effects of THC on Pituitary Hormones

There is evidence that hypothalamic nervous pathways that control the secretion of ganadotropins are inhibited by such CNS drugs as marijuana, narcotics, barbiturates and tranquilizers. The neuro-endocrine effects of marijuana and its constituent compounds are exceptimal in several ways and have provided interesting insights into the study of drug effects on hypothalamic-pituitary function. Since the initial report by Marks (1973) of the inhibitory effect of THC on luteinizing hormone levels in ovariectanized rats, a ber of studies have appeared which confirm this observation and attempt to describe the mechanism. Studies in the ovariectanized rhesus monkey have defined the dose-response relationship of the effect (Smith et al. 1979a). In these studies, THC (0.6-5.0 mg/kg) was given to ovaraiectomized monkeys by an intramscular injection. The result was a prompt and significant&crease in LH levels (average 50-80 percent decrease) that lasted for 12-24 hours depending upon the dose level of THC. These results are in agreement with studies in ovariectomized rats (Tyrey 1978) which showed a suppression in episodic LH secretion following THC administration. In both the monkey and rat studies, the magnitude of the decreases appeared not to be directly related to the dose of THC; but the duration of the responses was shown to be related to the dose, with larger doses of THC producing longer lasting depression in gonadotropin levels. The suppression of LH secre-tion appeared to be complete, but the effect was completely reversible.

A comparision of the effects of the various doses of THC on the levels of LH and the levels of FSH in ovariectanized monkeys showed great difference between the two gonadotropins (Smith et al. 1979b). For example, the average maximum decreases in LH and FSH following the 5.0 mg/kg dose of THC were 68 percent and 56 percent respectively. Comparison of the time course of the effects on gonadotropin levels in individual monkeys showed that the maximum decrease in hormone levels occurred at generally the same times for LH and FSH.

It is important to note, at this point, that these studies have examined the effect of delta-9-tetrahydrocannabinol on pituitary hormone levels. One study has compared the effects of THC and other marijuana derivatives, including the crude alcohol extract of marijuana (CME) containing 25 to 30 percent THC (Smith et al. 1980). THC and CME were administered to ovariectomized monkeys at equivalent dose levels (based on total ammount of THC). The decreases in both LH and FSH levels were equivalent in response to equal doses of THC and CME. That is, the 4.16 mg/kg dose of CME produced an average 40.8 percent inhibition of LH levels that lasted for 6 hours. The equivalent dose of THC (1.25 mg/kg) produced an average 35.9 percent decrease in LH that also lasted for 6 hours. Other cannabis derivatives that were examined for

effects on pituitary hormones included cannabidiol (CBD) and cannabinol (CBN). Neither CBD nor CSN had any statistically significant effect on gonadotropin levels at dose levels up to 10 mg/kl.

The comparison of the inhibition of gonadotropins produced by THC, marijuana extract, and the other cannabis derivatives indicates that the inhibitory action of marijuana on gonadotropin levels is producedby THC, andthatthe other cannabis derivatives contained in marijuana do not contribute to the effect (Smith et al. 1980). This is particularly evident because the relative doses of CBN and CBD used in this study were much larger than would be contained in the doses of marijuana extract. In addition, these results suggest, but do not prove, that the inhibitory effect of cannabis derivatives on gonadotropins is related to their psychoactivity. Further studies need to be done with other psychoactive cannabinoids and with cannabis derivatives devoid of psychoactive properties. It should also be noted that while certain cannabis derivatives may not contribute significantly to the endocrine changes caused by marijuana, they may have other direct effects on spermatogenesis, ovulation, and other reproductive functions.

The pharmacological site of action of a single dose of THC on gonadotropin lwels was also investigated in ovariectomized monkeys (Smith et al. 1979b). Synthetic gonadotropin releasing hormones (GnRH)was administered to the monkeys 6 hours after the administration of 2.5 mg/kg of THC. This dose of THC produces a statistically significant depression in gonadotropins that lasts for at least 12 hours. GnRH administration resulted in a stimulation of the blood levels of both LH and FSH. The stimulation of LH and FSH levels measured after GnRH administration could be considered as a reversal of the effect of THC since all of the stimulated gonadotropin levels were within the 95 percent confidence interval established for each monkey's control values. The response to releasing hormone in ovariectomized rats was the same as the response in monkeys (Tyrey 1978). Further, the suppression of ovulation in rats (Nir et al 1973) and in rabbits (Asch 1979a) caused by THC can be reversed by the administration of GnRH. These results show that the pituitary gland can respond to hypothalamic releasing hormones by releasing gonadotropins in the presence of THC. This indicates a hypothalamic site of action for THC, and while the exact mechanism of the inhibitory effect of THC on gonadotropin secretion remains unknown, the involvement of hypothalamic neuroendocrine processes seems very likely.

While there are a number of drugs, including narcotics, phenothiazine tranquilizers, and sex steroids, that will inhibit LH and FSH levels, these drugs have a stimlatory effect on prolactin levels. Studies in male and female rhesus monkeys have shown that the acute effect of THC on prolactin levels is a significant, but short-lived inhibition (Asch 1979b). THC or vehicle was administered to the monkeys, and blood was drawn at 30, 60, 90, 120 and 180 minutes after injection. While vehicle administration produced

no cmsistent change in prolactin levels, THC produced a prompt and sigficant decrease in prolactin level. The decrease in prolactin levels was significant for both male and female monkeys (average 84 percent decrease) at all of the time intervals from 30 to 180 minutes. The site of action of the inhibitory effect of THC on prolactin was determined by using thyrotropin releasing hormone (TRH). TRH administration stimulated the release of prolactin fran the pituitary by a direct actim on the pituitary gland. The results indicated that the effect of THC on prolactin levels is mediated by a hypothalamic site of actim, since the administration of TRH at 30 minutes after THC injectim reverses the inhibitory effect on prolactin levels. This inhibitory actim of THC on prolactin secretim has also been shown in the rodent (Daley et al. 1974 and Raine et al. 1978). Further, this respmse can be abolished by cyproheptadine, a serotonin antagonist or perphenazine and pimozine, dopamine antagonists (Kramer and Ben-David 1974). While the exact mechanism remains unclear, the evidence now shows that the acute effect of THC on prolactin is inhibitim.

It is frequently difficult to distinguish between direct drug actims on the hypothalamic-pituitary axis and subsequent effects on ggonadal hormones and sex accessory gland function. The distinction is an important one,however. Most neuroactive drugs produce only transient effects on the central nervous pathways necessary for normal gonadotropin secretion. The disruptive effects of these drugs are likely to be transient and canpletely reversible. Under these circumstances, normal adults may experience only subtle changes in sexual function. However individuals with compromised reproductive function may exhibit major problems. It is also likely that adolescents may risk reproductive damage, since the endocrine events associated with puberty are dependent upon the developing hypothalamic-pituitary axis.

Acute Effects of THC on Neuroendocrine Function

It has been known for several decades that the secretions of the anterior pituitary gland are regulated by substances generated by the hypothalamus. The best evidence for this came from the demonstration of the existence of a factor in the hypothalamus that causes the release of the gonadotropins from the anterior pituitary gland. This releasing factor actually brings about the release of both the gonadotropins LH and FSH, so it is also referred to as gmadotropin releasing hormone (GnRH). It is now recognized that all of the effects of the hypothalamus on the release of both FSH and LH can be explained by variations in dose, time course, and steroid hormone interactims at the pituitary level with a single releasing factor, CnRH. The mechanisms behind basal or tonic levels of secretion of gonadotropins, the generation of gonadotropin surges, or even the timing of the onset of puberty are not well understood.

There is considerable evidence in experimental animals that the

hypothalamic neurons that release GnRH are regulated by biogenic amines. For example, dopamine andnorepinephrine cmtent in the hypothalamus change with different stages of the estrous cycle in the rat, and dopamine can stimulate GnRH release when incubated with hypothalamic fragments in vitro (Schneider and McCann 1976). Little is know, however, about the role of biogenic amines in LH or FSH cmtrol in primates. Primates and rodents differ in the development of the cyclic LH release mechanism, in that androgen levels early in life suppress that resonse in the rodentbutnot in the primate. In the rhesus monkey, both thetmic and episodic secretary mechanisms appeartobelocalizedwithinthemedial basal hypothakus (Knobil 1974), while in rodents, neural path-waysthatariseelsewhere in the brain inpinge upon the GnRH system. These differences may help explain the differing suscepti-bility of gonadotropins in primates and rodents to pharmacological manipulation. In general, it can be said that the neuroptico-logical studies in primates indicate that norepinephrine, and perhaps dopanine, may be involved in GnFN release. Adrenergic and dopaminergic agonists probably enhance the release of GnRH, and adrenergic and dopaninergic antagonists probably inhibit the release of GnRH. More studies need to be dme in primates and humans before the exact role of these transmitters can be defined.

Of the hypothalamic mechanism for the control of pituitary hormones, the mechanisms for the control of prolactin have been most extensively studied. Prolactin secretion is probably con-trolled by two systems: a prolactin inhibitory factor (PIF) and one or more prolactin releasing factors. Current evidence indi-cates that dopamine is the inhibitory substance of hypothalamic origin that cmtrols prolactin secretion. The existence of a prolactin releasing factor or factors is less well established. Serotonin (5-HT), in cmtrast to dopamine, has been shown to produce an increase in prolactin release (Meites et al. 1972).

Current evidence gives no clear picture of the effects of THC on hypothalanic neurotransmitters and GnRH concentrations. Studies by Doms et al. (1981) show that acute admistration of THC causes an accumulation of GnRH containing granules in the medial basal hypothalamus in rats. In recently completed studies, Steger et al. (personal communication) examined levels of the hypothalamic transmitters dopamine, norepinephrine, and serotonin, and of GnRH, in ovariectomized rhesus mmkeys that were sacrificed 12 hours after a single dose of THC (2.5mg/kg) or vehicle. The brains were removed and frozen within 5 to 7 minutes. The median eminence, medial basal hypothalamus and preoptic area and a portion of the frontal cortex were dissected and prepared for neurotransmitter analysis. The results showed no effect of THC on norepinephrine or dopanine levels inanyofthebrainareas studied. GnRH levels were significantly elevated in the median eminence and the medial basal hypothalamus in the THC treated monkeys. There were no significant changes in serotonin levels in any of the brainareas studied but 5-hydroxyindoleacetic acid (5-HIAA) levels were significantly decreased in the preoptic area in the THC treated

monkeys. These results indicate that acute THC treatment muses an accumulation of GnRH in the hypothalamus when gonadotropin levels are markedly depressed. If the decline in 5-HIAA levels indicates decreased serotmergic activity, this may explain the decrease in prolactin caused by acute THC treatment. Additional experiments are needed before the mechanism of the effects of THC on hypothalamic function can be described.

Acute Effects of THC on Gonadal Hormones

The acute effects of THC on menstrual cycle hormones have been Studied in the rhesus monkey using both in vivo and in vitro techniques (Almirez et al. 1983). In the in vivo studies, THC or vehicle was administered by an intramuscular injection to rhesus monkeys on day 20, 21 or 22 of the menstrual cycle. Progesterone levels were measured at 6 hour intervals for the first 24 hours after treatment. THC caused a significant decrease in progesterone levels during this 24 hour period. This decrease was reversed by the administration of human chorionic gonadotropin (HCG) at 6 hours after THC administration. This stimulatory effect of HCG could be observed as early as 30 minutes after injection, and normal progesterone levels were observed from 180 minutes to 48 hours after the HCG injection. These results indicate that the acute effect of THC on progesterone levels is mediated by an indirect effect on pituitary gonadotropins rather than direct effects on the ovarian synthesis and secretion of progesterone. This cmclusimis supportedby in vitro studies of basal progesterme production by dispersed luteal cells from the rhesusmmkey. The corporaluteawere surgically removed from the ovaries six to eight days after ovulation. The dispersed cells will continue to produce progesterone for several hours without adding gonadotropin to the medium. THC or marijuana extract was added to the cell suspension to a final cmcentratim of up to 50 micromolar. Neither THC nor marijuana extract had any effect on progesterone production at any cmcentratim up to 50 micromolar (limit of drug solubility).

Other in vitro studies with cannabinoids have shown that these drugs disrupt gonadal steroidogenesis, protein and nucleic acid synthesis, glucose utilization and prostaglandin synthesis, and reduce cyclic AMP concentrations in various species including mice, rats and pigs and in various tissues including Leydig cells, granulosa cells and luteal cells (Ayalm et al. 1977; Burstein et al. 1979; Dalterio et al. 1976; Jakubovic and McGeer 1976; and Reich et al. 1982). Because of the cmflict of the in vivo studies that show no direct effect on gonadal steroid production with these in vitro results, special attentim to the details of the in vitro methods warranted. All of the cannabinoids studies in vitro systems are poorly water soluble. If the solubility of the compound is exceeded, the actual tissue level may be greater than the cmcentratim added to the medium. Unlike the studies with dispersed luteal cells from rhesus monkeys, most of the other in vitro systems use tissues that

require the addition of a gonadotropin to the incubation medium to stimulate steroid production. The integrity of the *in vitro* environment and the function of the gonadotropins (polypeptide hormones)added to the incubations may be compromised in the presence of these drugs. Until adequate informatim is available on the actual levels of these drugs in the media and in the tissues and on the chemical identity of the drugs throughout the incubatim period, the cmclusims drawn fran all *in vitro* studies will remain tentative.

SHORT-TERM EFFECTS OF THC ON FEMALE REPRODUCTIVE HORMONES

The effects of short-term administration of THC on the menstrual cycle in the rhesus monkey has been studied in our laboratories. One such study examined the effect of THC administration follicular development and ovulation (Asch el al. 1981). The rhesus monkeys used in these studies had nonral menstrual cycles. The first day of menses was designated as day 1 of the cycle, and ovulation normally occurs about days 15. In order to determine whether THC administration would affect ovulatim in these monkeys, THC or vehicle was injected daily from day 1 to day 18 of the cycle. Blood was drawn daily from day 8 to day 18 of the cycle and then every other day until the occurrence of menses. The hormones that were measured included total estrogens, LH, and progesterme. Serial lapsroscopies were dme twice weekly to observe follicular maturation, ovulation, and corpus luteun formation. None of the five THC-treated monkeys exhibited estrogen rises or LH surges, and nme of the THC-treated monkeys ovulated before the next menses. The period of disruptim that followed the short-term THC treatment ranged from 55 to 145 days. After this period, normal hormone levels and ovulation were observed in all monkeys. This study shows that the inhibitory effect of THC on gonadotropin secretion is sufficient to disrupt ovulatim and that the subsequent disruptim of the menstrual cycle may last as long as several months. When exogenous gonadotropins were administered to these monkeys treated with THC during the first 18 days of the cycle, ovulatim was restored and normal luteal function followed. The successful induction of ovulation and normal luteal function using exogenous gonadotropins in the presence of antiovulatory doses of THC clearly supports the hypothesis of a central action of the drug rather than an action directly at the gonad. These results are consistent with studies from other laboratories that show that THC treatment causes an inhibition of ovulation in rats (Nir- et al. 1973) and rabbits (Asch et al. 1979a) by a reversible inhibition of gonadotropin secretim.

Similar results were obtainedin studies in which THC was administered to rhesus monkeys during the luteal phase of normal ovulatory cycles (Asch et al. 1979c). The daily administration of THC (2.5 mg/kg) had no effect on serum progesterone levels or on the length of the luteal phase. Again, the post-treatment period was marked by an absence of normal levels of estrogens, gonadotropins, and progesterone. The prolactin levels recorded during the post-treat-

ment period were 4 to 5 times greater than prolactin levels in normal ovulatory cycles. An additional study was done in which increasing doses of HCG were administered from day 6 to day 10 after ovulation. This treatment with HCG during the luteal phase in cmtrol animals resulted in augmentation of the progesterone levels to 4 to 5 times greater than those of control cycles. The daily injections of THC (2.5 mg/kg) had no effect on the HCG-induced progesterone rise when compared to either vehicle treatment or cmtrol responses. These results show that THC has no direct effect on luteal functim during normal cycles. Further, THC has no direct effect on corpus luteum function stimulated by HCG administration. The lack of an effect of THC on HCG-stimulated corpus luteum function is particularly important, since this experimental condition mimics progesterone secretion in early pregnancy.

CHRONIC EFFECTS OF THC ON THE FEMALE REPRODUCTIVE HORMONES

Drug tolerance can be defined as a decrease in pharmacologic respmse that results from prior exposure to the drug. Tolerance to the behavioral and cardiac effects of THC administration has been reported in man and laboratory animals. Tolerance can develop by several mechanisms including metabolic tolerance or an increased metabolic clearance of the drug from the body. Cellular or adaptive tolerance occurs when the organ system through homeostatic mechanisms loses its sensitivity to the drug's actims. Either of these mechanisms could be involved in the development of tolerance to the reproductive effects of THC.

The chronic studies described here were designed to study the effects of chronic THC on the primate menstrual cycle and to examine the mechanisms involved in the development of tolerance (Smith et al. 1983). The study was designed to continue drug treatment for at least one year or until tolerance developed and normal cycles were restored. Five female rhesus monkeys with normal menstrual cycles were used in this study. Ovulation was detected by monitoring plasma estrogen, LH and progesterme levels and by laparoscopic examination. Daily vaginal swabbings were utilized to detect the onset of vaginal bleeding and duration of menses. Each monkey was followed for one control cycle and me vehicle treatment cycle before the THC treatment began. On day l of the third cycle the monkeys began to receive thrice weekly injections of 2.5 mg/kg THC or 1.25 mg/kg THC. The injections were given on a M-W-F schedule (at noon), and blood was sampled on each treatmnt day immediately before injectim. The drug or vehicle was administered by an intrarmuscular injectim. The blood level of THC obtained with the 2.5 mg/kg dose of THC 3 times per week in monkeys is equivalent to moderate to heavy use of marijuana (5 to 6 joints per day; 3 times per week). Blood levels of THC were measured by RIA (Cook et al. 1982) where adequate session was available after hormone measurements. The maximum blood level of THC was an average of 300 ng/ml at 60 minutes after injection. The blood level had decreased to an average of 20

446-384 0 - 84 - 7

ng/ml by 12 hours, and this through level was maintained until the next dose at 48 hours. These parameters did not change significantly throughout the studies. The average minimum THC blood level during the anovulatory cycles was 409 ng/ml (range 288-542 ng/ml) and 378 ng/ml (range 250-640 ng/ml) after cycles were restored. The average trough level of THC at 48 hours after drug injection was 15.3 ng/ml (range 12.1-16.5 ng/ml) during the anovulatory cycles and 18.8 ng/ml (range 13.0-24.0 ng/ml) after menstrual cycles were restored.

All monkeys exhibited normal hormone levels and ovulation during the control and vehicle cycles. Cycle lengths were within normal limits for the colony. After the drug injections began, none of the monkeys ovulated or shoved normal hormone levels. Results of this study are shown for five monkeys in Figure 1. The durations of the drug effects (days until next normal menstruation) were 135, 110 and 103 days for the monkeys treated with the 2.5 mg/kg dose and 120 and 70 days for monkeys treated with the 1.25 mg/kg dose of THC. After the tolerance to the drug effects developed, normal cycles were re-established. Ovulation was again detected by laparoscopy and normal hormone levels were observed. Comparisons were made between pre-treatment prolactin values from control and vehicle cycles, prolactin values during the period of disruption, and after menstrual cycles were restored. There appeared to be sane decrease in the average prolactin levels during the period of disruption produced by the chronic drug treatment for several of the monkeys. When the data for the 5 monkeys were combined, there was no significant decrease in prolactin levels. It was clear, however, that the previously observed elevations in prolactin levels following discontinuatian of short-term drug treatment are not observed with chronic drug treatment.

This chronic study demonstrates the disruptive effects of chronic THC administration on the primate menstrual cycle. Since both gonadotropins and sex steroids are at basal levels during the period of disruption, it is likely that there is a direct suppression of hypothalamic-pituitary activity. This study also demonstrates that with chronic drug treatment, tolerance develops to the inhibitory effect of THC and normal cycles are re-established.

The mechanism for the tolerance to the effect of THC is not known. Tolerance develops to other pharmacologic effects of THC including euphoria andtachycardia. Behavioral tolerance has been reported in rhesus monkeys and was observed in this chronic study as well. Preliminary data from this study and complete pharmacokinetic studies in man and laboratory animals indicate that increased drug metabolism or clearance is not a major factor in the development of tolerance (Lemberger and Rubin 1978). It is likely that the tolerance that develops to the reproductive effects of THC is due to adaptation of neural mechanisms in the hypothalamus rather than to increased metabolism of the drug.

The results of this chronic study are consistent withone clinical

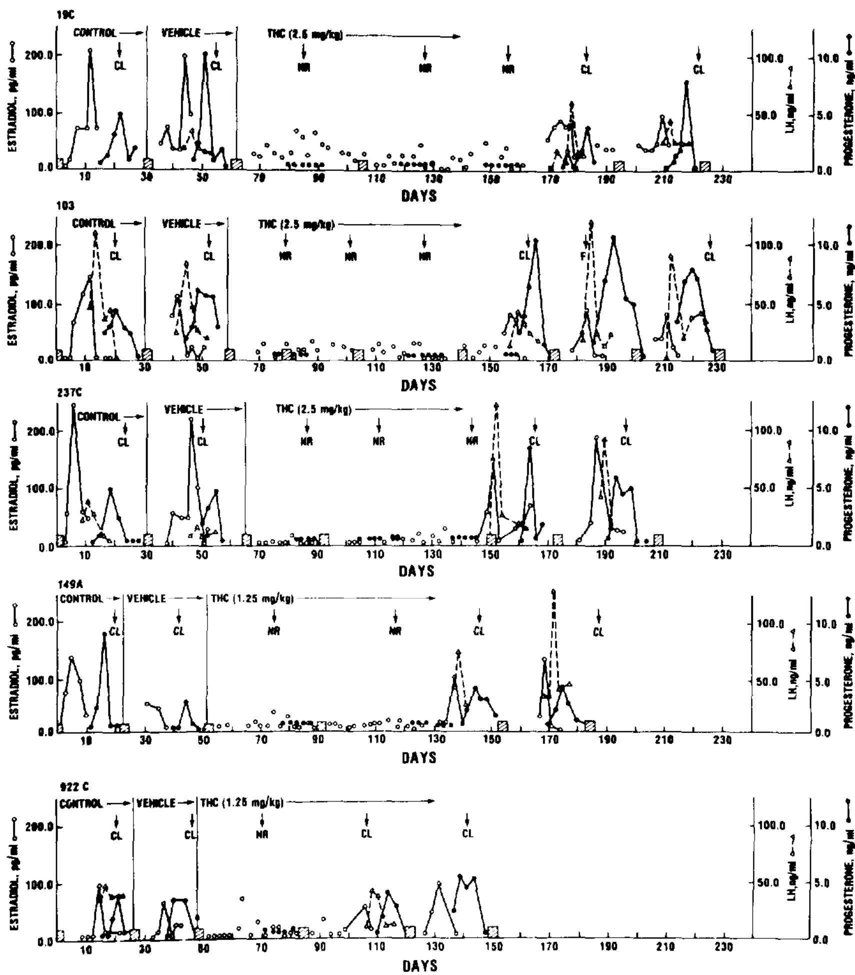


FIGURE 1. Tolerance develops to the disruptive effects of delta-9 tetrahydrocannabinol on the primate menstrual cycle.

study of young women who regularly used marijuana (Bauman 1980). These women experienced changes in menstrual cycles associated with decreased prolactin levels. However, the developnent of tolerance and return to apparently normal menstrual cycles may mean that normally fertile young women who use marijuana regularly may not notice much change in their menstrual cycles. Drug effects may be obvious during adolescence in young women who have some other menstrual irregularities, or if pregnancy occurs. This study also demonstrates that the development of tolerance must be considered available in reproductive studies in young men and women who use marijuana and may help to explain sane of the conflicting data in human and laboratory animal studies.

One proposed mechanism for the disruption of the reproductive system produced by marijuana suggests a direct action of THC at the cellular level of the reproductive target organs. Studies by Shoemaker and Harmon (1977) indicate that THC may compete with sex steroids for their receptor proteins in the target organ cells. The binding of THC to these receptors would either antagonize the trophic effects of steroids in these tissues or produce the same trophic effect as the sex steroid. Solomon and coworkers (1977) have shown a positive trophic effect of THC on reproductive tissues in ovariectomized rats when THC was administered by intraperitoneal injection. Their work, however, was criticized by Okey and Bondy (1978) who claim that experiments in which THC is administered by intraperitoneal injection give erratic and unreliable results, caused by inflammation of abdominal organs. Several of the cannabis derivatives including THC have been examined in the rhesus monkey for estrogenic activity. The compounds do not interact with estrogen receptors in reproductive tissues (Smith, R.G. et al. 1979) nor do they have any inherent estrogenic activity in vivo (Smith et al. 1979a). THC interferes with the growth and development of the sex accessory organs in both male and female animals but not by a direct action on the tissues (Husain and Lame 1982). The postulated direct effects of these and other drugs by a tissue receptor mechanism are not as unlikely as they may appear. A number of plant sterols and synthetic compounds have inherent estrogenic activity. One such synthetic compound, diethylstilbestrol (DES), has estrogenic activity that exceeds the most potent endogenous estrogen in many assays. Certainly, there are other chemicals in marijuana that may have hormonal activity, but none has yet been described. It is important to point out that the steroid receptor mechanism is highly stereospecific. Therefore, it is not likely that many drugs will interfere at this level of cellular activity.

SUMMARY

Studies with laboratory animals clearly show that the crude drug marijuana and delta-9-THC, the principal psychoactive ingredient, inhibit secretion of the pituitary hormones LH and FSH as well as prolactin. These changes in pituitary hormone levels produce decreases in sex steroid hormones and cause disruption of ovulation

and spermatogenesis. With chronic drug use, disruption of sex accessory organs has also been observed. A principal site of THC action is the hypothalamus, because THC effects on pituitary hormones productim can be reversed with hypothalamic releasing factors. It is now known that drug effects in sexually mature animals are reversible when drug treatment stops. In adults, tolerance develops to hormone changes brought on by the use of marijuana. Clinical studies on human subjects generally agree with the animal findings, although conflicting results have been reported as well. It is likely that the differences in results obtained in experiments with laboratory animals and with humans are caused, at least in part, by differences in experimental design. Further, it is not known how much disruption of reproductive hormone levels is necessary for changes in human fertility and sexual function to occur.

The use of marijuana by pregnant women or by women are attempting to became pregnant is cause for special concern. Studies with laboratory animals and retrospective studies on women who have used marijuana during pregnancy show that the risks of pregnancy loss and other adverse effects on the fetus are increased by marijuana use. THC crosses the placental barrier and while the potent teratogenic and mutagenic effects suggested for marijuana some years ago have not been confirmed, significant changes consistent with retardation of fetal growth and development have been observed. Effects of THC on the proper functioning of the placenta may be responsible for these effects on pregnancy. Pregnancy that occurs after the development of tolerance with chronic marijuana use may involve an ovum that has been damged by exposure to the drug during critical developmental stages. More studies need to be done before the mechanisms of toxic effects on pregnancy and fetal development can be described.

While there have been no clinical studies relating marijuana use to adolescent development, studies in laboratory animals show that the developing reproductive system during adolescence is particularly vulnerable. Single casereports in the clinical literature strongly suggest that the effects observed in animals may also occur in young people. Marijuana inhibits events during adolescence that are crucial for normal reproductive function, so that a delay in normal sexual development may result and the possibility of permanent infertility also exists.

REFERENCES

Almirez, R.G., Smith, C.G., and Asch, R.H. The effects of marijuana extract and delta-9-tetrahydrocannabinol on luteal function in the rhesus monkey. Fertil Steril 39:212-217, 1983.

Asch, R.H., Fernandez, E.O., Smith, C.G., anb Pauerstein, C.J. Blockage of the ovulatory reflex in the rabbit with delta-9-tetrahydrocannabinol. Fertil Steril 31:331-334, 1979a.

Asch, R.H., Smith, C.G., Siler-Khodr, T.M., Pauerstein, C.J. Acute decreases in serum prolactin concentrations caused by

delta-9-tetrahydrocannabinol in nonhuman primates. Fertil Steril 32:571-575, 1979b.
Asch., Smith, C.G., Siler-Khodr, T.M., and Pauerstein, C.J. Effects of delta-9-tetrahydrocannabinol adninistration on gonadal steroidogenic activity in vivo. Fertil Steril 32:576-582, 1979c.
Asch, R.H., Smith, C.G., Siler-Khodr, T.M., and Pauerstein, C.J. Effects of delta-9-tetrahydrocanna binol during the follicular phase of the rhesus monkey (macaca mulatta). J Clin Endocrinol Metab 52:50-55, 1981.
Ayalon, D., Nir, I., Cordova, T., Bauminger, S., Puder, M., Naor, Z., Kashi, R., Zor, U., Harell, A., and Lindner, H.R. Acute effect of delta-1-tetrahydrocannabinol on the hypothalamo-pituitary-ovarian axis in the rat. Neuroendocrinology 23:31-32, 1977.
Bauman, J.E. Marijuana and the fen-ale reproductive system. Testimony before the Subcomittee on Criminal Justice of the Comittee on the Judiciary, U.S. Senate. In: Health Consequences of Marijuana Use. Washington, D.C.: U.S. Govt. Print. Off., 1980.
Burnstein, S., Hunter, S., and Shoupe, T.S. Cannabinoid inhibition of rat luteal cell progesterone synthesis. Res Comm Chem Pathol Pharmacol 24:413-416, 1979.
Cook C.E., Seltzman, H.H., Schindler, V.H., Tallent, C.R., and Chin, M.M. The analysis of cannabinoids in biological fluids. National Institute on Drug Abuse Research Monograph No. 42, Washington, D.C.: U.S. Govt. Print. Off., 1982.
Daley, J.D., Branda, L.A., Rosenfeld, J., and Younglai, E.V. Increase of serum prolactin in male rats by (-)-trans-delta-9-tetrahydrocannabinol. J Endocrinol 63:415-416, 1974.
Dalterio, S., Bartke, A., and Burstein, S. Cannabinoids inhibit testosterone secretion by muse testes in vitro. Science 196:1472-1473, 1976.
Doms, R.W. Jr., Nichols, S.F., and Harclerode, J. The effects of delta-9-tetrahydrocannabinol and phencyclidine hydrochloride on the gonadotrophs of the rat anterior pituitary. Fed Proc 40(3):279, 1981.
Husain, S. and lame, M. Possible mechanism of the cellular effects of marihuana on male reproduction. Fran the Proceedings of the Cannabinoids 1982 Meeting (in press).
Jakubovic, A. and McGeer, P.L. In vitro inhibition of protein and nucleic acid synthesis in rat testicular tissue by cannabinoids. In: Nahas, G.G., Paton, W.D.M. and Idanpaan-Heikkila, J. (Eds.) Marihuana: Chemistry, Biochemistry and Cellular Effects, New York: Springer-Verlag, 1976, pp. 223-241.
Knobil, E. On the control of gonadotropin secretion in the Rhesus monkey Recent Prog Horm Res 30:1, 1974.
Kramer, J. and Ben-David, M. Suppression of prolactin secretion by acute administration of delta-9-THC in rats. Proc Soc Exp Biol Med 147:482-484, 1974.

Lemberger, L. and Rubin, A. Cannabis: Role of metabolisn in the development of tolerance. Drug Metab Rev 8:59-68, 1978.

Marks, B.H. Delta-1-THC andluteinizing hormone secretion. In: Zimmerman, E., Gispen, W.H., Marks, B.H., and de Wied, D. (Eds.) Progress in Brain Research - Drug Effects on Neuroendocrine Regulation Vol. 10, Amsterdam: Elsevier, 1973, pp. 331-338

Meites, J., Lu, K.K., Wuttke, W., Welsch, C.W., Nagasawa, H., and Quadri, S.K. Recent studies on functions in rats. Recent Prog Horm Res 28:471-490, 1972.

Nix, I., Ayalon, D., Tsafriri, A., Cordova, T., and Lindner, H.R. Suppression of the cyclic surge of luteinizing hormone secretiom and of ovulation in the rat by delta-1-tetrahydrocannabinol. Nature (London) 243:470-471, 1973.

Okey, A.B. and Bondy, G.P. Delta-9-tetrahydrocannabinol and 17-beta-estradiol bind to different macromolecules in estrogen target tissues. Science 200:312-314, 1978.

Reich, R., Laufer, N., Lewysohn, O., Cordva,.T., Ayalon. D., and Tsafriri, A. In vitro effects of cannabinoids on follicular function in the rat. Biol Reprod 27:223-231, 1982.

Raine, J.M., Wing, D.R., and Paton, W.D. The effects of delta-1-tetrahydrocannabinol on mammary gland growth, enzyme activity and plasma prolactin levels in the mouse. Eur J Pharmacol 51:11-17, 1978.

Schneider, H.P.G. and McCam, S.M. Possible role of dopamine as transmitter to promote discharge of LH-releasing factor. Endocrinology 85:121-132, 176.

Shoemaker, R.H. and Harmon, J.W. Suggested Mechanism for the demasculinizing effect of marihuana. Fed Proc 36:345, 1977.

Smith, C.G., Besch, N.F., and Asch, R.H. Effects of marihuana on the reproductive system. In: Thomas, J.A. and Singhal, R. (Eds.) Advances in Sex Hormone Research Vol. 4, Baltimore: Urban and Schwartzenberg, 1980, pp.373-294.

Smith, C.G., Smith, M.T., Besch, N.F., Smith, R.G., and Asch, R.H. Effect of delta-9-tetrahydrocannabinol (THC) on female reproductive function. In: Nahas, G.C. and Patton, W.D.M. (Eds.) Marihuana: Biolical Effects. Advances in the Biosciences, Vol. 22-23, New York: Pergamon Press, 1979a, pp. 449-461

Smith, C.G., Besch N.F. Smith, R.G., and Besch, P.K. Effect of tetrahydrocannibinol the hypothalmic-pituitary axis in the ovariectomized rhesus monkey. Fertil Steril 31:335-339, 1979b.

Smith, C.G., Almirez, R.G., Berenberg Asch, R.H. Tolerance develops to the disruptive effects of delta-9-tetrahydrocannabinol on the primate menstrual cycle. Science 219:1453-1455, 1983.

Smith., R.G., Besch, N.F., Besch, P.K., and Smith, C.G. Inhibition of gonadotropin by delta-9-tetrahydrocannabinol: mediation by steroid receptors. Science 204:325-327, 1979.

Solomon, J., Cocchia, M.A., and DiMartino, R. Effect of delta-9-tetrahydrocannabinol in uterine and vaginal cytology of ovariectomized rats. Science 195:875-877, 1977.

Tyrey, L. Delta-9-tetrahydrocannibinol suppression of episodic luteinizing hormone secretion in ovariectanized rat. Endocrinology 102:1808-1814, 1978.

AUTHORS

Carol Grace Smith, Ph.D.
Deparmtment of Pharmacology
Uniformed Services University School of Medicine
4301 Jones Bridge Road
Bethesda, Maryland 20814

Ricardo H. Asch, M.D.
Department of Obstetrics and Gynecology
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284

Effects of Marijuana on Neuroendocrine Hormones in Human Males and Females

Jack H. Mendelson, M.D., and Nancy K. Mello, Ph.D.

I. MARIJUANA EFFECTS ON PITUITARY-GONADAL HORMONES IN MALES

In 1971, our laboratory began clinical research on marijuana effects in humans following a request by the National Commission on Marijuana for acute and chronic studies of marijuana smoking in human males (Mendelson et al. 1972; Mendelson et al. 1974a). During the past 12 years we have examined the biological and behavioral consequences of marijuana use in over 200 human male volunteer subjects. Many of these studies have been multi-disciplinary and have involved investigators from internal medicine, psychiatry, psychology, biochemistry, physiology, sociology, neuropsychology and neuroradiology (Mendelson et al. 1974a and b; 1976a and b; Rossi et al. 1977, 1978; Kuehnle et al. 1977).

Numerous experimental animal studies, primarily conducted with rodents, have shown that cannabis compounds suppress plasma testosterone levels (Collu et al. 1975; Mascarinec et al. 1978; Symons et al. 1976; Thompson et al. 1974, 1973). Varying effects of cannabis have been reported in vitro studies of leydig cell testosterone synthesis from tissues obtained from rat and mice testes (Burstein et al. 1978, 1979; Dalterio et al, 1977; Jakubovic et al. 1979). The manner in which cannabis preparations affect leydig cell function in the intact animal remains unclear since leydig cell regression has been found in mice (Dixit et al. 1974) but no leydig cell changes were observed in pigeons (Vyas and Singh 1976). There are also conflicting data concerning the effects of cannabis compounds on HCG stimulation of plasma testosterone, with both inhibitory and lack of inhibitory effects reported (Jakubovic et al. 1979).

Luteinizing hormone levels have been found to be suppressed in male rodents following injection of cannabis compounds (Collu et al. 1975; Marks 1973; Symons et al. 1976), but it is not clear if luteinizing hormone levels were depressed antecedent to changes in plasma testosterone levels. It is unfortunate that parametric data are not available for cannabis effects on sequential determination of luteini zing hormone and testosterone levels in plasma

samples obtained from experimental animals. If cannabis compounds suppress testosterone levels via a central mechanism it would be anticipated that decrements in luteinizing hormone levels would occur prior to changes in plasma testosterone levels. On the other hand, if decrements in plasma testosterone levels occurred initially without an antecedent fall in LH levels the most parsimonious explanation for this phenomena is that cannabis acts directly to inhibit testosterone production, increase testosterone clearance, or both. But if cannabis compounds directly affect production or clearance rate of gonadal steroids, negative feedback stimulation should result in an increment rather than a decrement in LH values. An unfortunate lack of attention to well known feedback control mechanisms for regulation of hypothalamic-pituitary-gonadal function in cannabis related studies with experimental animals has been critically commented upon by Abel in his recent review on marijuana and sexual function (Abel 1981). An excellent comprehensive review of the effects of marijuana and cannabis on reproduction and endocrine function has been prepared by Dr. Eric Bloch (1983).

Studies of marijuana effects on pituitary gonadal hormones in human males were stimulated by Harmon and Aliapoulios' (1972) clinical observations of gynecomastia in 3 men who reported smoking large quantities of marijuana over a long period of time. Subsequently, Kolodny and his associates (1974) reported that plasma testosterone levels in 20 male marijuana smokers (aged 18-28) were significantly lower than testosterone levels in males who reported no past history of marijuana use. In 1974, we reported minimal effects of chronic marijuana smoking on testosterone levels in men studied under controlled conditions, on a clinical research ward (Mendelson et al. 1974b). Since antecedent use of other drugs (alcohol, opiates) has been shown to depress male plasma testosterone levels (cf. Mendelson et al. 1978a) we felt it was essential to evaluate the direct effects of marijuana in an otherwise drug-free environment. Twenty-seven men (aged 21-26) were designated as heavy or casual marijuana users on the basis of their past history of marijuana use. Twelve subjects reported smoking marijuana for an average of 5.3 years and smoking an average of 11.5 times per month during the year before the study. These subjects were designated as casual users. Fifteen subjects reported smoking marijuana for 5.6-years and smoked a mean of 42 marijuana cigarettes per month during the year before the study. These subjects were designated as heavy users.

The study consisted of 3 phases: a drug-free baseline period of 5 days; a 21-day period during which subjects could acquire and smoke marijuana cigarettes; and a postmarijuana smoking period of 5 days duration. During the 21-day marijuana smoking phase, subjects had an opportunity to work at a simple operant task to earn points that were exchangeable for either money or marijuana cigarettes. Blood samples were obtained daily between 8:30 and 9:30 a.m. throughout the 31-day study for analysis of plasma testosterone levels.

The effects of marijuana on plasma testosterone levels in casual and heavy marijuana smokers are shown in figures 1 and 2. Casual users smoked an average of 54.3 marijuana cigarettes during the 21 day period. Casual users smoked slightly more marijuana during successive 7 day periods of the study (figure 1). However, plasma testosterone levels were not significantly different from baseline, and no statistically significant differences in testosterone levels were found between any phases of the study (fig. 1). The heavy users smoked a mean of 119.5 marijuana cigarettes during the 21 day smoking period (figure 2). The heavy users smoked more marijuana cigarettes each day than the casual users and the heavy users also smoked progressively more marijuana over successive phases of the study (figure 2). Despite heavy marijuana smoking, plasma testosterone levels did not change appreciably from baseline. No statistically significant differences in plasma testosterone lwels of heavy smokers were found during any phases of the study. Moreover, there were no statistically significant differences in plasma testosterone levels between heavy and casual users during any phase of the study.

In 1976, Kolodny and his associates reported findings obtained in studies of acute changes of plasma testosterone levels in normal males after marijuana smoking. Plasma testosterone levels were measured at 15, 30, 60, 120 and 180 minutes after marijuana smoking as compared with values ohtained for the same individuals during a control period when no marijuana smoking took place. Kolodny and his colleagues (1976) also reported that statistically significant lower plasma luteinizing hormone values were found 180 minutes after marijuana smoking in the same group of men.

We subsequently undertook a more detailed investigation of the interrelationships between marijuana use, luteinizing hormone and testosterone levels in healthy adult males, studied under controlled research ward conditions (Mendelson et al. 1978b). A major technological advance allowed us to collect continuous plasma samples over 24 hours from a chronic intravenous catheter attached to a battery-operated portable non-thrombogenic pump. This technique permitted determination of integrated plasma values for testosterone and luteinizing hormone before, during, and after a period of chronic marijuana use. Again, no systematic relationships were found between marijuana smoking and increases or decreases in plasma testosterone levels and no correlation was obtained between changes in luteinizing hormone levels with marijuana use. Figure 3 (middle) shows the relationship between marijuana cigarette smoking and plasma testosterone and IH values in a representative subject. Comparison of these data with plasma testosterone and LH values prior to and following marijuana use (rows 1 and 3, figure 3) shows that there were episodic fluctuations in testosterone levels and smaller fluctuations in plasma LH levels on all study days. But these episodic variations could not be correlated with antecedent or concurrent marijuana use (Mendelson et al. 1978b). The episodic secretory patterns of these pituitary-gonadal hormones attest to the importance

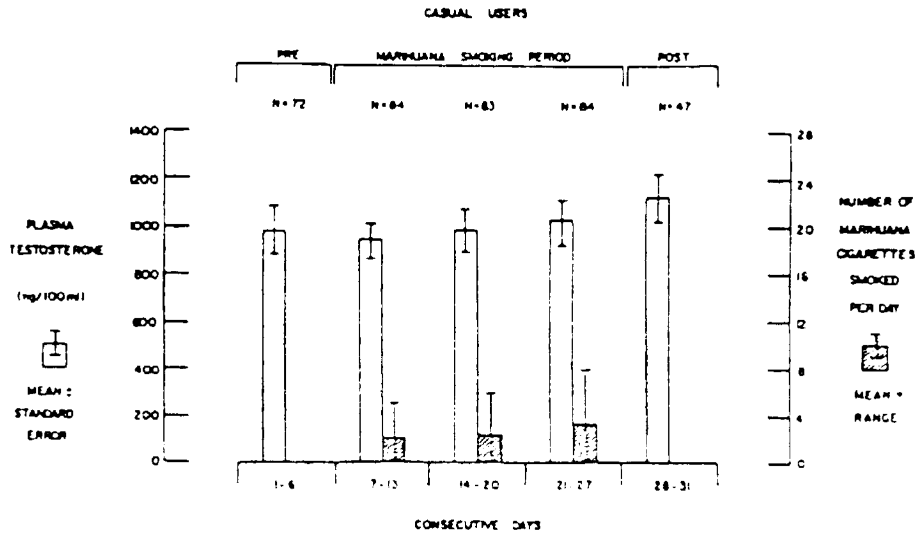


Figure 1. Plasma Testosterone Values for 12 “Casual Users before, during and after a 21 -Day Marihuana-Smoking Period

From: Mendelson, J.H., Kuehnle, J., Ellingboe, J., and Babor, T.F. Plasma testosterone levels before, during and after chronic marihuana smoking. N. Eng. J. Med., 291:1051-1055, 1974b. Copyright, The Massachusetts Medical Society. Reprinted by permission.

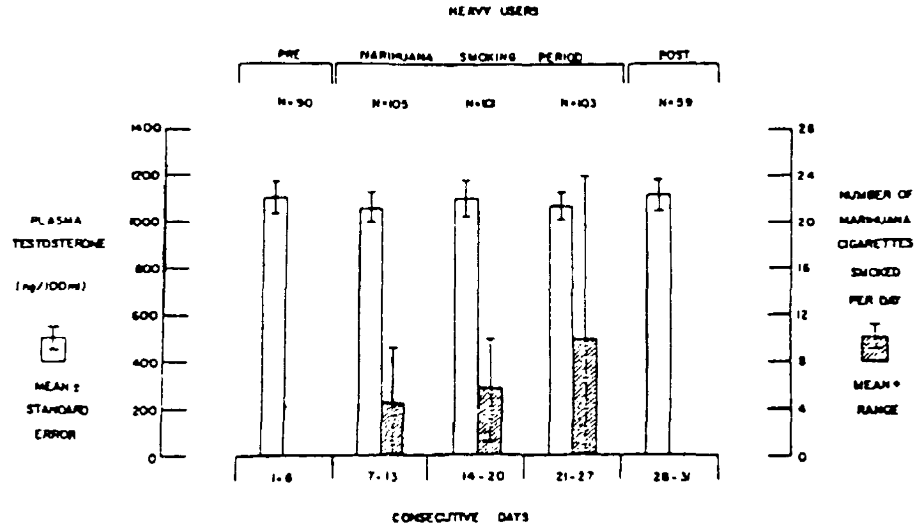


Figure 2. Plasma Testosterone Levels for 15 "Heavy Users" before, during and after a 21-Day Marihuana-Smoking Period

From: Mendeison, J.H., Kuehnle, J., Ellingboe, J., and Babor, T.F. Plasma testosterone levels before, during and after chronic marihuana smoking. N. Engl. J. Med., 291:1051-1055, 1974b. Copyright, The Massachusetts Medical Society. Reprinted by permission.

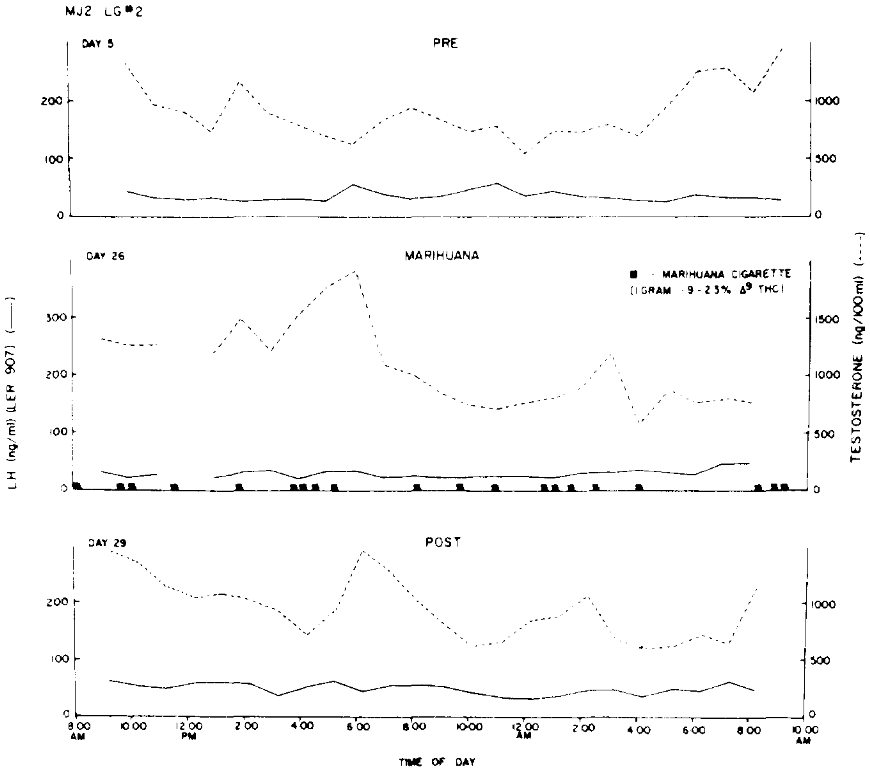


Fig. 3. Plasma lestosterone and luteinizing hormone values for a 26-year-old healthy adult male before during and after marihuana smoking Days 5. 26 and 29 refer to experimental day (day 26 was the 21st consecutive day of marihuana smoking) The 11:00 AM sample for testosterone and LH analysis on day 26 was lost and therefore not reported

Mendelson, J.H.; Ellingboe, J.; Kuehnle, J.C.; and Mello, N.K. Effects of chronic marihuana use on integrated plasma testosterone and luteinizing hormone levels. J Pharmacol Exp Ther, 207:611-617, 1978. © 1978, American Society for Pharmacology andrxperimental Theraputics. Reprinted by permission.

of using an integrated plasma sampling procedure which yields a true mean value for each collection interval.

II. MARIJUANA EFFECTS ON PITUITARY GONADAL HORMONES IN FEMALES

Despite the considerable clinical evidence that certain drugs of abuse (alcohol, heroin, methadone) suppress reproductive hormones and compromise sexual function in males (Mendelson et al. 1978a; Gordon et al. 1976; Elendelson et al. 1977) and disrupt menstrual cycles in females (Wallach et al. 1969; Mello 1980) there have been relatively few systematic studies of drug effects on pituitary gonadal hormones essential for reproductive function in women. Since disruption of normal hormonal function in women could adversely affect fetal growth and development, it is of particular importance to acquire objective information about the interaction between drug use and abuse and reproductive hormone function in human females.

Although precise estimates of incidence and prevalence of marijuana smoking among young men and women are not available, the presumed frequency of marijuana use among young women argues strongly for an empirical analysis of the direct effects of marijuana on reproductive hormones. Although studies of marijuana effects on male reproductive hormones have yielded conflicting data, there is accumulating evidence that THC, the psychoactive component of marijuana, adversely affects female reproductive function.

THC Effects on LH Secretion and Ovulation

The administration of THC (Δ^9 tetrahydrocannabinol) to female rodents has been consistently shown to suppress luteinizing hormone (LH) secretion (Marks 1973; Nir et al. 1973; Tyrey 1978, 1980). A dose dependent suppression of LH in ovariectomized rats occurred rapidly, within one hour after THC administration (Marks 1973). THC suppression of LH could be blocked by relatively small doses of estradiol which suggests that THC suppressed LH secretory activity at suprapituitary, and probably hypothalamic sites.

THC was found to suppress ovulation as well as cyclic luteinizing hormone secretion in the rat (Nir et al. 1973). However, normal patterns of spontaneous ovulation could be restored in THC treated rats by gonadotropin releasing hormone administration. This provides further evidence that the suppressant effects of THC on LH occur in the hypothalamus (Nir et al. 1973).

More recently Tyrey (1978, 1980) has reported a dose dependent suppression of LH secretion in ovariectomized rats at relatively high doses (0.5 to 8 mg/kg/bwt)(Tyrey 1978) and at doses more comparable to those attained by human smokers (62.5 to 500 mcg/kg/bwt) (Tyrey 1980). Administration of THC in doses of 62.5 mcg/kg/bwt or above rapidly suppressed episodic LH release and reduced serum LH concentration by 42 to 68 % (Tyrey 1980). The

disruption of LH secretion persisted longer at higher doses of THC, but the degree of LH suppression was comparable across the dose range studied (Tyrey 1980).

The administration of THC to ovariectomized rhesus monkeys has also been shown to significantly suppress LH secretion over 12-24 hours (Besch et al. 1977). These observations have been confirmed and extended in a series of well controlled studies in ovariectomized monkeys (Smith et al. 1979a). In contrast to findings in rats (Tyrey 1980), the degree of THC induced suppression of LH was not correlated with the THC dose. However, the dura tion of LH suppression was dose dependent in monkeys and III was reversed within 48 hours after THC administration.

In both rhesus monkey (Smith et al. 1979a) and rat (Tyrey 1978) administration of LH/RH reversed the inhibitory effects of THC on LH secretion. The investigators interpret these results to indicate that THC suppression of LH secretion occurred at hypothalamic sites.

Although chronic inject ion of Δ^9THC suppressed ovulation and decreased gonadotropin, gonadal steroids, and prolactin levels in mature rhesus monkey for a period of several months, this suppression did not persist (Smith et al. 1983). Continued expo-- sure of the female rhesus monkey to Δ^9 THC (beyond 2-3 months) was associated with a remission of menstrual cycle and pituitary gonadal hormonal abnormalities and thus female monkeys develop tolerance to disruptive effects of marijuana on reproductive function.

The rabbit model has been especially useful in studies of reproductive function since ovulation can be induced. Asch and coworkers (1979) attempted to determine if THC induced suppression of ovulation in the rabbit was due to hypothalamic, pituitary or gonadal mechanisms. It was found that THC administration did not directly affect ovarian function and did not directly suppress pituitary release of luteinizing hormone (Asch et al. 1979). Rather, gonadotropin secretory activity from the pituitary appeared to be inhibited by THC effects which were mediated at the hypothalamic level.

In summary studies of THC effects on LH episodic secretory pattern in several animal models agree that THC inhibits or suppresses the release of gonadotropin releasing hormone at the hypothalamus. THC does not appear to directly affect either the production or release of ovarian steroids (Asch et al. 1979; Smith et al. 1979b). The extent to which the duration and degree of LH suppression by THC is dose dependent has been inconsistent across species (e.g. Tyrey 1980; Smith et al. 1979b). Finally, tolerance to THC effects upon reproductive hormones occurs in monkey (Smith et al. 1983).

THC Effects on Prolactin

Studies of the effects of THC on prolactin have yielded incon-

sistent results. THC has been reported to increase serum prolactin levels (Daley et al. 1974) and to suppress prolactin secretion (Kramer and Ben-David 1974) in male rats. Subsequent studies in humans and rat have yielded comparable conflicting results (Lemberger et al. 1975; Raine et al. 1978).

Recent carefully controlled studies in monkey have shown that an acute dose of THC produces a significant decrease in prolactin levels in both male and female rhesus monkeys (Smith et al. 1979b). However, the THC induced decrease in prolactin is of relatively short duration. Further studies by Smith and associates indicate that THC induced suppression of prolactin levels is probably mediated at a suprapituitary site. The finding that THC produces a suppression of prolactin levels in male and female rhesus monkeys and also suppress LH levels in female monkey is of considerable interest since psychoactive drugs which have potent effects in suppressing LH levels, such as opiate agonists, (Tolis et al. 1975) stimulate rather than inhibit prolactin secretion.

Implications

It is clear from the foregoing that THC consistently produces significant changes in pituitary gonadal hormones which are essential for normal reproductive function in experimental animal models. The major unanswered question is what are the relevance of these data for human females? There are often marked species differences even within animal models and the degree to which THC induced disruption of pituitary gonadal hormones in animals can be extrapolated to humans is an empirical question. Despite the predictive values (and relative economy) of studying drug effects in animals, the ultimate significance of these findings can only be determined in human studies. Tyrey (1980) has argued for the importance of human studies in view of his observations in rats. Tyrey concludes that studies of LH patterns in human females "may reveal a more striking deviation from normal than thus far has been apparent. This possibility together with a broader implication of possible associated disturbances in reproductive function is worthy of further evaluation" (Tyrey 1980, p. 308).

In recent years animal models have appeared to gain some ascendancy over human studies and economic considerations have lent special weight to the familiar arguments often advanced in favor of rodent studies: i.e. activity, nutritional, genetic control; feasibility of surgical intervention (e.g. ovariectomy, castration); toxicological analysis of post mortem tissue, and relative expendability. Since both animal and clinical studies are essential in many spheres of biology and medicine, it seems appropriate to comment on some seldom acknowledged limitations of animal studies, which specifically apply to animal studies of marihuana. It is well known that differences in drug metabolism and disposition as well as species variations in hormonal mechanisms may limit the validity of ad hoc comparisons between animal and human pharmacology. The significance of experimental animal studies for human pharmacology may be limited by factors such as non-

446-384 0 - 84 - 8

physiological levels of drug dosage, differences in route of administration, and species differences in time and duration of peak drug dose effects. For example, Smith and co-workers (1979a) reported that parenteral administration of THC in single doses of 2.5 to 5.0 mg/kg/day significantly suppressed LH in female rhesus monkeys. However, a single parenteral dose of 2.5 mg/kg of THC is equivalent to an inhalation dose of 150 mg of THC for a 60 kilogram female. High quality marijuana contains approximately 2% THC and a 1 gram marijuana cigarette contains about 20 mg of THC. Therefore, a 60 kilogram female would have to smoke at least 7 to 8 one gram marijuana cigarettes containing 2% THC over a time course equivalent to a bolus injection of THC in order to receive a peak dose effect comparable to the acute administration of 2.5 mg/kg in monkey over a comparable time interval.

Investigators who are conducting studies to determine the effects of cannabis on pituitary gonadal hormones should be very careful to specify the rationale, for extrapolating the relevance of doses of THC which they have used with animals to humans. Obviously, they should be extremely careful in justifying doses employed in experimental animal studies to human pharmacology. Unfortunately, problems appear to consistently arise in this area. For example, in a recent study (Dalterio et al. 1983) the dose of THC administered to mice was 50 mg/kg of body weight. The authors rationalized selection of this dose as follows: "This dose would correspond to about 4 mg/kg orally in the human user or 3 marijuana cigarettes of 1% THC based on a body surface conversion of 12 for mouse." The selection of a body surface conversion factor of 12 for mouse in comparison to human may be considered appropriate although it should be emphasized that different conversion factors may be appropriate for different drugs based upon the pharmacokinetics, metabolism, and excretion of specific compounds. Nevertheless, if we accept the conversion factor of 12 we concur that 50 mg/kg for the mouse would be equivalent to 4 mg/kg for the human. But is 4 mg/kg the dose of marijuana which is contained in 3 marijuana cigarettes with 1% THC as the authors state? If a 70 kg human ingested 4 mg of THC/kg of body weight, the total amount ingested would be 280 mg. A 1 gram marijuana cigarette containing 1% THC contains 10 mg of THC. A simple calculation reveals that 3 marijuana cigarettes contain 30 mg of THC. However, 280 mg of THC would actually be present in 28 1 gram marijuana cigarettes which contain 1% THC. Surely there is a significant difference between human consumption of 3 vs. 28 marijuana cigarettes. Although the error in calculation of dosage was undoubtedly a computation problem, pharmacologists should exercise special care in computing dose equivalents for animal and human studies.

Given the problems associated with experimental animal studies it is important to determine if the acute and chronic use of marijuana by human females produces any alterations or abnormalities in those pituitary gonadal hormones which are essential for reproductive function. Moreover, we believe it is essential to study

marijuana self-administration by smoking and inhalation since this route of administration most closely approximates marijuana use in real life circumstances. It is also important to examine the effects of marijuana on female reproductive hormones under conditions where other psychoactive drugs which are known to significantly alter pituitary gonadal hormones in males (e.g. alcohol and opiates) do not confound data on marijuana effects on hormonal functions. It is likely that variations in the degree of other drug use may account, in part, for some of the discrepant findings concerning marijuana effects on male pituitary gonadal hormones.

Only one study has been published on the effects of marijuana on reproductive hormones in human females. Women were studied on an outpatient basis without control of other drug use, the results obtained were congruent with animal studies of the effects of THC. Bauman and co-workers (1979) compared 26 women who reported using marijuana at least 4 times a week with 16 age-matched control women who reported never using marijuana. A blood sample was collected on days 1, 5, 10, 12-18, 20, 25 and 30 of the menstrual cycle in each group. Thirty-seven of the 42 women were studied over 2 complete menstrual cycles. Samples were analyzed for estrone, estradiol, luteinizing hormone, follicle stimulating hormone, prolactin and testosterone. It was found that marijuana users had significantly shorter menstrual cycles and a significantly shorter luteal phase than non-marijuana smokers. Prolactin levels were consistently and significantly lower in marijuana users and testosterone levels were consistently and significantly higher. However, there were no statistically significant differences in other hormones measured. The two groups of women had an equivalent number of anovulatory cycles (Bauman 1979). These data further attest to the importance of determining the direct effects of marijuana use on pituitary gonadal hormones essential for reproductive function in healthy, young marijuana users, under controlled conditions.

At the time of preparation of this review article our laboratory is at approximately the midpoint of a series of studies designed to assess acute and chronic effects of marijuana on reproductive hormones in women. These studies involve comprehensive assessment of menstrual cycle function and pituitary gonadal hormone status in women who use marijuana daily, weekly, or less frequently. A novel portion of these studies is a 35-day residential research ward program where detailed evaluations can be carried out on marijuana use and covariance of menstrual cycle function. Hormonal data are currently being analyzed and results should be available for statistical analysis utilizing appropriate computer programs in the near future. Several behavioral findings which appear to be quite robust may be described at this time.

1) First, approximately 20% of all women who responded to advertisements of solicitation for participation in the study were found to have galactorrhea during initial medical screening examinations. The exact incidence of galactorrhea is unknown in

the general population. Whether or not galactorrhea present in 20% of a volunteer population represents a pathological phenomena attributable to regular marijuana use or is unrelated to marijuana or other drug use remains to be determined. The majority of the women who had galactorrhea did not have elevated or abnormal serum prolactin levels. However, hyperprolactinemia is not necessarily a concomitant or a unique causal factor in galactorrhea. Serial determinations of prolactin levels which were obtained during the course of chronic marijuana smoking in our studies should provide better information about possible concordance between marijuana use and galactorrhea.

2) Marijuana use by females as reported by past history, ongoing use in naturalistic settings assessed by daily diary reports and actual observed use on the research ward is less (both in terms of dose and frequency) than that found in males of similar age and socioeconomic status. This finding is not particularly surprising since drug use (including alcohol) by females is usually not as great as observed for males. However, it should be noted that important exceptions may exist. For example, the heaviest marijuana user observed in any of our studies conducted on our research ward with over 150 individuals was a female. Moreover, this subject developed unequivocal signs and symptoms of cannabis withdrawal following cessation of marijuana smoking. Thus, trends toward light or heavy marijuana use as a function of gender may misleadingly suggest that very heavy or exceptionally heavy use of marijuana is not likely to occur in female users. Given the very large number of women who smoke marijuana in contemporary American society it may be anticipated that very heavy or extreme use may occur in a small proportion of total female users but nevertheless may involve a surprisingly large number of individuals. For example, if our current findings that 10% of female marijuana users smoke 4-8 marijuana cigarettes per day are applied to the general population, there are perhaps 250,000 to 300,000 females who are engaging in this heavy degree of marijuana use.

3) We have completed preliminary analysis of acute effects of marijuana smoking on pituitary gonadal hormones during the periovulatory period of the menstrual cycle. The major purpose of discussing these data is to highlight some of the problems associated with interpretation of studies of acute marijuana effects on hormone function in women.

Table 1 presents LH levels prior to and following (at 0 time) smokin a 1 gram marijuana cigarette containing approximately 1.8% Δ^9 THC (standardized marijuana cigarettes were obtained from the National Institute on Drug Abuse). The women who participated in the study were carefully monitored over three consecutive menstrual cycle phases to precisely establish the timecourse of the periovulatory period. A criterion of 200 ng/ml commonly accepted in endocrinological practice was employed to define the lower limit of luteinizing hormone levels associated with the midcycle, periovulatory, LH surge. These studies were carried

TABLE I

TIME(minutes)	LH (ng/ml) $\bar{X} \pm$ S.D.
-120	380 ± 128
-90	351 ± 132
-60	345 ± 134
-30	335 ± 137
0	293 ± 110
1.5	322 ± 126
20	361 ± 135
25	343 ± 114
30	339 ± 113
45	298 ± 113
60	289 ± 125
90	281 ± 147
120	240 ± 118
150	223 ± 127 *
180	216 ± 83 *

*lower than 0 time value ($p < .05$)

out under research ward conditions to ensure adequate safety and comfort for research subjects and also to assure that no other substance use confounded interpretation of the III data.

Plasma specimens were collected from an indwelling intravenous catheter from 120 minutes prior to and 180 minutes following marijuana smoking. Subjects smoked an entire 1 gram marijuana cigarette containing 1.8% THC over a 15 minute period by deeply inhaling the pyrolized material at a frequency of once per 30 seconds.

Statistical analysis of LH levels shown in Table 1, revealed that statistically significant decrements in LH levels (when compared with 0 time values) occurred at 150 and 180 minutes ($p < .05$). Curiously these statistically significant findings of decrements in LH values are similar to data reported by Kolodny and his associates for marijuana induced decrements in LH levels in males (Kolodny et al. 1976). Do the findings we have obtained with females have any biological significance?

If we examine data for all LH values shown in Table 1, it is apparent that there is a steady decrement in LH levels from -120 minutes to 0 time prior to marihuana smoking (2 way ANOVA $p = 0.026$). Since the periovulatory period is relatively short (24 to 36 hours), it may be possible that we are simply observing a normal decrease in LH levels through time. We have attempted to carry out assessments of LH levels during the periovulatory period following placebo marijuana cigarette smoking (placebo marijuana cigarettes were provided by the National Institute on Drug Abuse). However, because of the short duration of the periovulatory LH surge, we have, as of this time, been only able to successfully study one female who was administered a marijuana placebo cigarette during the periovulatory LH surge. Findings obtained with this subject were almost identical to those observed for the 5 subjects who received the active marijuana cigarettes. We therefore believe that great caution must be exercised in interpreting changes in H levels measured at ovulation after concomitant use of marijuana.

Hopefully, discrepancies between putative cannabis effects on neuroendocrine function in humans as contrasted with data obtained in experimental animal studies will be resolved as a consequence of improved experimental design and methods. However, very recent studies suggest that the complexities in interpreting data obtained from basic studies may be increasing rather than diminishing. Sauer and his associates (1983) have studied the interaction of cannabis compounds with the estrogen receptor. They found that both crude marijuana extract and condensed marijuana smoke competed with estradiol for binding to the estrogen receptor of rat uterus cytosol. However, pure Δ^9 THC and ten Δ^9 THC metabolites did not compete with estradiol for receptor binding. Moreover in *in vivo* studies cannabis extract in large doses had neither estrogenic nor anti-estrogenic effects. Sauer and his

colleagues (1983) concluded, "thus, although estrogen receptor binding activity was observed in crude marijuana extract, marijuana smoke condensate and several known components of cannabis, direct estrogenic activity of cannabis extract could not be demonstrated in vivo." It therefore appears that lack of concordance of findings in experimental animal and human studies, (with respect to marijuana effects on hormonal function) is paralleled by equally discordant data obtained from in vitro basic receptor binding studies compared to effects observed the intact animal.

REFERENCES

Abel, E.L. Marihuana and sex: A critical survey. Drug Alcohol Depend 8:1-22, 1981

Asch, R.H., Fernandez E.O., Smith, C.G. and Pauerstein, C.J. Blockage of the ovulatory reflex in the rabbit with delta 9-tetrahydrocannabinol. Fertil Steril 31: 331, 1979.

Bauman, J.E., Kolodny, R.C., Dornbush, R.L. and Webster, S.K. Efectos endocrinos de1 uso cronico de la mariguana en mujeres. In: Cuadernos Cientificos CEMESAM (Centro Mexicano de Estudios en Salud Mental), Vol. 10, Mexico, D.F., Julio, 1979, pp. 85-97.

Besch, P.K. and Kaufman, R.H. The effect of marihuana (delta-Q-tetrahydrocannabinol) on the secretion of luteinizing hormone in the ovariectomized rhesus monkey. Am J Obstet Gynecol 128: 635, 1977.

Bloch, E. Effects of marihuana and cannabinoids on reproduction, endocrine function, development and chromosomes. WHO Fxpert Committee Report on Health Hazards of Cannabis Use. Toronto, 1983 (in press).

Burstein, S., Hunter, S.A. and Shoupe, T.S. Site of inhibition of Leydig cell testosterone synthesis by delta-1-tetrahydrocannabinol. Mol Pharmacol 15:633-640, 1979.

Burstein, S.A., Hunter, S.A., Shoupe, T.S., Taylor, P., Bartke, A., and Dalterio, S. Cannabinoid inhibition of testosterone synthesis by mouse Leydig cells. Res Commun Chem Pathol Pharmacol 19:557-560, 1978.

Collu, R., Letarte, J., Leboeuf, G. and Ducharme, J.R. Endocrine effects of chronic administration of psychoactive drugs to prepuberal male rats. Life Sci 16:533-542, 1975.

Daley, J.D., Branda, L.A. and Rosenfeld, J. Increase of serum prolactin in male rats by (minus)-trans-delta-9-tetrahydrocannabinol. J Endocrinol 63:315, 1974.

Daltcrio, S., Bartke, A. and Burstein, S. Cannabinoids inhibit testosterone secretion by mouse testes in vitro. Science 196: 1472-1473. 1977.

Dalterio, S., Mayfield, D.L., Michael, S.D., MacMillan, B.T. and Bartke. A. Effects of Δ^9-THC and castration on behavior and plasma hormone levels in male mice. Pharmacol, Biochem, Behav 18:81-86, 1983.

Dixit, V.P., Sharma, V.N. and Lohiya, N.K. The effect of chronically administered cannabis extract on the testicular function of mice. Europ J Pharmacol 26:111-115, 1974.

Gordon, G.G., Altman, K., Southren, A.L., Rubin, E. and Lieber, C.S. Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. N Eng J Med 295:793-797, 1976.

Harmon, J. and Aliapoulios, M.A. Gynecomastia in marihuana users. N Eng Med 287:936, 1972.

Jakubovic, A., McGeer, E.G. and McGeer, P.L. Effects of cannabinoids on testosterone and protein synthesis in rat testes Leydig cells in vitro. Mol Cell Endocrin 15:41-50, 1979.

Kolodny, R.C., Masters, W.H., Kolodner, R.M. and Toro, G. Depression of plasma testosterone levels after chronic intensive marihuana use. N Engl J Med 290:872-874, 1973.

Kolodny, R.C., Lessin, R.C., Toro, G., Masters, W.H. and Cohen, S. Depression of plasma testosterone with acute marihuana administration. In: Braude, M.C. and Szara, S. eds. The Pharmacology of Marihuana. New York: Raven Press, 1976, pp. 217-225.

Kramer, J. and Ben-David, M. Suppression of prolactin secretion by acute administration of delta-9-THC in rats. Proc Soc Exp Biol Med 147:482, 1974.

Kuehnle, J.C., Mendelson, J.H., Davis, K.R. and New, P.F.J. Computed tomographic examination of heavy marihuana smokers. JAMA 237:1231-1232, 1977.

Lemberger, L., Crabtree, R., Rowe, H., Clemens, J.: Tetrahydrocannabinols and serum prolactin levels in man. Life Sci 16: 1339, 1975.

Marks, B.H. TIIC and luteinizing hormone secretion. In: Zimmerman, E., Gispen, W.H., Marks, B.H. and DeWied, D., eds. Progress in Brain Research/Drug Effects on Neuroendocrine Regulation, Vol. 10. Amsterdam: Elsevier, 1973, pp. 331-338.

Mascarinec, M.P., Shipley, G., Novotny, M., Brown, D.J. and Forney, R.B. Different effects of synthetic delta-9-tetrahydrocannabinol and cannabis extract on steroid metabolism in male rats. Experientia 34:88, 1978.

Mello, N.K. Behavioral and biological aspects of alcohol problems in women. In: Kalant, O. et al, eds. Research Advances in Alcohol and Drug Problems, Vol. V, Alcohol and Drug Problems in Women. New York: Plenum Press, 1980, pp. 286-298

Mendelson, J.H., Meyer, R.E., Rossi, A.M., Bernstein, J.G., Patch, V.D., Babor-, T.F., Reed, H., Salzman, C. and Becker, D. Behavioral and biological concomitants of chronic marihuana smoking by heavy and casual users. In: Marihuana: A Signal of Misunderstanding. Technical Papers, Vol. 1. Washington, D.C. U.S. Government Printing Office, 1972, pp. 68-216.

Mendelson, J.H., Rossi, A.M. and Meyer, R.E. eds. The Use of Marihuana: A Psychological and Physiological Inquiry. New York: Plenum Press, 1974a, pp. 202

Mendelson, J.H., Kuehnle, J.C., Ellingboe, J. and Babor, T.F. Plasma testosterone levels before, during and after chronic marihuana smoking. N Eng J Med: 291:1051-1055, 1974b.

Mendelson, J.H., Babor, T.F., Kuehnle, J.C., Rossi, A.M., Bernstein, J.G., Mello, N.K. and Greenberg, I. Behavioral and biological aspects of marihuana use. Ann NY Acad Sci 282: 186-210, 1976a.

Mendelson, J.H., Kuehnle, J.C., Greenberg, I. and Mello, N.K. Operant acquisition of marihuana in man. *J Pharmacol Exp Ther* 198:42-53, 1976b.

Mendelson, J.H., Mello, N.K. and Ellingboe, J. Effects of acute alcohol intake on pituitary-gonadal hormones in normal human males. *J Pharmacol Exp Ther* 202:676-682, 1977.

Mendelson, J.H., Mello, N.K. and Ellingboe, J. Effects of alcohol on pituitary-gonadal hormones, sexual function, and aggression in human males. In: Lipton, M.A., DiMascio, A. and Killam, K.F. eds. *Psychopharmacology: A Generation of Progress*. New York: Raven Press, 1978a, pp. 1677-1692.

Mendelson, J.H., Ellingboe, J., Kuehnle, J.C. and Mello, N.K. Effects of chronic marihuana use on integrated plasma testosterone and luteinizing hormone levels. *J Pharmacol Exp Ther* 207:611-617, 1978b.

Nir, I., Ayalon, D., Tsafriri, A., Cordova, T. and Lindner, H.R. Suppression of the cyclic of luteinizing hormone secretion and of ovulation in the rat by Δ^9-tetrahydrocannabinol. *Nature* 243:470, 1973.

Raine, J.M., Wing, D.R. and Paton, W.D. The effects of delta-l-tetrahydrocannabinol on mammary gland growth, enzyme activity and plasma prolactin levels in the mouse. *Eur J Pharmacol* 51: 11, 1978.

Rossi, A.M., Kuehnle, J.C. and Mendelson, J.H. Effects of marihuana on reaction time and short-term memory in human volunteers. *Pharmac Biochem Behav* 6:73-77, 1977.

Rossi. A.M., Kuehnle. J.C. and Mendelson. J.H. Marihuana and mood in human volunteers. *Pharmac Biochem Behav* 8:447-453, 1978.

Sauer, M.A., Rifka, S.M., Hawks, R.L., Cutler, G.B. and Loriaux, D.L. Marijuana: Interaction with the estrogen receptor. *J Pharmacol Exp Ther* 224:404-407, 1983.

Smith, C.G., Almirez, R.G. and Barenberg, J.: Tolerance develops to the disruptive effects of delta 9 tetrahydrocannbinol on the primate menstrual cycle. *Science*, 1983 (in press).

Smith, C.G., Besch, N.F., Smith, and Besch, P.K. Effect of tetrahydrocannabinol on the hypothalamic-pituitary axis in the ovariectomized rhesus monkey. *Fertil Steril* 31:335, 1979a.

Smith, C.G., Smith, M.T., Besch, N.F., Smith, R.G. and hsch, R.H. Effect of Δ^9-tetrahydrocannabinol (THC) on female reproductive function. In: Nahas, C.G. and Patton, W.D.M., eds.. *Marihuana-Biological Effects*. Oxford: Pergamon. 1979b, pp. 449.

Symons, A.M., Teale, J.D. and Marks, V. Effects of delta-9-tetrahydrocannabinol on the hypothalamic-pituitary-gonadal system in the maturing male rat. *J Endocrinol* 67:43P-44P, 1976.

Thompson, G.R., Fleishman, R.W., Rosenkrantz, J. and Braude, M.C. Oral and intravenous toxicity of delta-9-tetrahydrocannabinol in rhesus monkeys. *Toxicol Appl Pharmacol* 27:648-665, 1974.

Thompson, G.R., Mason, M.M., Rosenkrantz, H. and Braude, M.C. Chronic oral toxicity of cannabinoids in rats. *Toxicol Appl Pharmacol* 25:373-389, 1973.

Tolis, C., Hickey, J. and Guyda, H. Effects of morphine on serum growth hormone, cortisol, prolactin, and thyroid stimulating hormone in man. J Clin Endocrinol Metab 41:797, 1975.

Tyrey, L. Δ^9-tetrahydrocannabinol suppression of episodic luteinizing hormone secretion in ovariectomized rat. Endocrinol 102 1808-1814, 1978.

Tyrey, L. Δ^9-tetrahydrocannabinol: A potent inhibitor of episodic luteinizing hormone secretion. J Pharmacol Exp Ther 212 306-308, 1980.

Vyas, D.K. and Singh, R. Effect of cannabis and opium on the testis of the pigeon, *Columba livia Gmelin.* Indian J Exper Biol 14:322-25, 1976.

Wallach, R.C., Jerez, E. and Blinick, G. Pregnancy and menstrual function in narcotics addicts treated with methadone. The methadone maintenance treatment program. Amer J Obstet Gynecol 105:1226-1229, 1969.

ACKNOWLEDGEMENT

Supported by NIDA Research Grant No. 5 R01 DA 02905, "Marijuana Effects on Reproductive Hormones in Women," and ADAMHA Research Scientist Award No. 5 K05 DA 00064, "Biobehavioral Studies of Narcotics Abuse in Humans."

AUTHORS

Jack H. Mendelson, M.D.
Nancy K. Mello, Ph.D.
Alcohol and Drug Abuse Research Center
McLean Hospital/Harvard Medical School
Belmont, Massachusetts 02178

Effects of Marijuana on Pregnancy and Fetal Development in the Human

Katherine Tennes, M.A.

The first systematic studies of the effects of marijuana on pregnancy and on fetal growth were published in the past two years. Results of the investigations are suggestive of an effect, but none of the investigators thus far has reported consistently significant effects upon pregnancy, nor have they found signs or symptoms which could be considered a syndrome characteristic of neonates exposed to marijuana. Major difficulties are encountered in conducting clinical studies of this nature which may account, in part, for the tentativeness of the results.

This paper will review the evidence linking maternal and fetal risk factors with marijuana use during pregnancy and consider the methodological problems inherent in the research designs.

PREGNANCY, LABOR, AND DELIVERY

One group of investigators conducted two studies to test the hypothesis that women who smoke marijuana during pregnancy will experience greater difficulty at delivery than non-users (Greenland et al. 1982a, 1982b, 1983 in press). The hypothesis was based upon evidence from animal studies that cannabinoids (or their metabolites) affect uterine contractility.

In the first study, conducted at three prenatal care centers in the Los Angeles area, Greenland et al. (1982a, 1982b) compared 35 women who used marijuana more than once a month during pregnancy with 36 non-users. A significantly higher proportion of users was found to have precipitate labor (37% users versus 3% non-users, $p < .01$) and meconium passage (57% users versus 25% non-users, $p < .05$). They also examined differences between marijuana users who tested positive and those who tested negative for THC at delivery. Precipitate labor was observed in half of the 16 women who had positive urine or serum tests for THC and none of the women who had negative results. This finding suggested that the effect upon delivery was related to recency of use before delivery.

Other apparent differences between users and non-users, not reaching statistical significance, were: more prolonged or arrested labor (31% users, 19% non-users), operative deliveries (31% users, 14% non-users), abnormal fetal tests (11% users, 0% non-users), and manual removal of the placenta (17% users, 8% non-users).

In an attempt to verify these findings, 313 women enrolled in a low-risk home-delivery program, were studied (Greenland et al. 1983 in press). In this group, precipitate labor was again more common among the 41 marijuana users (13% users, 8% non-users) as was dysfunctional active labor (43% users, 35% non-users), meconium passage (17% users, 13% non-users), and manual removal of the placenta (10% users, 3% non-users). However, the magnitude of the differences was much smaller than in the first study and statistical significance was reached only in the case of manual removal of the placenta ($p < .05$).

The investigators considered the results inconclusive due to the small sample size and the possibility that chance alone could account for finding a few significant relationships when several hundred variables were examined. Nevertheless, they pointed out that there appeared to be a consistent pattern indicating that marijuana users generally experienced more difficult labor and their infants passed more meconium.

The predominant effect of marijuana on uterine contractility is a clinically relevant but currently unresolved issue. *In vitro*, studies have shown both an inhibitory and a stimulatory effect on prostaglandin synthesis (Burstein and Raz 1972; Burstein et al. 1973; Burstein and Hunter, 1981). Since prostaglandins have been implicated in the onset of labor (Quilligan et al. 1981) and are used clinically as uterine stimulants, further research is warranted to clarify the overall effect of marijuana on labor.

FETAL GROWTH AND DEVELOPMENT

Two studies designed to investigate the effect of maternal alcohol consumption upon fetal growth and development considered marijuana as one of the variables in the data base.

From 1977 to 1979, a sample of 1,690 women and infants were surveyed at Boston City Hospital (Alpert et al. 1981; Hingson et al. 1982). The primary focus of the infant examinations was upon growth retardation and physical anomalies associated with the Fetal Alcohol Syndrome. Examination of birthweight by multivariate analysis indicated that 43% of the variance was accounted for by gestational age, mother's age, mother's weight change during pregnancy, weight prior to pregnancy, race, cigarettes per day, infant sex, and marijuana. The variables entered the equation in the order given and all were significant at $p < .05$. It was of interest that marijuana but not alcohol made a significant contribution to the variance in birthweight. If the difference in birthweight was calculated by comparing 181 marijuana users with non-users, without accounting for confounding factors, the infants were found to weigh less by 300 grams ($p < .001$). When the effect of marijuana upon infant weight was calculated from the regression equation, the infants exposed to marijuana were lighter by 105 grams ($p < .01$). Thus, the investigators pointed out that multivariate analyses are essential for estimating the effects of a single variable upon outcome measures in order to avoid attributing to marijuana an effect partially accounted for by confounding factors.

A second investigation of the effects of prenatal exposure to alcohol was conducted at about the same time in 1978-1979 in Denver on a smaller population of 278 women (Tennes and Blackard 1980). The variance in birthweight was analyzed by stepwise multiple regression. The variables entered in the regression were: gestational age, mother's pre-pregnancy weight, number of minor physical anomalies, nicotine, parity, marijuana, caffeine, mother's age, socio-economic status, and alcohol. Only the first five variables were significant at $p < .05$. Thus, marijuana was not regarded as making a significant contribution to the variance in birthweight although it was the sixth variable accepted by the regression. Nevertheless, since the Boston City Hospital study indicated that marijuana may contribute to a reduction in fetal weight gain, the Denver study was considered to be suggestive of a similar trend but limited by an inadequate sample size.

TERATOGENIC EFFECTS

A second effect of marijuana upon fetal development suggested by the Boston City Hospital study was an increase in dysmorphology. Of 1,341 infants, 31 were found to have features compatible with the Fetal Alcohol Syndrome (Hanson et al. 1978). Women who smoked marijuana were found to be five times more likely than non-users to deliver a child with these features ($p < .001$). Low maternal weight gain during pregnancy and exposure to roentgenograms also contributed to the risk but at a lower rate than marijuana.

Recently, Qazi et al. (1982 and personal communication), during clinical examinations of infants for congenital anomalies, found six infants with fetal-alcohol-syndrome-like features. The infants' mothers denied using alcohol but smoked tobacco moderately and used marijuana regularly, from two to 14 times daily. The infants also were of low birthweight for gestational age, These retrospect - ive observations corroborate the findings in the Boston City Hospital prospective study. The finding that criteria developed to judge an alcohol effect was associated with marijuana exposure suggests a lack of specificity for either alcohol or marijuana. Furthermore, the presumptive evidence implicating marijuana does not rule out the possibility that the causative factor may be an unidentified variable common to some heavy alcohol and some heavy marijuana users.

Retrospective observations by clinicians have been an important meam of detecting the teratogenic potential of a drug. The likelihood of noticing a cause-and-effect relationship between a specific malformation and a drug is increased if the abnormality is rare, if the drug usage is widespread, or if there is a sudden incremental change in the incidence of drug use. Despite the reported increased use of marijuana by young women in the past decade, teratogenic effects have rarely been reported by clinicians. Three cases of infants with malformations described in the literature (Carakushansky et al. 1969; Bogdanoff et al. 1972; Hecht et al. 1968) were born to polydrug users and the contribution of marijuana to the defects could not be isolated.

NEUROBEHAVIORAL EFFECTS

The influence of prenatal exposure to a drug upon neurological and behavioral functioning is difficult to measure in the neonatal period because of instability due to the birth process, to extrauterine adjustments and to rapid neonatal growth (Lancioni et al. 1980). Nevertheless, examinations of infants born soon after birth are useful in isolating acute intrauterine effects, for monitoring withdrawal symptoms and for establishing a baseline for later developments. The seriousness of intrauterine drug exposure upon central nervous system functioning can be determined only by assessments of the children at older ages.

Before neurobehavioral effects can be evaluated, gestational age and birthweight need to be taken into account. In an ongoing prospective study in Ottawa (Fried et al. 1980a, 1980b, 1981), 420 women were interviewed and only those who said they used no illicit drugs other than marijuana were accepted into the study. Of 89 infants who were examined, nine were born to women who were heavy users of marijuana throughout pregnancy. Fried compared the nine heavy marijuana users with controls matched for nicotine and alcohol. He found that women who used marijuana gained significantly less weight during pregnancy than non-users ($p < .02$) and a significantly higher proportion delivered before 40 weeks' gestation ($p < .05$). No effect of marijuana exposure upon infants' birthweight was detected when gestational age was taken into account. Given the number of variables that influence birthweight and the relatively small impact expected from marijuana, an effect of marijuana on birthweight would be difficult to detect in a sample this small.

In a preliminary report of neurobehavioral examinations of the infants based upon 27 items in the Brazelton Neonatal Assessment Scale (Brazelton 1973), Fried found three items that distinguished infants of 11 heavy and moderate marijuana users from an equal number of controls matched for nicotine and alcohol. First, there was an alteration in response to a visual stimulus, second, an increase in tremulousness, and third, a peculiar high-pitched cry.

When a light is directed at the eyes of the sleeping infant, the expected response is a large body movement or marked blinking, without awakening, followed by diminishing responses or "habituation" as the light is presented repetitively. Five of the infants born to the moderate or heavy users, as compared with two of the controls, failed to give the motoric response to the light; and two of the users but none of the controls failed to habituate.

Fried speculated that the infants' altered visual responses might be a manifestation of effects similar to those reported in rabbits and primates immediately after injection of THC. In rabbits, the absolute visual thresholds were found to be elevated (Rose et al. 1979). The primate study suggested that THC inhibits light sensitive neurons in the lateral geniculate nucleus of the thalamus (McIsaac et al. 1971). Though the association to animal studies is intriguing, the finding of both greater sensitivity and greater insensitivity to a light in the infants with heavy marijuana exposure is puzzling.

By contrast with the measurement of sensitivity, no difference was found between the infants of users and non-users in visual orientation, focusing or following during awake, alert states.

Heightened tremulousness was observed in infants of users during crying, wakefulness, or sleep. Based on the entire sample of 89 infants, a significant dose response relationship between tremors and marijuana use was found ($r = .25$, $p < .002$) and the relationship did not change when nicotine and alcohol were partialed out. The third difference, a high-pitched cry similar to a "cri de chat," was noted among one-third of the infants of regular marijuana users and never among the controls. Tremors and a peculiar cry are characteristics of infants undergoing narcotic withdrawal (Lipsitz 1975). However, infants undergoing narcotic withdrawal have an adversive reaction to light, rather than insensitivity, and they are extremely irritable and hypertonic, characteristics not found in infants of marijuana users.

Repeated examinations of the infants at later ages in Fried's study suggested that diminished responsiveness to visual stimuli and heightened tremulousness persisted in half the infants of heavy users at nine days of age but were no longer present at 30 days. No difference between infants of seven users and seven matched controls was found in scores on an infant development test at one year of age.

Because of the large number of variables being considered and the small sample size, these results may not be regarded as conclusive. Elaboration of the findings is expected to result from the investigation still in progress in Ottawa and from a similar study being conducted in Denver. In addition to assessments of growth, neurological status and behaviors, infants in both studies are being evaluated for the presence of major and minor physical anomalies.

METHODOLOGICAL PROBLEMS

Naturalistic clinical studies are subject to a number of methodological problems that need to be considered in designing research and interpreting results. This paper will discuss problems with data collection and verification, and treatment of confounding variables with reference to the studies reviewed above.

Self-Report of Drug Use

A major concern of clinical studies dependent upon self-reported data of illegal drug use is that underreporting will obscure real effects. Greenland et al. (1982a) made an important contribution toward establishing the veracity of self-report as a method of data collection by radioimmunoassay of blood and urine specimens for cannabinoids. He found no positive tests among those denying usage.

Both Greenland et al, (1982a) and Fried et al. (1980a) prescreened subjects for use of illegal drugs other than marijuana during preg- nancy and confirmed the self-reports by chemical determinations for

amphetamines, opiates, etc. No contradiction between self-reports and bioassays was found.

The validation of self-report data may be applicable only to subjects enrolled in research studies. In the majority of studies, the women choose whether or not to participate. Refusal rate in public prenatal clinics in Boston and Denver was approximately 15%. Women choosing not to participate may be different from participants in their willingness to reveal actual drug use.

Not only is the data base dependent upon the woman's report, but in naturalistic studies, the woman is in control of administration of the drug. A proportion of women who use marijuana prior to pregnancy and in the first trimester decrease use in the course of the pregnancy. In the Denver studies we found that about one-third of the heavy users of marijuana sharply decreased their use early in pregnancy when they learned they were pregnant. A second abrupt drop in use occurred in some women after they were interviewed for the study although no advice was given regarding its use. Since the effects during embryogenesis may be different from later effects on fetal growth and brain development, separate assessments need to be made of early and chronic exposure.

An additional problem in data gathered by self-report is in estimating actual amounts of fetal exposure to cannabis. Assay of the infants' cord bloods at time of birth for THC is of very limited value since blood levels do not reflect tissue levels in the infant and assays are limited to Δ-9-THC and its metabolites which may not be the only effective components of marijuana.

Confounding Factors

Perhaps the most difficult methodological problem confronting investigation of marijuana use during pregnancy is the need to account for potentially confounding effects.

Variables known to co-vary with marijuana use include age, cigarette smoking, alcohol, and socio-economic status. Greenland et al. (1982a) addressed the issue when selecting subjects by matching a group of marijuana users with a group of non-users for age, number of children, and the medical center providing care. To examine the effect of other covariants of marijuana use, the investigators calculated the proportion of difference in each outcome variable that could be attributed to the other risk factors. Confidence in the results of the study was heightened by the finding that in no instance was the association between marijuana and the outcome variable altered by the adjustment.

In the Boston City Hospital study, identification of marijuana as a factor contributing to the variance in birthweight was a result of analysis by multivariate techniques. The usefulness of this excellent method, used to account for confounding variables, is limited by the need for a relatively large sample size, in order to fulfill the assumptions underlying the statistic.

A different set of confounding variables may be appropriate when examining specific effects of marijuana use on delivery or on neonatal characteristics. Correlates of these outcome measures need to be considered in addition to correlates of maternal marijuana use. Meconium-stained fluid during delivery, for example, is known to be associated with small and with large infants and to be influenced by postmaturity. To gain clinical credibility, the association between marijuana use and meconium passage require systematic examination of all known correlates of meconium staining. Likewise, other factors co-vary with tremulousness in the neonate. One of the conditions associated with tremulousness is infection which may vary as a function of life-style rather than directly with marijuana use.

One benefit of the pioneering studies of marijuana use during pregnancy is that they suggest specific effects which can then be subjected to systematic indepth scrutiny. As was pointed out by Hingson et al. (1982), studies that attempt to relate a specific drug such as marijuana to abnormal fetal development without examining a host of confounding variables "may best be regarded as identifying factors that are symptomatic of a higher risk pregnancy, rather than a definite cause of pregnancy risk."

CONCLUSION

The rate of marijuana use by pregnant women is sufficient to represent a health hazard if marijuana is found to have a deleterious effect upon maternal well-being or fetal development. The reported use among pregnant women, varying in the localities sampled from 10% to 37%, is comparable to an estimated 26% of women between ages 18 and 25 surveyed in the general population (Fishbume et al. 1980). Inconclusive evidence from the first exploratory studies in the human suggest that marijuana use may alter the delivery process, reduce intrauterine weight gain by the fetus or affect visual and neurological excitatory responses. Confirmation of these findings among the studies is lacking as is evidence for the unconfounded or direct action of marijuana. Nevertheless, the studies serve an important function in providing directions for new and ongoing investigations.

Finally, three issues might be considered of importance for future investigations of the effects of marijuana as used by pregnant women. First, the degree of placental transfer of Δ-9-THC or its metabolites, including the possibility that rates are variable over the course of pregnancy, has not been determined in the human (Abel 1980). Second, the synergistic or interactive effects of illicit drugs or medications with marijuana as taken by pregnant women has been given little attention. Third, the immediate clinical relevance of findings as well as the long-term effects need to be assessed in evaluating the impact of the drug.

REFERENCES

Abel, E. Prenatal exposure to cannabis: a critical review of effects on growth, development, and hehavior. Behav Neural Biol, 29:137-156, 1980.
Alpert, J., Day, N., Dooling, E., Hingson, R., Oppenheimer, E., Rosett, H., Weiner, L., and Zuckerman, B. Maternal alcohol consumption and newborn assessment: methodology of the Boston City Hospital prospective study. Neurobehav Toxicol Teratol, 3:195-201, 1981.
Bogdanoff, B., Rorke, L., Yanoff, M., and Warren, W. Brain and eye abnormalities. Amer J Dis Child, 123:145-148, 1972.
Brazelton, T. Neonatal behavioral assessment scale. Clinics in Developmental Medicine. No. 50. Philadelphia: Lippincott, 1973, 66 pp.
Burstein, S. and Raz, A. Inhibition of prostaglandin E 2 biosynthesis by delta-1-tetrahydrocannabinol. Prostaglandins, 2(5):369-374. 1972.
Burstein S., Levin, E., and Varanell, C. Prostaglandins and cannabis - II. Inhibition of biosynthesis by the naturally occurring cannabinoids. Biochem Pharmacol, 22(4):2905-2910, 1973.
Burstein, S. and Huntcr, S. Prostaglandins and cannabis - VIII. Elevation of phospholipase A_2 activity by cannabinoids in whole cells and subcellular preparations. J Clin Pharmacol 21:240S-248S, 1981.
Carakushansky, G., New, R., and Gardner, L,. Lysergide and cannabis as possible teratogens in man. Lancet l, 586:150-151, 1969.
Fishbume, P., Abelson, H., and Cisin,. National Survey on Drug Abuse: Main Findings 1979. National Institute on Drug Abuse. DHHS Pub. No. (ADM) 80-976. Rockville, Maryland: Office of Medical and Professional Affairs.
Supt. of Documents, U.S. Government Printing Office, 1980. 250 pp.
Fried, P. Marijuana use by pregnant women: neurohehavioral effects in neonates. Drug Alcohol Depend, 6:415-424, 1980(a).
Fried, P., Watkinson, B., Grant, A., abd Knights, R. Changing patterns of soft drug use prior to and during pregnancy: a prospective study. Drug Alcohol Depend, 6:323-343, 1980(b).
Fried, P. Marihuana use by pregnant women and effects on offspring: an update. Neurobehav Toxicol Teratol, 3:195-201, 1981.
Greenland, S., Staisch, K., Brown, N., and Gross, S. The effects of marijuana use during pregnancy - I. A preliminary epidemiologic study. Am J Obstet Gynecol, 143:408-413
Greenland, S., Staisch, K., Brown, N., and Gross, S. Effects of marijuana on human pregnancy, labor: and delivery. Neurobehav Toxicol Teratol, 4:447-450, 1982(b).
Greenland, S., Richwald, G., and Honda, G. The effects of marijuana use during pregnancy - II. A study in a low-risk home-delivery population, Drug Alcohol Depend, 1983 (in press)
Hanson, J., Streissguth, A., and Smith, D. The effects of moderate alcohol consumption during pregnancy on fetal growth and morphogenesis. J Pediatr, 92:457-460, 1978.
Hecht, F., Beals, R., M., Jolly, H., and Roberts, P. Lysergic-acid-diethylamide and cannabis as possible teratogens in man. Lancet 2, 577:1037, 1968.

Hingson, R., Alpert, J., Day, N., Pooling, E., Kayne, H., Morelock, S., Oppenheimer, E., and Zuckerman, B. Effects of maternal drinking and marijuana use on fetal growth and development. Pediatrics, 70:539-546, 1982.

Lancioni, G., Horowitz, F., and Sullivan, J. The NBAS-K: I. A study of its stability and structure over the first month of life. Infant Behav Dev, 3:341-359, 1980.

Lipsitz, P. A proposed narcotic withdrawal score for use with newborn infants. Clin Ped, 14:92-594, 1975.

McIsaac, W., Fritchie, G., Idänpään-Heikkilä, J., Ho, B., Englert, L. Distribution of marihuana in monkey brain and concomitant behavioral effects. Nature, 230:593-594, 1971.

Qazi, Q., Marlane, Beller, E., Milman, D., and Crumbleholme, W. Is marijuana smoking fetotoxic? Pediatr Res, 16:272A, 1982.

Quilligan, E., Little, A., Oh., W., Catz, C., Alexander, D., and Cornblath, M. Pregnancy, birth and the infant. Child Health and Human Development: An Evaluation and Assessment of the State of the Science. NIH Pub.

Rose, S. Dwyer, W., and Yehle, A. Delta 9-tetrahydro-cannabinol: elevation of absolute visual thresholds of rabbits. Pharmacol Biochem Behav, 10:851-853, 1979.

Tennes, K. and Blackard, C. Maternal alcohol consumption, birthweight, and minor physical anomalies. Am J Obstet Gynecol, 138: 774-780, 1980.

AUTHOR

Katherine Tennes, M.A.
Department of Psychiatry
University of Colorado Health Sciences Center
4200 East Ninth Avenue
Box C-264
Denver, Colorado 80262

Discussion and Recommendations

Monique C. Braude, Ph.D.

Historically, the National Institute on Drug Abuse (NIDA) and its predecessor, the National Institute of Mental Health (NIMH) have been committed to research on the biomedical effects of marijuana and its major constituents, the cannabinoids. This commitment has extended through the last 15 years to become an essential part of the preclinical and clinical program of marijuana research.

A significant part of this marijuana program has been devoted to studies of the effects of marijuana on the endocrine and reproductive systems in view of its great potential impact for the health of future generations. Early preclinical as well as clinical studies, reviewed in the previous chapters, pointed out significant effects of marijuana or its constituents on the hormonal and reproductive systems usually after acute or short-term administration. After long-term administration, the hormonal changes seen after acute administration often disappear, probably due to the development of tolerance.

A great deal of discussion during the meeting centered on the doses or concentrations which should be given to experimental animals versus those used by man. One should not forget that animals are usually less susceptible to drugs than humans as shown in behavioral and pharmacological testing. Furthermore, early studies could not correlate plasma levels of cannabinoids with biological effects as the analytical methodology to assay cannabinoids in biological fluids was not yet sufficiently developed. Today, the availability of rapid and economical assays, such as the EMIT assay and the RIAs allow aualitative and quantitative assessment of marijuana constituents in biological fluids. It should therefore be easier to correlate plasma levels across species to endocrine effects in future studies.

Much of the important early research on the effects of marijuana on the reproductive system was done on the male in part due to the relative simplicity of the male system compared to the more complex hormonal interactions that occur in the female. The early work which focused attention on the fact that marijuana depresses reproductive hormones, such as LH and testosterone, has led to

important observations of changes in the sexual organs such as decreases in testis, seminal vesicles and prostate weights. The reports of reduction in reproductive organ weights are often accompanied by reports of oligospermia and sperm abnormalities, in rodents as well as in primates. Chronic use of marijuana can produce decrements in the function of many endocrine organs including the pituitary, testis, thyroid, and adrenal cortex. The initial fears of prolonged depression in LH and testosterone have been tempered with time, as it now appears that some degree of tolerance to the effect of marijuana on the endocrine system in the adult animal may occur.

Studies of the effects of marijuana on the endocrine system in vivo have been often narrowed to studies of its major psychoactive ingredient, delta-9-THC (THC). In vitro, it appears that many organ systems respond not only to the direct addition of THC to the incubation medium but also to the addition of various other cannabinoids as well. Whether such actions of the cannabinoids occur in vivo yet remain to be proven.

The elucidation of the mechanisms involved in the effects of cannabinoids on the target organs (ovary, testis) have led to numerous discussions, some believing that these effects are produced by a direct action on the target organs, others believing that the cannabinoids exert an indirect action via the hypothalamic pituitary gonadal (HPG) axis. There is some evidence to indicate that there might be a direct action of the cannabinoids on the reproductive tissues themselves in addition to the reproductive decrements caused by the altered hormonal environment, but the debate is still open.

The search for the site of cannabinoid action on the reproductive system has led to a number of studies of the effects of cannabinoids on various parts of the HPG axis and the brain. It is now believed that THC does not act directly on the pituitary to alter hormone secretion of LH and prolactin, for instance. A major site of action is the hypothalamus, as it has been shown that THC effects on pituitary hormone production can be reversed by hypothalamic releasing factors. It now also appears that higher neural centers may be affected, which could alter the neuronal input to the hypothalamus.

RECOMMENDATIONS

The members of the review panel were asked to present their ideas for future research which would build upon our current knowledge base. Most of the question and answer sessions following the oral presentation of each paper, as well as most of the discussion session at the end of the meeting, centered on this issue.

The following is a synthesis of the highlights of some of their recommendations, which is not intended as, and should not be considered to be, an exhaustive list.

1. Basic research on the effects of cannabinoids on the genome and on gene expression

Cannabinoid-induced modifications in cell structure and function have been well documented as have a series of physiological effects resulting from such drug-induced cellular changes. Two pivotal processes which have been shown to be significantly influenced by cannabinoids are endocrine function and cell proliferation. Moreover, these are not unrelated processes since in many cases proliferation is responsive to hormone control. In view of recent advances in molecular biology, an understanding of the manner in which drug-induced alterations in gene expression are brought about is now possible and should provide insight into the molecular basis of cannabinoid-related modifications in cellular functions. Cannabinoid-related alterations in gene expression can result from changes in the organization of genetic sequences and/or in the manner in which genetic information is transcribed and processed. Therefore, a critical and systematic evaluation of the influence of cannabinoids on the structure and expression of specific genetic sequences, particularly in human cells, should be a high priority. Understanding cannabinoid effects on human gene organization and expression at doses/concentrations approximating usual human use is prerequisite to evaluating possible short-term or long-term inheritable disorders that may arise from the use of these drugs either therapeutically or as abused substances. Of particular importance, despite the history of fragmentary and often contradictory reports of cannabinoid-induced modifications in genome-related phenomena (e.g. chromosomal changes, alterations in RNA metabolism, etc.), we are now in a position to address these issues directly and definitively. The availability of a series of cloned human genes permits evaluation of drug-induced effects on specific genes and defined regions of genes as well as on the transcription and processing of genetic information. Cloned genetic sequences can be used as high resolution probes for identification and quantitation of specific human gene transcripts in several human cell types following cannabinoid administration.

It would be instructive to focus where possible on human studies since, for example, drug-induced effects on the organization of specific genetic sequences or regions thereof can be performed utilizing DNA from white blood cells isolated from only a 20 ul blood sample. Thereby, the opportunity is available to determine the effects of cannabinoids on the genome of subjects participating in endocrine function and/or behavioral studies. A number of normal and tumor-derived human cell lines are available and should be utilized to complement such an approach. By combining both intact organism and cell culture approaches it is possible to draw on the physiological reality of the organism and the biochemical simplicity of isolated cells. It would also be appropriate to concentrate efforts on evaluating drug-induced effects on a limited series of genetic sequences, those related to proliferation and endocrine function, where definitive effects are well understood and the information obtained from such studies can be integrated with ongoing investigations. This approach can be implemented for assaying unfractionated marijuana extracts, psychoactive and

nonpsychoactive components of marijuana, natural and synthetic cannabinoids, and cannabinoid metabolites, as well as other abused substances individually or in conjunction with cannabinoids.

2. Effects in the female

Up to 1978, studies of cannabinoids with women who were or might become pregnant were not allowed by the Food and Drug Administration in view of the early preclinical reports of malformations in offspring of cannabis treated animals. However, as reviewed in this volume and also in the proceedings of an ARF/WHO scientific meeting published in 1983 on adverse consequences of cannabis use, further studies indicate that cannabinoids are, at most, weakly teratogenic in rodents and rabbits and there are so far no reports of gross malformations in children of marijuana-using parents. As studies in women are now allowed, the following recommendations were made:

a. Surveys of pregnant women have shown that the incidence of marijuana use among pregnant women is approximately the same as in young adult women in the general population. Because of the high incidence of marijuana use by pregnant women, it is now important to investigate the effects directly upon pregnancy and upon the development and functioning of their offspring's reproductive function.

b. Dosage and time of marijuana use during gestation are difficult to control in clinical studies of pregnant women, but correlation of marijuana with the cannabinoid content of biological fluids should be made periodically. Research using the primate as a model appears to be the most direct approach to investigating physiologic effects upon maintenance of pregnancy, initiation of delivery, and neonatal risk factors.

c. Naturalistic studies of pregnant women's use of marijuana are needed to establish patterns of use and to identify confounding variables. Since a proportion of women who use marijuana regularly prior to pregnancy reduce their use after they become aware of being pregnant, research should be directed toward determining effects upon development of the embryo early in pregnancy as well as the effects upon fetal development. Since a proportion of pregnant marijuana users are polydrug users, it is important to investigate the incidence and patterns of specific illegal drugs of choice used in conjunction with marijuana by pregnant women.

d. Because of the difficulties involved in identifying a cohort of women who are chronically heavy users of marijuana throughout pregnancy, it might be appropriate to organize a consortium of researchers who have such a cohort identified in order to pool information from medical records of the women and neonates born to them.

e. Knowledge is scarce regarding the effects of marijuana upon the mother's postnatal care of the infant in women who are chronic users after pregnancy. Both the exposure of the

infants through breastfeeding and the effect upon women's motivation to give maternal care need to be examined. Physiologic effects may be investigated through animal models but only research involving mother-infant dyads will be able to account for effects upon the child's behavior and subsequent development.

3. Effects of THC in the male

Although studies in the last 10 years have concentrated on male subjects, there are still a number of questions to be resolved about the effects of marijuana and its constituents on the male endocrine and reproductive systems. For instance:

a. Are there any specific direct effects of THC on the testis and other accessory reproductive organs? There are many reports in the preclinical literature of reduced weight of seminal vesicles, prostate, and epididymis of animals which have been treated chronically with THC. There is also some evidence to indicate that even after cessation of treatment and a return to normal hormonal levels, the reproductive organs never make up the weight deficit that is incurred during the period of drug treatment. Is this due to hormonal involvement or are nutritional factors involved?

b. How does THC and/or marijuana affect sperm production and auality? Many possibilities exist here. Do cannabinoids have a direct effect on the germinal epithelium of the seminiferous tubules or are the effects indirect, i.e., produced by altered hormonal and nutritional environment induced by THC within the seminiferous tubules themselves?

c. What is the effect of THC on FSH output by the pituitary and what effect does this have on spermatogenesis in the male? In essence, changes in FSH have been poorly documented. More information is needed about the effect of THC on FSH production by the pituitary.

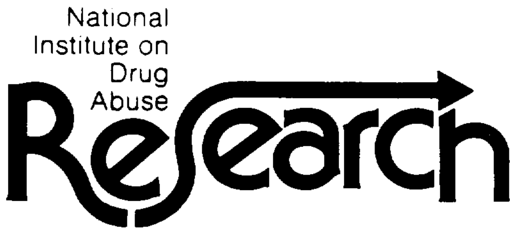
d. What are the specific sites of action of cannabinoids within the central nervous system which could alter the secretion of the hypothalamic releasing hormones that regulate pituitary hormone production? Recent evidence, as reviewed in this volume by Tyrey, has indicated that THC is probably acting through higher CNS sites to alter the neural transmitters in the hypothalamus that are responsible for secretion of releasing hormones.

e. What are the long-term effects of exposure of juvenile males to marijuana or THC? How does exposure affect them during maturation? Are there changes incurred in juveniles exposed to marijuana which alter reproductive function as adults? What changes occur through perinatal exposure to marijuana? This search must be at all levels including neuroendocrine, change in function of reproductive structures, and perhaps alterations in sexual behavior.

In conclusion, the acute and short-term effects of THC and marijuana on the neuroendocrine and reproductive systems have been fairly well elucidated, as reviewed in this monograph. However, the effects of long-term marijuana smoking and of marijuana components other than THC need to be further investigated and should be pursued with vigor.

AUTHOR

Monique C. Eraude, Ph.D.
Biomedical Branch
Division of Preclinical Research
National Institute on Drug Abuse
Rockville, Maryland 20857



While limited supplies last, single copies of the monographs may be obtained free of charge from the National Clearinghouse for Drug Abuse Information (NCDAI). Please contact NCDAI also for information about availability of coming issues and other publications of the National Institute on Drug Abuse relevant to drug abuse research.

Additional copies may be purchased from the U.S. Government Printing Office (GPO) and/or the National Technical Information Service (NTIS) as indicated. NTIS prices are for paper copy. Microfiche copies, at $4.50, are also available from NTIS. Prices from either source are subject to change.

Addresses are:

NCDAI
National Clearinghouse for Drug Abuse Information
Room 10A-43
5600 Fishers Lane
Rockville, Maryland 20857

GPO
Superintendent of Documents
U.S. Government Printing Office
Washington, D.C. 20402

NTIS
National Technical Information Service
U.S. Department of Commerce
Springfield, Virginia 22161

1 FINDINGS OF DRUG ABUSE RESEARCH. Not available from NCDAI.
Vol. 1: GPO out of stock NTIS PB #272 867/AS $32.50
Vol. 2: GPO out of stock NTIS PB #272 868/AS $29.50

2 OPERATIONAL DEFINITIONS IN SOCIO-BEHAVIORAL DRUG USE RESEARCH 1975. Jack Elinson, Ph.D., and David Nurco, Ph.D., eds. Not available from NCDAI.
GPO out of stock NTIS PB #246 338/AS $16

3 AMINERGIC HYPOTHESES OF BEHAVIOR: REALITY OR CLICHE? Bruce J. Bernard, Ph.D., ed. Not available from NCDAI.
GPO Stock #017-024-00486-3 $6.50 NTIS PB #246 687/AS $16

4 NARCOTIC ANTAGONISTS: THE SEARCH FOR LONG-ACTING PREPARATIONS. Robert Willette, Ph.D., ed.
GPO out of stock NTIS PB #247 096/AS $8.50

5 YOUNG MEN AND DRUGS: A NATIONWIDE SURVEY. John A. O'Donnell, Ph.D., et al. Not available from NCDAI.
GPO Stock #017-024-00511-8 $6.50 NTIS PB #247 446/AS $16

6 EFFECTS OF LABELING THE "DRUG ABUSER": AN INQUIRY. Jay R. Williams, Ph.D. Not available from NCDAI.
GPO Stock #017-024-00512-6 $4.75 NTIS PB #249 092/AS $8.50

7 CANNABINOID ASSAYS IN HUMANS. Robert Willette, Ph.D., ed.
GPO Stock #017-024-00510-0 $6.00 NTIS PB #251 905/AS $14.50

8 Rx: 3x/WEEK LAAM - ALTERNATIVE TO METHADONE. Jack Blaine, M.D., and Pierre Renault, M.D., eds.
Not available from GPO NTIS PB #253 763/AS $14.50

9 NARCOTIC ANTAGONISTS: NALTREXONE PROGRESS REPORT. Demetrios Julius, M.D., and Pierre Renault, M.D., eds. Not available from NCDAI.
GPO Stock #017-024-00521-5 $7.00 NTIS PB #255 833/AS $17.50

10 EPIDEMIOLOGY OF DRUG ABUSE: CURRENT ISSUES. Louise G. Richards, Ph.d., and Louise B. Blevens eds. Not available from NCDAI.
GPO Stock #017-024-00571-1 $6.50 NTIS PB #266 691/AS $22

11 DRUGS AND DRIVING. Robert Willette, Ph.D., ea. Not available from NCDAI.
GPO Stock #017-024-00576-2 $5.50 NTIS PB 8269 602/AS $16

12 PSYCHODYNAMICS OF DRUG DEPENDENCE. Jack D. Blaine, M.D., and Demetrios A. Julius, M.D., eds. Not available from NCDAI.
GPO Stock #017-024-00642-4 $5.50 NTIS PB #276 084/AS $17.50

13 COCAINE: 1977. Robert C. Petersen, Ph.D., and Richard C. Stillman, M.D., eds. Reports the extent and limits of current knowledge about cocaine, its use and misuse.
GPO Stock #017-024-00592-4 $6.00 NTIS PB #269 175/AS $19

14 MARIHUANA RESEARCH FINDINGS: 1976. Robert C. Petersen, Ph.D., ed. Technical papers on which the 6th Marihuana and Health report to Congress was based.
GPO out of stock NTIS PB #271 279/AS $22

15 REVIEW OF INHALANTS: EUPHORIA TO DYSFUNCTION. Charles Wm. Sharp, Ph.D., and Mary Lee Brehm, Ph.D., eds. Review of inhalant abuse, including an extensive bibliography.
GPU Stock #017-024-00650-5 $7.50 NTIS PB #275 798/AS $28

16 THE EPIDEMIOLOGY OF HEROIN AND OTHER NARCOTICS. Joan Dunne Rittenhouse, Ph.D., ed. Not available from NCDAI.
GPO Stock #017-024-00690-4 $6.50 NTIS PB #276 357/AS $20.50

17 RESEARCH ON SMOKING BEHAVIOR. Murray E. Jarvik, M.D., Ph.D., et al., eds. Includes epidemiology, etiology, consequences of use, and approaches to behavioral change. From a NIDA-supported UCLA conference.
GPO Stock #017-024-00694-7 $7.50 NTIS PB #276 353/AS $29.50

18 BEHAVIORAL TOLERANCE: RESEARCH AND TREATMENT IMPLICATIONS. Norman A. Krasnegor, Ph.D., ed. Theoretical and empirical studies of nonpharmacologic factors in development of drug tolerance.
GPO Stock #017-024-00699-8 $5.50 NTIS PB #276 337/AS $16

19 THE INTERNATIONAL CHALLENGE OF DRUG ABUSE. Robert C. Petersen, Ph.D., ed. Papers from the VI World Congress of Psychiatry .
GPO Stock #017-024-00822-2 $7.50 NTIS PB #293 807/AS $28

20 SELF-ADMINISTRATION OF ABUSED SUBSTANCES: METHODS FOR STUDY. Norman A. Krasnegor, Ph.D., ed. Techniques used to study basic processes underlying abuse of drugs, ethanol, food, and tobacco.
GPO Stock #017-024-00794-3 $6.50 NTIS PB #288 471/AS $22

21 PHENCYCLIDINE (PCP) ABUSE: AN APPRAISAL. Robert C. Petersen, Ph.D., and Richard C. Stillman, M.D., eds. For clinicians and researchers, assessing the problem of PCP abuse.
GPO Stock #017-024-00785-4 $7.00 NTIS PB #288 472/AS $25

22 QUASAR: QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS OF ANALGESICS, NARCOTIC ANTAGONISTS, AND HALLUCINOGENS. Gene Barnett, Ph.D.; Milan Trsic, Ph.D.; and Robert Willette, Ph.D.; eds. Not available from NCDAI.
GPO Stock #017-024-00786-2 $8.00 NTIS PB #292 265/AS $35.50

23 CIGARETTE SMOKING AS A DEPENDENCE PROCESS. Norman A. Krasnegor, Ph.D., ed. Discusses factors involved in the onset, maintenance, and cessation of the cigarette smoking habit. Includes an agenda for future research.
GPO Stock #017-024-00895-8 $6.00 NTIS PB #297 721/AS $19

24 SYNTHETIC ESTIMATES FOR SMALL AREAS: STATISTICAL WORKSHOP PAPERS AND DISCUSSION. Jos. Steinberg, ed. Papers from a workshop on statistical approaches that yield needed estimates of data for States and local areas. Not available from NCDAI.
GPO Stock #017-024-00911-3 $8.00 NTIS PB #299 009/AS $23.50

25 BEHAVIORAL ANALYSIS AND TREATMENT OF SUBSTANCE ABUSE. Norman A. Krasnegor, Ph.D., ed. Papers on commonalities and implications for treatment of dependency on drugs, ethanol, food, and tobacco.
GPO Stock #017-024-00939-3 $5.00 NTIS PB #80-112428 $22

26 THE BEHAVIORAL ASPECTS OF SMOKING. Norman A. Krasnegor, Ph.D., ed. Reprint of the behavioral section of the 1979 Report of the Surgeon General on Smoking and Health; introduction by editor.
GPO out of stock NTIS PB #80-118755 $17.50

27 PROBLEMS OF DRUG DEPENDENCE, 1979: PROCEEDINGS OF THE 41ST ANNUAL SCIENTIFIC MEETING, THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE, INC. L.S. Harris, Ph.D., ed. Not available from NCDAI.
GPO Stock #017-024-00981-4 $9.00 NTIS PB #80-175482 $37

28 NARCOTIC ANTAGONISTS: NALTREXONE PHARMACOCHEMISTRY AND SUSTAINED-RELEASE PREPARATIONS. Robert Willette, Ph.D., and Gene Barnett, Ph.D., eds. Papers report research on sustained-release and long-acting devices for use with the narcotic antagonist naltrexone.
GPO Stock #017-024-01081-2 $7.00 NTIS PB #81-238875 $23.50

29 DRUG ABUSE DEATHS IN NINE CITIES: A SURVEY REPORT. Louis A. Gottschalk, M.D., et al. Not available from NCDAI.
GPO Stock #017-024-00982-2 $6.50 NTIS PB #80-178882 $17.50

30 THEORIES ON DRUG ABUSE: SELECTED CONTEMPORARY PERSPECTIVES. Dan J. Lettieri, Ph.D.; Mollie Sayers; and Helen Wallenstein Pearson, eds. Volume presents summaries of major contemporary tneories of drug abuse by each of 43 leading theorists.
GPO Stock #017-024-00997-1 $10.00 Not available from NTIS

31 MARIJUANA RESEARCH FINDINGS: 1980. Robert C. Petersen, Ph.D., ed. The text of the 8th Marijuana and Health report to Congress and the background scientific papers on which it was based.
GPO out of stock NTIS PB #80-215171 $20.50

32 GC/MS ASSAYS FOR ABUSED DRUGS IN BODY FLUIDS. Rodger L. Foltz, Ph.D.; Allison F. Fentiman, Jr., Ph.D.; and Ruth B. Foltz. A collection of methoas for auantitative analysis of several important drugs of abuse by gas chromatography- mass spectrometry.
GPO Stock #017-024-01015-4 $6.00 NTIS PB #81-133746 $19

33 BENZODIAZEPINES: A REVIEW OF RESEARCH RESULTS, 1980. Stephen I. Szara, M.D., D.Sc., and Jacqueline P. Ludford, M.S., eds. A RAUS (Research Analysis and Utilization System) Review Report on the aouse liability of the Denzodiazepine “tranquilizers.”
GPO Stock #017-024-01108-8 $5.00 NTIS PB #82-139106 $13

34 PROBLEMS OF DRUG DEPENDENCE, 1980: PROCEEDINGS OF THE 42ND ANNUAL SCIENTIFIC MEETING, THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE, INC. Louis S. Harris, Ph.D., ed. Not available from NCOAI.
GPO Stock #017-024-01061-8 $8.00 NTIS PB #81-194847 $34

35 DEMOGRAPHIC TRENDS AND DRUG ABUSE, 1980-1995. Louise G. Richards, Ph.D., ed. Estimates of probable extent and nature of nonmeoical drug use, 1980-1995, based on age structure and other characteristics of U.S. population.
GPO Stock #017-024-01087-1 $4.50. NTIS PB #82-103417 $13

36 NEW APPROACHES TO TREATMENT OF CHRONIC PAIN: A REVIEW OF MULTI-DISCIPLINARY PAIN CLINICS AND PAIN CENTERS. Lorenz K.Y. Ng, M.D., ed. Discussions by active practitioners in the treatment of pain.
GPO Stock #017-024-01082-1 $5.50. NTIS PB #81-240913 $19

37 BEHAVIORAL PHARMACOLOGY OF HUMAN DRUG DEPENDENCE. Travis Thompson, Ph.D., and Chris E. Johanson, Ph.D., eds. Presents a growing body of data, systematically derived, on the behavioral mechanisms involved in use and abuse of drugs.
GPO Stock #017-024-01109-6 $6.50 NTIS PB #82-136961 $25

38 DRUG ABUSE AND THE AMERICAN ADOLESCENT. Dan J. Lettieri, Ph.D., and Jacqueline P. Ludford, M.S., eds. A RAUS Review Report, emphasizing use of marijuana: epidemiology, socio-demographic and personality factors, family and peer influence, delinouency, and biomedical consequerces.
GPO Stock #017-024-01107-0 $4.50 NTIS PB #82-148198 $14.50

39 YOUNG MEN AND DRUGS IN MANHATTAN: A CAUSAL ANALYSIS. Richard R. Clayton, Ph.D., and Harwin L. Voss, Ph.D. Examines tne etiology and natural history of drug use, with special focus on heroin. Includes a Lifetime Drug Use Index.
GPO Stock #017-024-01097-9 $5.50 NTIS PB #82-147372 $19

40 ADOLESCENT MARIJUANA ABUSERS AND THEIR FAMILIES. Herbert Hendin, M.D., Ann Pollinger, Ph.D., Richard Ulman, Ph.D., and Arthur Carr, Ph.D. A psychodynamic study of adolescents involved in neavy marijuana use, to determine what interaction between family and adolescent gives rise to drug abuse.
GPO Stock #017-024-01098-7 $4.50 NTIS PB #82-133117 $13

41 PROBLEMS OF DRUG DEPENDENCE, 1981: PROCEEDINGS OF THE 43RD ANNUAL SCIENTIFIC MEETING, THE COMITTEE ON PROBLEMS OF DRUG DEPENDENCE, INC. Louis S. Harris, Ph.D., ed. Not available from NCDAI.
Not available from GPO NTIS PB #82-190760 $41.50

42 THE ANALYSIS OF CANNABINOIDS IN BIOLOGICAL FLUIDS. Richard L. Hawks, Ph.D., ed. Not available from NCDAI.
GPO Stock #017-024-01151-7 $5 NTIS PB #83-136044 $1643

43 PROBLEMS OF DRUG DEPENDENCE, 1982: PROCEEDINGS OF THE 44TH ANNUAL SCIENTIFIC MEETING, THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE, INC. Louis S. Harris, Ph.D., ed. Not available from NCDAI.
GPO Stock #017-024-01162-2 $8.50 NTIS PB #83-252-692 $40

44 MARIJUANA EFFECTS ON THE ENDOCRINE AND REPRODUCTIVE SYSTEMS. Monique C. Braude, Ph.D., and Jacqueline P. Ludford, M.S., eds. A RAUS Review Report of animal studies and preclinical and clinical studies of effects of cannabinoids on human endocrine and reproductive functions.

45 CONTEMPORARY RESEARCH IN PAIN AND ANALGESIA, 1983. Roger M. Brown, Ph.D.; Theodore M. Pinkert, M.D., J.D.; and Jacqueline P. Ludford, M.S., eds. A RAUS Review Report on the anatomy, physiology, and neurochemistry of pain and its management.
GPO Stock #017-024-01191-6 $2.75 NTIS PB #84-184670 $11.50

46 BEHAVIORAL INTERVENTION TECHNIQUES IN DRUG ABUSE TREATMENT. John Grabowski, Ph.D.; Maxine L. Stitzer, Ph.D., and Jack E. Henningfield, Ph.D., eds. Reports on a variety of behavioral contingency management procedures used in research/treatment environments.
GPO Stock #017-024-01192-4 $4.25 NTIS PB #84 184688 $16

47 PREVENTING ADOLESCENT DRUG ABUSE: INTERVENTION STRATEGIES. Thomas J. Glynn, Ph.D.; Carl G. Leukefeld, D.S.W.; and Jacqueline P. Ludford, M.S., eds. A RAUS Review Report on a variety of approaches to prevention of adolescent drug abuse, how they can be applied, their chances for success, and needed future research.
Not available from GPO NTIS PB to be assigned

48 MEASUREMENT IN THE ANALYSIS AND TREATMENT OF SMOKING BEHAVIOR. John Grabowski, Ph.D., and Catherine S. Bell, M.S., eds. Based upon a meeting cosponsored by NIDA and the National Cancer Institute to delineate necessary and sufficient measures for analysis of smoking behavior in research and treatment settings.
GPO Stock #017-024-01181-9 $4.50 NTIS PB 84-145-184 $14.50

IN PRESS

49 PROBLEMS OF DRUG DEPENDENCE, 1983: PROCEEDINGS OF THE 44TH ANNUAL SCIENTIFIC MEETING, THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE, INC. Louis S. Harris, Ph.D., ed. A collection of papers which together record a year's advances in drug abuse research; also includes reports on tests of new compounds for efficacy and dependence liability.

51 DRUG ABUSE TREATMENT EVALUATION: STRATEGIES, PROGRESS, AND PROSPECTS. Frank M. Tims, Ph.D., ed. A state-of-the-art review of drug abuse treatment evaluation, identifying research needs, promising approaches, and emerging issues.

52 TESTING DRUGS FOR DEPENDENCE POTENTIAL AND ABUSE LIABILITY. Joseph V. Braay, Ph.D., and Scott E. Lukas, Ph.D., eds. Describes animal and human test procedures for assessing dependence potential and abuse liability of opioids, stimulants, depressants, hallucinogens, cannabinoids, and dissociative anesthetics.

IN PREPARATION

50 COCAINE: PHARMACOLOGY, EFFECTS, AND TREATMENT OF ABUSE. John Grabowski, Ph.D., ed. Content ranges from an introductory overview through neuropharmacology, pharmacology, animal and human behavioral pharmacology, patterns of use in the natural environment of cocaine users, treatment, through commentary on societal perceptions of use.

U.S. Government Printing Office: 1984 O - 446-384!

DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
Alcohol, Drug Abuse, and Mental
Health Administration
Rockville MD 20857

Official Business
Penalty for Private Use $300

Postage and Fees Paid
U.S. Dept. of H.H.S.
HHS 396

THIRD CLASS

NOTICE OF MAILING CHANGE

☐ Check here if you wish to discontinue receiving this type of publication

☐ Check here if your address has changed and you wish to continue receiving this type of publication. (Be sure to furnish your complete address including zip code.)

Tear off cover with address label and publication number still affixed and send to

Alcohol. Drug Abuse. and Mental Health Administration Publications
PHS Printing and Reproduction Management Banch
5600 Fishers Lane (Room 5B-19)
Rockville Maryland 20857

DHHS Publication No. (ADM) 84-1278
Printed 1984