MARIJUANA AND HEALTH HAZARDS

METHODOLOGICAL ISSUES IN CURRENT RESEARCH

Edited by

Jared R. Tinklenberg

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Jared R. Tinklenberg
Drug Abuse Council
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List of Contributors

Thomas F. Babor Alcohol & Drug Abuse Research Center, Department of Psychiatry, Harvard Medical School — McLean Hospital, Belmont, Massachusetts

Jonathan O. Cole Boston State Hospital & McLean Hospital, Boston, Massachusetts

Rhea L. Dornbush New York Medical College, Flower and Fifth Avenue Hospitals, New York, New York

James Ellingboe Alcohol & Drug Abuse Research Center, Department of Psychiatry, Harvard Medical School — McLean Hospital, Belmont, Massachusetts

Arthur Falek Georgia Mental Health Institute, Department of Psychiatry and Anatomy, Emory University, Atlanta, Georgia

Daniel X. Freedman Department of Psychiatry, University of Chicago, Chicago, Illinois

Donald W. Goodwin Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri

Louis S. Harris Department of Pharmacology, Medical College of Virginia,Richmond, Virginia

Leo E. Hollister Departments of Medicine and Psychiatry, Palo Alto Veterans Administration Hospital, & Stanford University School of Medicine, Palo Alto, California

Jerome H. Jaffe Department of Biological Psychiatry, Columbia University & New York State Psychiatric Institute, New York, New York

Reese T. Jones Department of Psychiatry, University of California Medical Center, San Francisco, California

Elvin A. Kabat Department of Microbiology & Department of Human Development & Human Genetics, Columbia University, New York, New York

Robert C. Kolodny Endocrine Research Section & Infertility Program, Reproductive Biological Research Foundation, St. Louis, Missouri

John Kuehnle Alcohol & Drug Abuse Research Center, Department of Psychiatry, Harvard Medical School — McLean Hospital, Belmont, Massachusetts

Steven S. Matsuyama Department of Psychiatry, University of California at Los Angeles, Los Angeles, California

Jack H. Mendelson Alcohol & Drug Abuse Research Center, Department of Psychiatry, Harvard Medical School — McLean Hospital, Belmont, Massachusetts

Roger E. Meyer Alcohol & Drug Abuse Research Center, Department of Psychiatry, Harvard Medical School — McLean Hospital, Belmont, Massachusetts

Albert E. Munsen Department of Pharmacology, Medical College of Virginia,Richmond, Virginia

Gabriel G. Nahas Department of Anesthesiology, College of Physicians and Surgeons of Columbia University, New York, New York

William Pollin Division of Research, National Institute on Drug Abuse Rockville, Maryland

Homer B.C. Reed, Jr. Neuropsychology Laboratory, Tufts University School of Medicine, New England Medical Center, Boston, Massachusetts

Robert M. Rose Department of Psychosomatic Medicine, Boston University School of Medicine, Boston, Massachusetts

Morton A. Stenchever Department of Obstetrics & Gynecology, University of Utah Medical Center, Salt Lake City, Utah

Jared R. Tinklenberg Drug Abuse Council, Inc., Washington, D.C. and Department of Psychiatry, Stanford University School of Medicine & Palo Alto Veterans Administration Hospital, Palo Alto, California

PREFACE

In response to the many controversial reports of direct links between the use of marijuana and various health hazards, the Drug Abuse Council Inc. sponsored a conference in Washington, D.C. on methodological issues involving recent marijuana research. Participating in the conference were 19 scientists, recognized internationally as experts on the numerous research techniques presently employed by those who are studying the effects of marijuana. These investigators provided their critical, state of the art assessments of what is presently known about the human health consequences of marijuana consumption.

This book, which combines those assessments and the ensuing discussions, focuses on five areas of current controversy and concern: 1) marijuana and genetics, 2) marijuana and immunity, 3) marijuana and testosterone, 4) marijuana and the central nervous system, and 5) marijuana and psychiatric problems. The validity and implications of present research findings in each of these five areas are discussed and attention is given to what types of investigations are required to further elucidate the many important, yet unresolved issues regarding the effects of marijuana.

Acknowledgements for making the conference and this book possible are due many people, but especially to the Board of Directors and members of the staff of the Drug Abuse Council. I thank Dr. Thomas E. Bryant, President, Drug Abuse Council, and Dr. Peter G. Bourne, Special Consultant, Drug Abuse Council, for realizing the critical timeliness of these important issues and for assisting in the organization of the conference. I am indebted to Dr. Leo E. Hollister and Dr. Daniel X. Freedman for skillfully co-chairing the conference. Thanks are due Ms. Hillary Mayell for her excellent job preparing the transcripts of the discussions and supervising the preparation of this manuscript. Thanks are due also to Ms. Margot Backas, Ms. Ginny Baldwin, and Ms. Genevieve Jones for their efficient assistance. It has been a pleasure to work with the staff of Academic Press. Finally, and most importantly, I thank the participants for their superb contributions to this volume.

Jared R. Tinklenberg

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Chapter 1

Genetic Studies of Marijuana: Current Findings and Future Directions

Arthur Falek

The first evidence that chemicals could induce mutations and chromosome damage was established in 1947 by investigations into the aberrations caused by mustard gas (Darlington & Kohler, 1947; for review, see Auerbach & Robson, 1974). In addition, Muller (1947) demonstrated genetic damage as a result of ionizing radiations. These early studies were conducted on the fruitfly drosophila and in the plant tradescanthia.

From 1958 through 1962, evidence accumulated that the presumably mild tranquilizer thalidomide was a teratogen. The ear and limb deformities to the fetus that resulted alarmed the general population and most forcefully alerted the medical community of the potential harm to man of other chemical agents. Concern was voiced in many quarters about the need to evaluate the effects of all drugs on fertility, production of malformations in the offspring, and carcinogenic potential to the individual himself.

Standard Procedures

Until the last decade, all that was available to determine whether a drug would cause alterations in humans at the genetic level were viability studies in animals and man and chromosome studies on some plants and lower animals. Information on chromosome damage in higher animals and in man is of more recent origin, as techniques to examine these chromosomes were not available until 1956 (Tjio & Levan, 1956). In fact, it was not until 1960 that relatively rapid procedures using peripheral blood leukocytes from human subjects for chromosome analysis were first described (Moorhead et al., 1960). Human chromosome damage by chemical agents was reported in the early 1960's (Cohen et al., 1963), but it was only in 1967 that the phenomena of chemical mutagenesis in the human

chromosome was dramatically brought to the attention of the public with the finding that the hallucinogen lysergic acid diethylamide (LSD-25) was capable of causing aberrations to leukocyte chromosomes (Cohen et al., 1967).

This discovery led to many cytogenetic investigations of various drugs of abuse including marijuana. Both *in vivo* and *in vitro* cytogenetic testing systems were employed to investigate the actions of these chemical agents. While initial reports were frequently considered conclusive, further studies often indicated equivocal results. Many studies brought allegations that the drug under investigation caused chromosome damage. However, it should be emphasized that all studies to date have been based on staining procedures employed prior to those of chromosome banding, and thus only gross chromosomal aberrations could be identified.

Since the detected chromosome alterations were most frequently of the unstable variety, both theoretical and practical knowledge indicated that such alterations could be expected to prevent the cells which contained them from completing in normal fashion either mitosis or meiosis. The finding of unstable chromosome alterations also indicated that stable chromosome rearrangements were occurring. Such stable alteration would not interfere with cell division. The geneticist is, of course, primarily interested in the occurrence and frequency of stable chromosome alteration-which persist from generation to generation, or in continuously recurring unstable alterations which signal possible malignancy. When there are no persisting chromosome rearrangements, the action of chemical agents has been considered not to be mutagenic but rather consistent with non-specific cytotoxicity which may indirectly cause chromatid breakage by a variety of mechanisms.

In addition to cytogenetic studies, other standard methods to determine the occurrence of chemical mutagens include those of the dominant lethal and host mediated assay investigations. The dominant lethal method permits an evaluation of the germ cell (egg or sperm) response to chemicals and drugs through an examination of offspring at early cleavage stages (Rohrborn, 1970). Rodents, with a relatively short period of pregnancy, are the animals of choice. Indications of a dominant lethal gene are based on evidence of a preimplantation loss of eggs, while a significant increase in the number of dead implant in the experimental over the control population is good evidence of an induced lethal mutation. While this method enables a test of the potency of a presumptive mutagen at all stages of spermatogenesis and oogenesis, the disadvantages of this system are (1) that the number of observable point mutations to be evaluated is generally very small and (2) that the mutations which produce early lethality in mice or

other rodents may be totally unrelated to those which cause damage to man.

The host mediated assay system was devised to overcome some of the limitations of the *in vitro* cytogenetic test (Legator, 1970). It has enabled evaluation of the effects of the chemical or drug after it was (a) concentrated in the serum, (b) distributed to the body tissues, or (c) detoxified by the liver. This system is based on a comparison of the kinds and frequency of mutations found in indicator microorganisms after their innoculation into test animals previously administered a potential mutagen. The mutagenic activity of the agent when given directly to the microorganism as compared with the indirect results when the agent is given to the host, indicates the host's ability either to detoxify the compound or, as a result of metabolic activities in the test animal, to form mutagenic compounds which affect the microorganism. This indirect test could not account for (1) DNA repair mechanisms in the host, (2) differences in the metabolism of the rodent hosts as compared with man, or (3) dissimilarities in the mutagenic response to an agent by man and the test microorganism. Recently, in an effort to improve this test system, Legator (in press) also included analyses of the mutagenic response of the test microorganism to urine and blood from host animals and human subjects.

Methodological Difficulties

All of these test systems contain methodological difficulties related to their design and analysis. There is, in fact, no reliable method for early detection of a genetic catastrophe which may be introduced into the environment by new chemicals and drugs. In a review of the then current methods, Shaw (1970) indicated that they only produced indirect experimental genetic information. She noted that such procedures would not detect a rapid increase in damage to the human gene pool that was not anticipated. Furthermore, Auerbach (1971) warned that sufficient knowledge was not available to determine whether it was possible to extrapolate from a system which resulted in genetic effect at a high dose of a potential mutagen to a possible effect at a lower dose. Physiologically acceptable doses might be well below the threshhold for genetic effects. It was Auerbach's belief that relevant information might be obtained from determination of the dose-effect curves for weak mutagens.

Crow (1971) was also concerned about weak mutagens. He stated that the most frequent class of mutants produced in the human population by environmental mutagens consisted of those with mild effects. From drosophila data he suggested that the spontaneous mutation rate for mildly deleterious mutants was about 15 times as high as the rate for lethal mutants. The average effects of these mutants when homozygous, according to

Crow, could reduce the probability of survival into adulthood by 2 to 3 percent.

He commented that such mutants were often not completely recessive but that they had an effect on the heterozygote. If in the heterozygote these mildly deleterious mutants did not produce significant genetic disorders, they would be able to remain undetected in the population for many generations. To identify these mutants, and in particular those whose effects were the most deleterious and of the greatest danger to the population, Crow suggested the following monitoring systems: (1) cytogenetic screening of cord blood from newborns; (2) birth defect screening with a few specific dominantly inherited traits whose incidence is a direct reflection of the mutation rate; and (3) chemical methods to determine changes in particular proteins which might be attributable to mutations.

It was Crow's opinion that while the impact of an increased mutation rate may be felt by the human population as a statistical increase in birth defects, adequate technology was not available to develop an extremely precise indicator system. Crow believed that we should go ahead with relatively poor and immediately ready systems, which in many ways might be unsatisfactory, while we developed, with basic research and pilot experiments, more sophisticated methods of evaluation. A similar report on a program to detect increased mutation rates in human populations was presented by Neel (1971). Although he considered the magnitude of the sample to be evaluated a most formidable one, he also emphasized that the risks to society of increased mutation rates were such that geneticists could not draw back from this priority undertaking. Concern about an increase in mutation rates stemmed from the belief of geneticists that optimal rates for a species had been established by many generations of selection and that most new mutations were deleterious.

Auerbach, in her discussion of the dilemma caused by current test procedures, also noted problems that arose in the attempt to extrapolate plant, bacterial, and other animal test data to man. The difficulties engendered were threefold: (1) specificity of response, (2) correlation between types of damage, and (3) dose-effect relationships. With regard to specificity of response, she and other researchers (Burns, 1970; Kato et al., 1970; Auerbach, 1971) noted that chemicals have a wide range of different genetic effects based on differences between species, strains, sex, and individuals. Even in the same individual, different cells in different stages of the cell cycle might vary in response.

The chemical urethane, for example, when tested in rabbits and mice, induced tumors only in mice. If these related mammalian species showed such a striking difference in response to this chemical mutagen, it would be hazardous to extrapolate between such distantly related species as mouse

and man. Furthermore, both the vehicle employed to administer the test agent (water, alcohol, sesame seeds, etc.) and the method of administration (intravenous, oral, smoke, epigastic, topical, etc.) are variables which need to be evaluated and controlled in drug studies. These variables, as they effect marijuana research, have been of concern to investigators at the National Institute on Drug Abuse (Thompson et al., 1973; Rosenkrantz et al., 1974).

Auerbach indicated the difficulty at the chromosome level of correlating stable with unstable chromosome damage. She emphasized, that theoretically, chromosome breaks not followed by reunion should only result in the formation of a dominant lethal and early abortion, but that there were no procedures to evaluate whether actually such animals could also produce more stable mutants which could survive and reproduce. Despite all of the difficulties, particularly in the identification of weak mutagens, Crow indicated that if they also affected the physical, physiological, and mental ability of the individuals, to ignore them would be to ignore the submerged part of an iceberg.

On the other hand, marijuana, as a "social drug" of abuse, is on the most visible portion of the iceberg. What evidence is there that marijuana is a carcinogen or results in genetic damage to the individual or affects his offspring?

Marijuana Investigations

Animal Studies

The earliest studies of marijuana, starting in the mid 60's, presented evidence of limited fertility and teratogenic effects of marijuana when given at relatively high levels and by different modes of administration to rats, mice, rabbits, and hamsters (Miras, 1965; Persaud & Ellington, 1967, 1968; Geber & Schramm, 1969a, 1969b). A preliminary report implied that pregnant rats and mice who inhaled marijuana smoke had normal offspring but that serious defects were observed in the second generation. Such an across-generation response would suggest that marijuana was mutagenic; however, more systematic work on this particular issue has not yet been reported in scientific journals.

On the other hand, a more recent animal study (Pace et al., 1971) indicated that tetrahydrocannabinols (THC) given to hamsters at various dosages from low to high produced no significant increase in chromosome damage or offspring abnormalities in comparison to a control group. An observed higher incidence of neonatal mortality in the experimental group was found to be due to insufficient maternal milk production for litter survival. Based on labeling experiments, THC and its metabolites were

reported to have found their way into the fetus only to a limited extent because of placental interference in the transfer of the drug (Idanpaan-Hakkila et al., 1969; Harbison, 1971).

Because of conflicting reports, the National Institute on Drug Abuse (NIDA) conducted studies with pregnant rats and rabbits at markedly lower levels of THC than those used in previous experiments, but at a range of 10 to 100 times the effective human dose. At those levels the NIDA studies confirmed the finding that marijuana did not appear to have serious deleterious effects during pregnancy on either the fetus or the mother or after birth on the newborn (Haley et al., 1973; Keplinger et al., 1973).

In a recent review of the animal data for the International Conference on the Pharmacology of Marihuana, Rosenkrantz and Braude (in press) reaffirmed the generally negative findings on reproductive damage to the offspring of experimental animals with behaviorally effective dose levels of marijuana. At that meeting, however, Mantilla-Platta and Harbison (in press) presented evidence that at high dose levels marijuana was teratogenic in mice and rabbits, and suggested that such information made it essential that parameters for the possible production of abnormality in man be established, based on similar animal experiments (Mantilla-Platta et al., 1973).

In a comparative study of squirrels and rhesus monkeys given delta-9-THC, species differences in response to marijuana were observed (Scheckel et al., 1968; Wursch et al., 1972). The results indicated that the response in these monkeys was similar to the pattern found in man. However, not only were there species differences, but in the rhesus monkey the results varied according to the method of drug administration (Thompson et al., 1974). Intravenous administration resulted in marked toxicity at low dose levels, but no deaths were observed with oral administration at extremely high dose levels. This review points out that despite the many studies and the relative consistency of the data, difficulties ensue when the attempt is made to extrapolate animal findings to man.

Finally, there is the battery of studies with microorganisms, rodents, and man conducted by Legator (in press) in his attempt to achieve a global approach and detect any alteration in the mutation rate as a result of marijuana use. No evidence of an increase in the mutation rate was noted in any of the studies. His tests included an analysis of micronuclei in polychromatic erythrocytes which was designed to evaluate low mutagenic effects. His negative findings are not sufficient to terminate further research investigations. Neither are Martin's *in vivo* and *in vitro* cytogenetic studies in rats (1969) which also produced negative results.

Human Studies

In vitro cytogenetic studies by Neu and associates (1970) with delta-8 and delta-9-THC and by Stenchever and Allen (1972) with delta-9-THC showed no evidence of a significant increase in chromosome damage to treated cells as compared with a control sample or reported laboratory standards.

Retrospective *in vivo* studies by Dorrance, Janiger, and Teplitz (1970) on 9 persons, by Gilmore and colleagues (1971) on 13 light marijuana users, and the Jamaica study on 30 matched marijuana users (Rubin & Comitas, 1972) all showed no evidence of an increased frequency of chromosome damage. Unfortunately, the Jamaica study had a high incidence of culture failure (almost 50 percent) and only a small number of cells analyzed for each subject.

Prospective studies conducted by Matsuyama and his co-workers (Matsuyama et al., 1973; Matsuyama & Jarvik, in press) and Nichols and associates (1974) were based on regular sampling of volunteers who had reported previous use of marijuana. In none of the studies was there evidence of an increase in chromosome breakage frequency with time, but this may have been due to the fact that at the initiation of the program the frequency of chromosome damage in these men was already higher than that which would have been found in a non-marijuana using control population. In none of the studies was a control population evaluated for comparative purposes. Also, in the Nichols study, delta-9-THC was administered orally. Based on evidence of differences in toxicity as a consequence of the method of administration, it is possible that the oral route was also an important factor in the observed result.

In contrast, an increase in the mean frequency of chromosome damage as compared with controls was reported by Stenchever, Kunysz, and Allen (1974). Although the form data in the patient and control groups appeared to be different, the authors stated that their numbers were too small to be significant. Their observation that the majority of abnormal cells seen were tetraploid is of interest. Though it is conceivable that these abnormal cells were the result of technical difficulties in cell culture, a similar observation is reported by the Leuchtenbergers, based on the DNA content in lung tissue exposed to marijuana smoke. The caution of Stenchever and his colleagues that it would be prudent to conduct further studies to confirm their observations is, of course, sensible. However, at this relatively gross level of chromosome analysis, other studies usually result in equivocal findings. Other positive findings from *in vivo* include the report on heavy marijuana users by Kumar and Kunwar (1972) as well as the findings by Nahas and his group (Nahas et al., 1974; U.S. Senate, 1974; Morishima & Nahas, in

press; Morishima et al., in press) of a small increase of chromosome damage in marijuana smokers.

The few single case reports of birth defects in children of parents who smoked marijuana are confounded by the finding that these parents usually used multiple drugs (National Institute on Drug Abuse, 1973). No birth defects have thus far been associated with marijuana use alone. According to the reports of Crow and Neel, population studies to detect such possibly low-level frequencies of alteration would have to be designed as long-term investigations.

Current Basic Analyses

There is indication from more basic biomedical investigations of the potential of marijuana for mutagenic and carcinogenic effects. The observed increase in cytogenetic damage reported by Stenchever and his associates is supported by cytologic and cytochemical studies. For these elegant studies the Leuchtenbergers and their associates used two model systems (Leuchtenberger & Leuchtenberger, 1971, 1973; Leuchtenberger et al., 1973; U.S. Senate, 1974; Davies et al., in press; Leuchtenberger & Leuchtenberger, in press). The first system was one in which human and animal lung tissue in culture were exposed to fresh smoke of marijuana cigarettes. This made possible an assessment of any alterations in the cells and tissues after short and long term exposure to smoke. In a second system mice were exposed to a constant dose of marijuana cigarette smoke. Changes in the respiratory and other systems of these animals were evaluated after inhalation of the marijuana smoke. In addition to chromosome studies, DNA analysis was conducted by autoradiography, microspectrography, and microfluorometry.

In summary, the data revealed (1) that marijuana and tobacco smoke together produced more abnormalities to the lung cells of mice than did tobacco smoke alone, (2) that in human lung cultures marijuana smoke produced more anomalies in cells than was found after exposure of the cultures to tobacco smoke, and (3) that there was a similar enhancement of aberrant transformation in hamster lung cell cultures after exposure to whole smoke from either tobacco or marijuana. The researchers observed not only marked morphologic changes in the exposed cells but also found consistent evidence of (1) a decrease in the mitotic index, (2) an increase in cells with 4n DNA, and (3) after a period of time, a decrease in DNA synthesis.

These results reinforced Stenchever's observations of an increase in tetraploid cells in the leukocyte cultures of persons smoking marijuana. Furthermore, the findings in cells of lung tissue in culture were also observed by Morishima and his associates in cultured human leukocytes

(Morishima, 1974; Nahas et al., 1974; Morishima et al., in press; Morishima & Nahas, in press). They found that *in vivo* and *in vitro* exposure of human leukocytes to marijuana or its derivatives produced a marked decrease in the mitotic index rate and a significant increase in the prevalence of micro-complements of chromosomes at metaphase (less than 30 chromosomes/complement). From a cytological point of view, the latter discovery was supported by the Leuchtenbergers' observations of tripolar bodies in lung cultures.

Further support for the mutagenic capacity of marijuana is based on evidence from the Leuchtenbergers that in mice inhalation of marijuana smoke or exposure of cultured testis to marijuana smoke produced a decrease in the number of spermatids with normal haploid amounts of DNA. In addition, from the finding by Kreuz and Axelrod (1973) that the localization of delta-9-THC is in the body fat, particularly in the liver, lung, and testes, and only disappears slowly from the plasma in man, it would seem that these tissues should be particularly vulnerable to damage. As a first step, cells from aberrant cultures should be introduced into these regions in test animals to determine the potential of the altered cells to initiate carcinogenesis *in vivo*.

Future Investigations

Based on the varied findings reported above, it is clear that the final word with regard to marijuana and genetic hazards is not yet in. Difficulties reside in several areas.

One major problem is that conclusions are often based on single laboratory reports, and confirmation is frequently the result of a revised study from that laboratory. To determine whether there is consistency in the findings, studies should be established in several laboratories utilizing standardized protocols, supplies, and even aliquots of the same tissue. Only then will it be possible to overcome the public doubts which resulted from the variable findings of laboratories involved in the LSD studies.

The cooperating laboratories should be organized to conduct a program of studies. The experimental design, the sample size, and the necessary statistical tests to evaluate the data and determine their significance need to be developed prior to the start of the investigation. Currently, it seems that sample size is considered appropriate if the data produce statistically significant results, but too small for proper evaluation if statistical analyses are not significant. These studies need to be organized at both the preclinical and the human level. At the preclinical level the test battery should incorporate procedures similar to those presented by Braude (in press) and Legator (in press) in their reports to the International Conference on the Pharmacology of Marihuana.

To afford those working with the test battery the opportunity to constantly update the program and to incorporate the most recent techniques, investigators with different technical skills should be included. A consultant research group representing a broad range of scientific opinion has recently been established under the direction of Dr. Monique Braude at NIDA.

At the human level, *in vivo* studies should include data ascertained from birth defect investigations. Although Crow and Neel in their papers indicated that large sample size and long-term investment were required for such screening purposes, they agreed that initial attempts should be made with appropriately designed smaller population studies. For example, one such program to identify birth defects in a geographically defined population is the investigation of all newborns — approximately 27,000 per year, born in the five county area which comprises Atlanta, Georgia. This study has been going on for the past seven years (Oakley et al., in press).

At present it would only be possible in our program to obtain information on marijuana use from parents of newborns exhibiting particular kinds of birth defects. One problem to be faced is that in the political climate of our times a more detailed or more refined screening program for a total population would probably stimulate resistance and the possible elimination of the study. Furthermore, birth defect programs are usually based on retrospective prenatal information. The limitations in the collection of accurate drug histories, particularly for reasons of the respondent's self-protection, would produce limited results. Another problem to be considered in a birth defect study such as the one conducted in Atlanta is the marked possibility of overlooking very mild malformations. To obtain as much information as possible, the program would have to be expanded to include regular reports on malformations detected after the first year of birth.

The necessity for, as well as the many difficulties in establishing appropriate control samples for *in vivo* drug abuse studies are readily apparent. Family, medical, and drug histories are never complete, and definitively proving that marijuana represents no genetic risk, particularly with regard to the use of this readily available social drug of abuse, is almost impossible. For marijuana studies, smoking is the important method of administration and not to be overlooked in the development of controls for the study. One of the control groups, therefore, should be composed of tobacco smokers.

At present, cytogenetic *in vivo* and *in vitro* studies on drugs of abuse are based on observations of chromosomes stained in the classic manner. The need, of course, is to establish programs which will also use the relatively new G, Q, R, and C banding procedures. These procedures are now employed successfully in most established laboratories. The value of chromosome banding studies, though methodologically more difficult, is

the opportunity for more definitive analyses to detect any alterations in specific portions of the chromosomes.

In addition to chromosome banding studies, investigations should be conducted on the cells and chromosomes of marijuana smokers and controls stressed by alterations in ph temperature, or growth media requirements. No information is presently available on the survival abilities of treated cells under traumatic conditions.

Morishima suggested that the cytogenetic changes he observed were possibly the result of increased fragility of cells in cultures from persons who have used marijuana. A similar statement has been made for other drugs of abuse (Falek et al., 1972; Falek, 1974).

If this effect is a mutagenic rather than a toxic one, it would seem useful to establish long-term lymphocyte cultures and to sample them for a period of time after the standard 72-hour culture period. The fragility of the cells exposed to marijuana may be demonstrated if subtle changes increase the frequency of chromosome damage over prolonged periods of time similar to the results reported for lung cultures, which are exposed to marijuana smoke for extended periods.

To establish long-term lymphoid cell lines, the most frequent approach has been to use the Epstein-Barr virus (EBV) and phytohemagglutinin (PHA) to initiate blastogenic response. This procedure is based on the viral transformation of the cells in culture. However, there is a report, confirmed in our laboratory, of a method to establish long-term cultures by using only PHA (Beratis & Hirschhorn, 1973). Comparative analysis of long-term lymphoid cell lines from persons smoking marijuana and control cultures from non-smokers should be investigated. Evidence of diminished PHA activation of cells exposed to marijuana in culture may possibly limit the potential of this procedure, but at least an attempt should be made to establish and analyze these cells over a period of time (Morishima, 1974; Nahas et al., 1974; Morishima et al., in press; Morishima & Nahas, in press).

In addition to cytogenetic studies from lymphocyte cultures, chromosome and cell culture analyses using material from lung, skin, and other tissue should be conducted to confirm and advance the Leuchtenberger findings. For example, are the results obtained by them for lung cells exposed to marijuana smoke also produced when the cells from other tissues are exposed to the smoke? Certainly, as noted previously, the aberrant tissue grown in culture should be reintroduced into test animals to evaluate at the *in vivo* level whether such cells would initiate carcinogenesis.

Evaluation is now possible at the level of DNA base pair substitution (Clarkson & Evans, 1972; Cleaver, 1972; Fox & Fox, 1973). Chemical lesions in DNA treated or affected by tetrahydrocannabinols can now be evaluated by investigating their repair capability according to unscheduled

DNA synthesis. Repair replication investigations include density gradient experiments in which the frequency of tritium-labeled BUdR substitution for thymidine may be measured after the cells are irradiated by X-ray or ultraviolet light. With this procedure, repair replication frequencies at the DNA base pair level may be explored for cells treated with marijuana smoke as compared with untreated control cell cultures. Furthermore, autoradiographic studies and cell survival experiments after irradiation should give evidence of the repair replication ability of cells exposed to marijuana smoke.

Three types of repair replication are described. In addition to excision repair and photorepair, one further repair replication procedure, post-replication repair, is demonstrated in the mammalian cells by the new technique for differential staining to identify sister chromatid exchanges (Latt, 1973; Craig-Holmes, 1974; Latt, 1974; Perry & Wolff, 1974). This procedure requires the addition of BUdR to the cultures for approximately 48 hours prior to harvest (2 replications). Staining the chromosomes with acridine orange or Hoechst #33258 stain enables fluorescence identification of sister chromatid exchanges based on intensity of the dye during fluorescence. It would be of interest to determine whether there is an alteration in the frequency of sister chromatid exchanges in the chromosomes of individuals using marijuana as well as those using other drugs of abuse. At the recent 13th Annual Somatic Cell Genetics Conference, Latt (1974) reported that sister chromatid exchanges were more sensitive indicators of chromatid damage than chromosome breaks for such exgenous agents as mitomycin C and nitrogen mustard. A direct evaluation of drug damage at a basic molecular level is now possible.

At present, genetic findings on drugs of abuse including marijuana are open to question. The development of standardized procedures and the planned testing of marijuana in several cooperating programs are necessary steps to improve reliability and enhance confidence in the laboratories conducting such investigations. Possibly the equivocal findings are due to the relatively gross methods of analysis now employed. To detect low levels of mutagenesis, more refined techniques are required. These are available with tissue culture biochemistry and the new procedures to investigate chromosomes at the DNA strand level.

A carefully planned program to study marijuana with the new methodologies would be a first step in the development of a model to be exploited for genetic studies of many drugs and chemicals.

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Chapter 2

Cytogenetic Studies of Marijuana

Steven S. Matsuyama

The prospect of genetic damage from the use of marijuana and other cannabis preparations is of concern not only to geneticists and other investigators in the scientific community but also to the individuals who consume these drugs. An evaluation of genetic risk must include the question of potential mutagenic effects on both germinal and somatic cells. Since deleterious effects on germinal cells will manifest themselves in future generations, our heirs are the ultimate losers. Of primary concern to the individual is the risk from somatic genetic change, the greatest danger being carcinogenesis. Though not all mutagens are necessarily carcinogens, there does seem to be a relationship. A large number of known carcinogens, when tested in non-mammalian systems, have been found to be mutagenic (Ames, 1972; Ames et al., 1973). There is, then, the possibility of an increase in neoplastic diseases which may affect today's young people many years from now, as well as the possibility of an increase in deformed infants in the next or successive generations as a result of present day marijuana use. In addition, adverse genetic effects from the consumption of marijuana and related compounds during the organogenesis stage of pregnancy may cause teratogenic consequences.

Numerous experimental test systems are available to assess the genetic effects of marijuana and its metabolites (AMBIO Report, 1973). Non-mammalian systems and mammalian systems other than man are being extensively employed (see Falek's overview in Chapter One). However, since it is difficult to make reliable extrapolations of the data from these systems to man, it would seem best to perform genetic tests on man. One such test is to examine human chromosomes. At the present time, cytogenetic analysis is the best available procedure for assessing the effects of drugs and/or other environmental agents on the genetic material of man. The human peripheral leukocyte culture technique has been used almost exclusively for both *in vivo* and *in vitro* testing of drug effects on human chromosomes. Two other cell types, bone marrow cells and fibroblasts, are also used in the

cytogenetic assessment of drug effects. *In vivo* effects are assessed by direct examination of bone marrow cells, and skin fibroblast cultures are utilized in long-term *in vitro* testing. However, in both cases the procurement of cells for analysis requires special procedures (bone marrow aspiration or skin biopsy). We are hesitant to recommend bone marrow examinations for patients who do not need them for diagnostic purposes other than chromosome analysis. Indeed, the exposure of patients to such a distinctly unpleasant procedure, even in the interest of science, is not justified. However, some subjects may be eager to volunteer, and under such circumstances we will be happy to accommodate them. By contrast, blood samples for leukocyte cultures can be readily obtained and repeat determinations easily made with little discomfort to the subjects. It is for this reason that leukocyte cultures for chromosome analysis have been preferred. Unfortunately, chromosome analysis is laborious, time-consuming, and expensive; hence, only small sample sizes can be studied, a distinct disadvantage in any scientific endeavor.

Cytogenetic testing relies on the ability to detect microscopically visible morphologic changes in the metaphase chromosomes. Detectable chromosome aberrations may be of two types. One type is an alteration of chromosome structure by chromosome breaks which results in structural rearrangements; the second type is a change in chromosome number as a result of interference with cell division. This paper will focus on the first type.

The advent of the G, Q, R, and C banding techniques (for a review, see Miller et al., 1973) has further refined the investigator's ability to detect chromosome aberrations, including inversions, translocations, and deletions, provided that the chromosome segment involved is large enough to be detected. Despite these advances we are still at the gross chromosomal level, and no information can be obtained on the effects at the molecular level, i.e. the point mutations which can be as minute as a base-pair change in the DNA molecule. Moreover, leukocytes are somatic cells and do not provide information on germ cells, which are the cells of *primary* genetic importance. However, any evidence of genetic damage may be indicative of similar damage in gonadal cells and therefore potentially deleterious to future generations.

Precise information on the significance of chromosome breaks detected in cultures is lacking. Extensive chromosome aberrations often lead to non-viable cells, which are eliminated; or, breaks may be repaired by rejoining, so that the cells survive intact and unchanged. If either of these occurs, there should be no demonstrable long-term effects. We do know that breaks become important if cells with damaged chromosomes are sequestered and later come to expression as malignant neoplasia, or, in the

case of germinal cells, eventually give rise to offspring with congenital abnormalities. Because of these potentially deleterious effects of chromosome damage to the individual and to his/her offspring, cytogenetic testing is recommended for early detection of potentially harmful agents.

Present Evidence With Marijuana

The potential of marijuana to damage human chromosomes has been tested *in vitro* and *in vivo*. The two naturally occurring tetrahydrocannabinols (THC), delta-8-THC and delta-9-THC, and cannabis resin have been studied *in vitro* in human leukocyte cultures. Neu and associates (1970) found no increase in breaks with increasing concentrations of delta-8-THC, and they reported that preliminary results with delta-9-THC gave similar results. Stenchever and Allen (1972) also detected no increase in breaks following the *in vitro* addition of delta-9-THC. Finally, Martin et al. (1973) reported that dosage and duration of exposure to cannabis resin did not affect the frequency of chromosome breaks. The dose dependent mitotic inhibition seen in all three studies indicates that the compounds did indeed enter the cells; but whether they were able to penetrate the nuclear membrane is not known. However, in *in vitro* studies, the compound is directly available to the cells, and metabolic alterations that may impart chromosome damaging activity to the compound are not taken into account. Hence, direct applicability to man of these negative findings *in vitro* is not possible.

Investigations on the *in vivo* cytogenetic effects of marijuana have for the most part been retrospective, with break frequencies compared between "users" and non-users. Seven such studies have been published, and the findings are contradictory. Negative findings were reported in a study carried out in Jamaica (Martin et al., 1973) and by Dorrance and colleagues (1970) on light and heavy marijuana users. Gilmour et al. (1971) studied light marijuana users and failed to find an increase in the frequency of cells with chromosomal aberrations. However, for subjects who used a variety of drugs, of which marijuana was the only one common to all, they did note an elevated frequency of cells with chromosomal aberrations. The use of other drugs makes it difficult to properly evaluate this increase. Nahas et al. (1974) reported increased chromosome damage in chronic marijuana smokers, but the increase was not statistically significant. The three positive findings which have been published must be interpreted with caution. A significant increase in cells with breaks among light and heavy marijuana users, as compared to controls, was reported by Stenchever et al. (1974). However, since the marijuana was street-obtained, and drug history information was gathered through interviews, some imprecision is possible. Herha and Obe (1974) also reported a significant increase in exchange-type

aberrations (dicentrics and chromatid translocations) in chronic marijuana users. Yet, when one includes chromatid and chromosome breaks in the analysis, there is no difference between users and controls. Usually both breaks and exchange-type aberrations are reported together. The third study, and the only one to examine chromosomes in direct bone marrow preparations, is by Kumar and Kunwar (1972). They found a significant increase in breaks in heavy cannabis users. However, this increase is accounted for by only two of the seven subjects examined; the remaining five showed no breaks.

In retrospective *in vivo* studies, a number of uncontrolled variables make definitive interpretation difficult. These variables include nutrition, radiation exposure, health care, and the unknown composition of illegally procured marijuana. Furthermore, since most drug users use a variety of drugs, it is difficult to relate chromosome damage to any one specific drug. In general, "users" tend to show a higher frequency of chromosomal abnormalities than non-users (Matsuyama & Jarvik, 1975). There is also the possibility of individual differences in susceptibility to chromosome damage; in other words, different people may vary significantly in their vulnerability to the same agent. Harris and Hopkinson (1972) report that as many as 20 percent of the genetic loci of man are heterozygous. Therefore, inter-individual variability at the biochemical level is not surprising. Even if the pathways of drug metabolism are similar, the rates of metabolism may differ so that equivalent drug dosages may not necessarily mean equal serum levels. Thus, inter-individual differences may operate at several levels to produce differences in susceptibility to chromosome damage.

Only through well-controlled prospective studies can the controversy over genetic effects of marijuana be resolved. However, for ethical and moral reasons, naive subjects cannot be used to determine the chromosome damaging potential of marijuana; prospective studies can be carried out only with subjects who have already used the drug.

Recently, results from three prospective studies have become available, each reporting essentially negative results. Two are from our laboratory and the third is by Nichols and colleagues. Nichols et al. (1974) could not detect an increase in breaks after oral administration of hashish extract (contains THC and appreciable amounts of CBN and CBD), marijuana extract (delta-9-THC only), and synthetic delta-9-THC. In our first study (Matsuyama et al., 1973), three groups of volunteers smoked either placebo, 1 percent, or 2 percent marijuana cigarettes, one per day, for 28 days. We could not state conclusively that marijuana caused chromosome damage. Pre- and post-exposure break frequencies were identical in the placebo group as expected. In the 1 percent group, there was a two-fold increase, while in the 2 percent group there was a slight *decrease* in the fre-

quency of breaks. Our most recent study (Matsuyama & Jarvik, in press), a 94-day study with 72 days of unlimited smoking of marijuana cigarettes containing approximately 2.2 percent delta-9-THC, found no increase in the frequency of breaks when baseline and post-exposure values were compared. Further, there was no relationship between the number of cigarettes smoked ($X = 4.2 + \text{or} - 1.2$ to $10.0 + \text{or} - 3.1$ per day; range 1-28) and the frequency of chromosome breaks.

However, appropriate control groups of non-drug users were not included in the above studies, and the frequencies of breaks found before experimental marijuana administration cannot be properly evaluated. All of the subjects had some experience with marijuana prior to the study, and the frequency of breaks found in these groups may well be higher than that found in a control group of non-drug users.

Future Directions

Scientific information is based on replicability of reported findings. Future investigations should be well-designed prospective studies with appropriate control groups, and not further retrospective studies, which only clutter the literature and confuse the issue. These studies should employ a double-blind methodology, with blood samples sent to various collaborative laboratories for culturing and analysis. Identical methods should be used by the different laboratories, with observer differences as the only variable. Repeat determinations are essential since day to day variations have been reported by a number of laboratories (Tjio et al., 1969; Littlefield & Goh, 1973). Thus, one can ascertain the chromosome damaging potential of marijuana, and the design of the study can serve as a prototype for future cytogenetic investigations on the effects of drugs. However, in prospective studies there is an ethical dilemma that needs to be resolved. Ideally, one would like to collect chromosome data prior to the use of marijuana, as a baseline for later comparisons. Yet there is much apprehension in giving a socially disapproved and illegal compound to subjects who have not previously sought it, as they may subsequently search for it.

An opportunity for truly prospective studies with individuals who have not been previously exposed to marijuana may be possible in the near future. Other cannabinoids (cannabinol and cannabidiol) which do not have the psychoactive effects of delta-9-THC are being tested for potential therapeutic effects recently attributed to delta-9-THC (National Institute on Drug Abuse, 1974). The participants in these studies represent a valuable sample of naive individuals for prospective studies. Indeed, it behooves us now to take advantage of any ongoing investigations for the purposes of chromosome studies.

An alternative approach may be to select a random sample of healthy individuals at an early age, before any drug use has been initiated (e.g., elementary school students), and to obtain blood samples. At a later time, the use of marijuana by this sample would be ascertained and marijuana users would be compared to non-users. Both groups would be followed over a long term with periodic cytogenetical and biochemical (protein structure changes) screening for the mutagenic effects of marijuana or other potential environmental mutagens (Sutton, 1971; Neel et al., 1973). This study design also readily lends itself to evaluating potentially detrimental effects on future generations. Long-term observations, with accurate records of abortions, still-births, and viable births as well as the frequency of congenital malformations, would give an indication of genetic alterations.

Cytogenetic monitoring is time-consuming, tedious, very expensive, and requires highly trained individuals to carry out the analyses. Furthermore, we often deal with low aberration frequencies requiring large sample sizes, so that a large number of cells per subject need to be analyzed to obtain meaningful results. A procedure that would reduce the number of cells to be analyzed by increasing the frequency of aberration would greatly facilitate cytogenetic screening. One method might be to place cells from experimental subjects and controls under stress, i.e. to challenge cells with known chromosome damaging agents which by themselves produce extensive chromosome damage (e.g. streptonigrin or Mitomycin-C). Under these conditions, it may be expected that while increasing the frequency of chromosome damage in cells from control subjects, drug users may be even more susceptible to chromosome damage and hence manifest a higher break frequency than that seen in controls.

Recently, a new and relatively simple technique has become available to detect sister chromatid exchanges in lymphocyte cultures (Latt, 1973; Korneberg & Freedlender, 1974; Perry & Wolff, 1974; Wolff & Perry, 1974). This procedure involves the molecular alteration of the genetic material by 5-bromodeoxyuridine (BrdU) substitution for thymidine during DNA replication. The affinity of the chromatids for stain is effected by this technique. Since chromatids containing BrdU in both chains stain lightly while those with BrdU in only one chain stain heavily, exchanges that occur as a result of breakage and reunion can be easily detected by the non-homogeneity of stain intensity along the chromatid. This technique has potential applicability in the assessment of drug-induced damage. However, there is the question of using a known chromosome damaging agent, BrdU, in the detection of drug-induced sister chromatid exchanges, since BrdU may, by itself, induce such exchanges. Furthermore, great care must be exercised in carrying out the experiments, which must be done in the dark, since light causes photolysis of BrdU-substituted chromosomes and has

been shown to increase the frequency of sister chromatid exchanges (Ikushima & Wolff, 1974).

In summary, the available cytogenetic data provide no definitive evidence for chromosome damage as a result of marijuana use. However, an increasing number of individuals are using marijuana, and this poses a potentially widespread mutagenic danger to future generations. Since it is of utmost importance that definitive answers be obtained, further studies are warranted. Future investigations should include both well-controlled collaborative studies using a double-blind methodology and basic research to develop screening procedures with applicability not only to marijuana but to other drugs as well.

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Chapter 3

Observations on the Cytogenetic Effects of Marijuana

Morton A. Stenchever

Being the anchor man of this three-man presentation, I find there is little left for me to say, especially since I'm in agreement with most of the points previously made. However, my background is different from Dr. Falek's and Dr. Matsuyama's; I am an obstetrician, so I can analyze the data from a slightly different perspective. I'd like to summarize briefly a few of the methodological problems from the standpoint of my clinical and laboratory experience.

Effects of Marijuana on the Biology of the Cell

A number of studies have shown that marijuana, or certain ingredients in marijuana, have an effect on normal biological processes of cells. Our work would imply that a chromosome damaging agent is present in marijuana or that some agent in marijuana is convertible to a breaking agent by some metabolic process *in vivo*. Our observations and the observations of others with respect to cell damage make further investigation into the effects of marijuana and related agents on the cell an important area of research for the future. We are most anxious to evaluate as many agents in marijuana as possible, in an effort to discover what causes chromosome damage. We realize that we may be dealing with a metabolite rather than with a specific ingredient of marijuana; and if we are unsuccessful with our original screening process, we will need to begin to look at some of the metabolites formed in the body. However, we feel that the current evidence is sufficient to make this a reasonable quest, and that the implications of chromosome damaging potential of some marijuana substances are great enough to make this a worthwhile endeavor. For instance, a chromosome damaging agent could have a teratogenic effect on the developing fetus, or a damaging effect on germ cells which could lead to a mutagenic response, or it could effect a normal somatic cell to produce a neoplastic change. Even

without these serious implications, direct damage to normal cell metabolism could lead to a malfunction of an organ system in and of itself. A number of studies on a variety of organ systems, specifically the nervous system, imply that this may indeed be the case.

Assessment of a Chromosome Damaging Agent

In order to determine whether or not an agent has the ability to damage chromosomes, strict criteria must be applied. The definition of chromosome damage with respect to breaks, gaps, and abnormal forms has been established and must be utilized if studies are to be comparable. Another extremely important criterion is the need to carry out all evaluations in a blind fashion, thereby eliminating observer bias.

The use of standardized methodology and collaborative studies between laboratories are essential in the future. We cannot go it alone any longer.

It is also essential that we use newer techniques, such as banding techniques, in studying chromosome damage. But here a word of caution is needed. These new techniques are very tedious to carry out, since screening for chromatid exchanges is more time-consuming than searching for breaks. The amount of work will increase a hundred-fold over the previous experiments. However, I think it is necessary because the information obtained will be useful in developing insight into basic mechanisms of damage.

I believe that most investigators have sought large percentages of cells with damage to prove the agent dangerous. It has been our experience that chromosome damage is a very rare occurrence in our laboratory and that most agents do not break chromosomes. As a matter of fact, in most of the studies we have evaluated agents at levels toxic enough to kill the cells without finding evidence of chromosome damage. Therefore, any statistically significant variation in chromosome damage from control cultures would seem enough to report. To the surprise of many investigators, this may involve a relatively small percentage of damaged cells.

Additional Methodological Considerations

The problems involved in collecting drug use histories have been alluded to. I don't think many drug users will give a completely accurate history. Our own experience bears this out. I was very anxious to know how long damage would last after marijuana use was stopped. Naively, I believed that once an individual was shown to have significant breaks he would immediately stop using marijuana. I decided that if the research subjects chose, I would agree to restudy them in six months to see whether there was residual damage. Out of forty-nine subjects in our study, only

five returned for follow-up studies, and they all had breakage similar to that which they had had six months before. As I interviewed them I developed serious doubts that they had ever stopped using marijuana. I don't know how we are going to get at this problem of accurately quantifying any continued marijuana use, although quantification can be done with other drugs such as tranquilizers. In any case we must be very careful of reports that rely on *precise* histories of marijuana consumption.

The question of whether the cannabinoids get through the smoking filter systems was raised earlier. These are proper matters to be concerned with in our experimental approach. A number of water-soluble cannabinoid compounds and some volatile oils have been isolated by various workers. We really don't know what causes cell or chromosome damage: whether it is delta-9-THC or delta-8-THC or any of the other many ingredients of marijuana.

A word of caution about research involving delta-9-THC: While delta-9-THC is undoubtedly one of the more important active ingredients of marijuana in terms of psychic effect, we have no proof that this is the active biological ingredient responsible for all or any of the medical and biological responses ascribed to the drug. I believe that research with crude marijuana and a number of isolated ingredients needs to be carried out in concert with the delta-9-THC studies. As one reads the literature it becomes apparent that marijuana and delta-9-THC are used interchangeably by a number of researchers, and I do not believe this is biologically correct. We may yet find that the culprit in marijuana is something other than delta-9-THC, depending on what effect we are observing.

In our laboratory we have decided to look at as many compounds as we can, singly in cell systems. This again is very tedious, and if the techniques of banding are considered, it may take years to get through all these agents. I think these are necessary studies, nevertheless, because although we have casually interchanged marijuana with delta-9-THC, I don't think it has ever been proved that delta-9 induces the chromosomal changes we are seeing. That's another methodological problem to be dealt with.

Teratogenic Agents

Although the literature is full of anecdotal information concerning both LSD and marijuana as teratogenic agents in human experience, the data are still insufficient to draw specific conclusions. A number of animal studies have been carried out, and in my opinion none prove conclusively that marijuana or ingredients in the marijuana plant are teratogenic. Still, enough data are available that the teratogenicity of the agent cannot be ruled out. I rather suspect that with careful studies we will indeed find that the agent does damage the developing fetus. I do not believe we have those

data now; therefore, I believe that such studies should be carefully planned and executed in the near future.

When considering malformations, the thalidomide tragedy comes to mind, and with it the vision of babies born without arms and legs or with other severe defects. Such defects are only the extreme end of the spectrum of malformations that spreads from very severe malformations to very mild ones. The very mild ones may not be found for several years, if at all.

We should not seek only structural damage as evidence for teratogenicity, but also the malformations which can't be seen, e.g. malformations that involve learning. Elegant rat studies done several years ago indicated that rats born to mothers treated with meprobamate had learning difficulties (Werboff & Kesner, 1963). These studies should be repeated with the various marijuana substances.

It is possible that a drug that has an affinity for brain tissue in adults may have a greater affinity for brain tissue in the developing fetus, and that it may induce malformations in the development of small areas of the brain. Marijuana certainly has this potential since it is lipophilic and does cross both the placental and blood-brain barriers. The work that has been published by Heath supports these fears (Heath, 1974). These studies should be expanded experimentally in animals.

To return to the example of meprobamate, this was a drug used widely throughout the 1950's. Its use at that time could probably be compared to the use of marijuana today in that large numbers of people were exposed, among them pregnant women. Although the drug didn't cause gross thalidomide-like abnormalities, in December 1974, Milkovich (Milkovich & van den Berg, 1974) indicated that if meprobamate or chlordiazepoxide were given to mothers in the first forty-two days of gestation, there was close to doubling of the malformation rate in the infants that were born. It has taken over fifteen years to produce these data.

The same delay between widespread use of a substance and identification of adverse effects has been shown for a number of other drugs.

We are interested in diazepam, which has been shown by our technique to induce chromosome changes very similar to those induced by marijuana. The interesting thing is that no one has really investigated whether these changes are involved in malformations. Perhaps diazepam will not pose a significant problem in that the manufacturer has warned against its use by women who are pregnant, but it is used so commonly, some women who are pregnant may use it inadvertently.

The reason for discussing other drugs is that I feel marijuana fits into the spectrum of drugs taken by people to feel good, which are therefore used very widely. Thus, undoubtedly a large number of babies are exposed *in utero*. As with other drugs, it is going to take ten or fifteen years to begin

to find out whether marijuana is teratogenic in humans. However, it certainly doesn't cause the same problems as thalidomide. I think animal experiments with marijuana can and should be designed. Animal experiments can also be designed which will throw light on marijuana's effects on miosis, and this, I feel, is a fruitful area to pursue.

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Chapter 4

Marijuana and Genetics: A Discussion

Dr. Cole: To what extent have these newer genetic techniques such as sister chromatid exchange and repair replication, which sound awfully elaborate to me, ever predicted anything that could be directly associated with a real clinical problem?

Dr. Falek: They have provided useful predictions. From studies on chromosome 21 trisomy or translocation to recent data from Chicago about leukemia and specific chromosomal abnormalities (Rowley, 1975), there is evidence of chromosome alterations related to clinical abnormalities. The data on solid tumors have suggested that even before the onset of some chemical disorders there is evidence of chromosomal anomalies. These include alteration in chromosome number as well as chromosome abnormalities of other types that manifest themselves before the onset of the clinical disorder itself (Grandberg, 1971). With new banding techniques we are beginning to get some definite information on this.

Dr. Matsuyama, Dr. Stenchever, and I are making the same point. Up to now investigators have been looking at unstable chromosome damage. However, now there are newer methods for chromosome banding, sister chromatid exchange, and repair replication which enable relatively precise investigation of stable chromosome alterations. (See chapter 1.) We are moving toward the assessment of mildly deleterious mutants that have been able to remain undetected in the population for many generations.

By taking advantage of new techniques we can explore more refined methods of assessing chromosome damage. The classic technique should not be discarded entirely, but rather de-emphasized and included with the newer procedures to determine if the new methods are more likely to give us information that has predictive value.

One other point. The value of Dr. Leuchtenberger's investigations is that she has opened a door to further studies of cells in culture after treatment with drugs of abuse. I think the techniques that she utilized should be repeated in other laboratories to evaluate reliability.

Dr. Harris: I would like to raise a philosophical point here, because sometimes we forget that not all mutations are necessarily bad. That's how species evolve. Looking at very small changes of gene pools, or even macromolecular levels doesn't tell us the whole story. It is what those changes produce in the on-going species that is important. Sometimes we get carried away looking at the minutiae and don't see the broader pattern. We should all keep that in mind, particularly in trying to interpret the results of laboratory findings.
Dr. Falek: There is one problem with your point, Dr. Harris. I don't know of any geneticist who has reported good mutations occurring over a period of time. Almost all mutations we know of to date are damaging to the species rather than enhancing.
Dr. Jones: You're not going to find good mutations with present research studies because they are designed to measure adverse consequences.
Dr. Falek: The relevant research studies have not been conducted on humans but have been carried out with drosophila. If you look for mutations in drosophila you find all changes are lethal or sublethal.
Dr. Harris: May I point out that from the standpoint of the organism this is not true? That goes for bacteria which very quickly change in response to drugs, to protect themselves. And you have mutant strains of bacteria.
Dr. Falek: That's resistance, not mutation.
Dr. Hollister: Dr. Harris, what you are saying is genetic heresy. That's why Dr. Falek is excited.
Dr. Harris: I will get out of this. I am not a geneticist, but I have, somewhere in my background, some instilled education about mutations being important in the survival of a species.
Dr. Falek: General variability is important in survival of species.
Dr. Harris: How do the genes change? How do you distinguish general variability from a change in the genes which is a mutation?
Dr. Falek: You know what the frequency of alteration is and you can learn about mutation rates by following populations over a period of time. But you know that within a population there is general variability. That's what one finds in bacteria as the response to different kinds of environmental factors.
Dr. Hollister: There have probably been spontaneous mutations by transduction and the like. It is possible that one could make beneficial genetic changes in this way.
Dr. Kabat: Mutation can be a beneficial process in evolution. A change occurs. This may be an amino acid substitution in a hemoglooin which helps a particular species. As a consequence, that species has a higher survival value than others, so that over evolutionary time there is a directional

change. On the other hand, the mutations which we have produced in the laboratory have generally been of an essentially deleterious character.

Incidentally, the effect of some drugs is merely to select out spontaneously-occurring mutations in bacteria. For example, it has been shown that in bacteria and penicillin the mutation that makes the organism resistant to penicillin can occur in the absence of the penicillin. Then if the penicillin-resistant organism is placed in the penicillin medium, it will grow out because everything else is suppressed. So the tendency for mutations is essentially a spontaneous tendency. The drug per se is not necessarily producing the mutation.

Dr. Pollin: It sounds as though in the coming decade we need to conceptualize and implement new kinds of studies to get more definitive answers about the health hazards of marijuana. One of the important types would seem to be the large-scale prospective study such as the Framingham study, or the study which finally showed definitively the relationship between cigarette smoking and lung cancer. On a smaller scale, there is the longitudinal follow-up that Dr. Stenchever described in relationship to meprobamate anomalies. It would probably be difficult to fund prospective studies of the necessary magnitude if they were restricted to marijuana. I am wondering if there are plans for similar studies being undertaken in other government programs.

Dr. Hollister: Finland and Australia have birth defect registries.

Dr. Pollin: Have the Environmental Protection Agency or any of the other programs considered truly large-scale prospective studies where they will attempt to evaluate simultaneously the impact of a whole series of compounds in large populations over a decade or so?

Dr. Harris: The EPA people are carrying out several prospective studies, but they are concentrating mostly on carcinogens and incidences of cancer. There is a large vinyl chloride and polyvinyl chloride study now underway. Are you thinking of adding to or tailing onto an on-going study like that?

Dr. Pollin: I'm thinking of a large-scale birth defect registry which might have as part of the data collection system a total drug history, despite all the problems Dr. Stenchever has pointed out. But it is one thing to do this in a country of 6 million or 12 million people, as in the case of Finland or Australia, and another thing to do it with 220 million people.

Dr. Stenchever: But you could split the country into regions and do it in a limited area like Dr. Falek's five-county study.

Dr. Meyer: One of the problems that came up at a previous conference dealing with birth defects from hallucinogens is that so many of the drugs cause chromosomal damage. When we focus on drugs like marijuana or LSD we obscure other issues, which include the great number of other agents that can be responsible for causing birth defects.

Given the fact that with marijuana we are not dealing with obvious thalidomide-type abnormalities, I think it is important that we try to identify the conditions under which risks are maximal and study marijuana from that point of view. We are not identifying a specific agent like vinyl chloride which causes a particular kind of liver cancer, or a specific drug such as thalidomide which is bound to cause teratogenicity. We are dealing with a fairly low order relationship which is probably shared by a large number of substances.

Dr. Falek: One possible basis for the observed low order of relationship is that the techniques used for evaluation are indirect and not sufficiently sensitive to the teratogenic potential of the drug. We need to devise techniques which will give us both more sensitive and more direct evidence of long-term, low-level damage. To reiterate, I think Dr. Matsuyama and Dr. Stenchever agree that the techniques to measure chromosome damage must be improved, and that we must utilize the newer techniques which will give us direct evidence for long-term damage, and possibly evidence on whether or not the observed alterations are effecting DNA base pairs. The sister chromatid exchange and the replication repair studies are aimed at the development of that level of evidence.

It disturbs me to hear expressed the point of view that while these new techniques are very interesting, we ought to continue with our former methods to investigate chromosome damage from drugs of abuse. If we continue with our present methods, which have resulted in ambiguous findings, when will we begin to employ the newer techniques which may possibly lead to more definitive information?

Dr. Pollin: Even with the newer kinds of studies, if one doesn't follow up with the type of investigation Dr. Stenchever described, won't there continue to be important questions unanswered regarding the clinical significance of what you see under the microscope?

Dr. Falek: Up to now the cytogenetic findings have been controversial because of the inconsistencies in the data obtained by different investigators. I am suggesting that this may be due to the kinds of alterations we have been measuring. To continue in the collection of such information will never improve the results.

Dr. Freedman: Have these new, more direct testing systems usefully predicted what known deleterious agents will do? For example, what will these new techniques predict on tobacco smoke? If these techniques usefully predict the effects of potent mutagens, then they might help in studying mild agents or relatively unknown drugs like delta-9-THC. I think this is one thing that would at least provide some perspective while one gropes for the most useful methods of detecting mutagens.

Dr. Falek: I don't know what these new techniques will do with a fog of smoke produced by tobacco, since they are too new for such evaluations to have been conducted. However, there is evidence of increase in sister chromatid exchanges with some chemical mutagens, and in Bloom's syndrome (Chaganti et al., 1974; Kato, 1974). For repair replication studies there are data demonstrating unscheduled DNA synthesis in cells after exposure to UV light, X-rays, and chemical mutagens (Clarkson & Evans, 1972), as well as in patients with Fanconi's anemia (Poon et al., 1974) and xeroderma pigmentosa (Cleaver, 1965). The new techniques, therefore, identify the effects of some chemical mutagens in addition to certain genetic disorders. Therefore, they should be used in studying agents such as delta-9-THC.

Dr. Kabat: What do widely used drugs such as aspirin or saccharin do in terms of chromosome breakage? I would like to know whether the slight incidence of chromosome breakage which may occur with marijuana consumption may not be something we are overemphasizing?

Dr. Matsuyama: In answer to the first question, aspirin, saccharin, cyclamate and its major metabolite cyclohexylamine have been tested *in vitro* for their chromosome damaging effects. Three studies have been published regarding aspirin. Jarvik and Kato (1968) reported that aspirin at extremely low concentrations (0.1 and 1.0 microgram/ml) caused a doubling in the frequency of breaks over control values. Mauer and associates (1970) did not confirm this, but a re-evaluation of the data did substantiate a doubling (Jarvik & Fleiss, 1971). Finally, Meisner and Inhorn (1972) reported an increased incidence of chromosome rearrangements. In the only *in vivo* study, no increase was reported (Mauer et al., 1970). The addition of saccharin to leukocyte cultures does not increase the frequency of breaks (Stone et al., 1969). In contrast, the artificial sweetener cyclamate and its metabolite cyclohexylamine have been shown to cause chromosome damage *in vitro* (Stone et al., 1969; Stoltz et al., 1970; Brewen et al., 1971; Meisner & Inhorn, 1972). However, these results are from *in vitro* studies, and direct extrapolation cannot be made to their possible *in vivo* effects. For agents that are as widely used as these, further studies are warranted to assess the *in vivo* consequences.

With regard to the second question, I do not believe that we can dismiss any increase in chromosome breakage. Chromosomal aberrations induced by chemicals and other environmental agents are similar to those produced by viruses and radiation, two agents directly linked to the development of various forms of malignant neoplasia and congenital malformations. Further, cytogenetic analysis is limited to the detection of gross alterations at the chromosome level and we are in total ignorance of changes that may occur at the molecular level. Therefore, the increased fre-

quencies of aberrations that we observe are most likely an underestimate of the total mutagenic effect of the compound under investigation. The development of new techniques is necessary to fill this gap, and they appear to be near at hand. However, at this moment, cytogenetic screening is the best method available to assess the effects of various agents on the hereditary material of man.

Dr. Harris: A high incidence of chromosome change has also been reported with caffeine (Ostertag, 1965).

Dr. Mendelson: In response to earlier questions, the older techniques which simply demonstrate damage are not specific enough to tell us very much. The problem I have with the plea for techniques that have greater specificity is related to Dr. Freedman's question. It seems to me that there should be a significant accumulation of data utilizing these techniques with a number of conditions before one starts introducing specific drugs like delta-9-THC. The biologically relevant system—the human being—uses marijuana with an inhalation technique which produces a considerable amount of tar in contact with the alveolar membrane, much more than is contained in cigarette smoke. Therefore, it seems to me that the newer, more specific techniques should be utilized first in looking at things one would expect to find frequently in life, such as the effect of high content tars, rather than separating out various components of marijuana and testing them one by one. My question is when will this kind of basic data be generally available? For example, have any of these techniques been used to determine the effects of high tar concentration?

Dr. Falek: That I can't answer. The techniques are relatively new and the basic data are just being gathered.

Dr. Mendelson: It would seem to me that an individual who smoked a lot of marijuana would be a higher risk for developing carcinoma of the lung, just as a heavy cigarette smoker is a higher risk.

Dr. Nahas: I would like to ask Dr. Falek whether certain drugs like diphenylhydantoin (Dilantin), which have to be taken chronically by those who have epilepsy, are not accompanied by an increased incidence of birth defects? My reason for asking about fat soluble drugs is, of course, related to the fact that THC is highly fat soluble.

Dr. Falek: Dilantin, I believe, does result in an increased frequency of birth defects in women who use it during pregnancy.

Dr. Stenchever: In epileptics using Dilantin and other antiepileptics, the incidence of birth defects is almost twice what might be expected by chance (Speidel & Meadows, 1972). However, it is felt that this is mediated by an antifolic acid effect of these drugs rather than a direct cellular effect, though this has not been conclusively proved.

The other thing I would like to note is that we are getting the impression that just about everything breaks chromosomes, and this is not true. In our laboratory we have studied 35 to 40 specific compounds that supposedly do or potentially could have chromosome damaging effects. Just about all of these have been negative in *in vitro* systems; these include certain anti-epileptic drugs (Stenchever & Jarvis, 1971; Stenchever & Allen, 1973). According to our *in vitro* techniques, barbiturates and caffeine do not break chromosomes.

Aspirin also has been assumed to be a very safe drug, but evidence is now accumulating that it isn't innocuous. One of the things aspirin does is to interfere with the prostaglandin system, and this has a marked effect on reproduction. There are a lot of agents whose safety we have taken for granted which perhaps may not be safe. But I don't think we should give the impression that everything we study breaks chromosomes, because in our laboratory only a handful of drugs has been chromosome damaging.

I have never studied meprobamate and I don't know whether studies show that it causes breaks or not. As I mentioned, diazepam (Valium) was one of the very few substances we found to break chromosomes. Chloridiazepoxide (Librium) did not break chromosomes in our studies, nor did most of the other tranquilizers and barbiturates we have studied. That doesn't mean that they are safe for the fetus, but at least they don't work through the chromosome damaging mechanism.

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Chapter 5

Marijuana and Immunity

Albert E. Munson

Before attempting a critical analysis of immunologic methods which have been employed in marijuana research, I would like to state that immunology has taken on a more restricted definition than it had two decades ago when it was more synonymous with what may now be considered host defense mechanisms. For instance, immunology deals more with lymphocytes, their interactions, reaction products, macrophages, and complement, and has little interest in such host defenses as wound healing, the cough, vomiting, intravascellular clearance of foreign materials, etc. In our concern about the public health and social implications of marijuana, we should, perhaps, be more interested in its overall actions on host defense mechanisms. However, one aspect of these mechanisms certainly is lymphocyte function.

The best approach to the question of marijuana and immunity would be to conduct a well-structured prospective investigation into the susceptibility of marijuana users to disease. One such study is now being carried out by Coggins (in press) in Costa Rica. Since human epidemiologic studies are cumbersome, difficult to control, and require years of data collection to draw definite conclusions it is necessary to take alternative interim approaches to appraise the potential problems which may result from use of this currently very important drug.

A reasonable alternative is to investigate the susceptibility to various pathogens of animals treated with marijuana. As an example, in collaboration with Dr. P. Morahan, we have initiated experiments to see if mice treated with delta-9-THC have a decreased resistance to encephalomyocarditis virus. Other representative pathogens, i.e. fungus, bacteria, etc., should also be the challenge. This line of investigation taxes the host defense mechanisms in total and should provide a reasonable prediction of what could happen in the human population.

Another approach is to select specific host defense mechanism tests and determine if marijuana alters them sufficiently so as to predict future

health problems. It is the selection of the diagnostic test and its interpretation that may cause the investigator to judge a drug or clinical situation incorrectly. Studies by Penn (1974) show that immunosuppression in humans, whether congenital or iatrogenic, results in cancer rates many times greater than those of the general population at a comparable age range. Most of the subjects in the Penn study can be considered immunosuppressed because of immunosuppressant therapy and acceptance of an organ transplant. I hasten to add that, thus far, there is no epidemologic or clinical evidence to suggest that chronic users of marijuana are more susceptible to neoplastic or infectious diseases.

A number of recent reports (Gupta et al., 1974; Lefkowitz et al., 1974; Levy et al., 1974; and Nahas et al., 1974) have been concerned with the immunosuppressant aspects of marijuana and its major psychoactive constituents. In an investigation on 51 human subjects, Nahas et al. (1974) demonstrated an impairment of cellular immunity in chronic users of marijuana as assessed by inhibition of lymphocyte blastogenesis. Blastogenesis was induced by the use of the T-cell specific mitogen, phytohemagglutinin (PHA), and with allogeneic cells (mixed lymphocyte culture). Employing rosette-formation as a method of quantifying lymphocytes, Gupta et al. (1974) showed that 9/23 chronic marijuana smokers had a lower number of T-lymphocytes but a similar number of B-lymphocytes. The absolute lymphocyte count of the two groups was not significantly different. Silverstein and Lessin (1974) used *in vivo* skin response to 2,4-dinitrochlorobenzene (DNCB) along with a battery of common antigens to study the overall immunocompetence in chronic marijuana smokers. With DNCB no difference was seen with the 22 chronic marijuana subjects and the 279 normal controls. It should be noted that lymphocyte reactivity to PHA in these patients was depressed (Silverstein & Lessin, in press).

Thus far only two studies have been reported on the action of delta-9-THC on the immune response in animals. Levy et al. (1974) showed that delta-9-THC prolonged allogenic skin graft survival, inhibited primary antibody production to sheep erythrocytes (SRBC) and inhibited both T- and B-cell reactivity as measured by the blastogenic response to PHA and E. coli lipopolysaccharide (LPS). Leftkowitz et al. (1974) also showed a reduced response to SRBC in delta-9-THC-treated mice as measured by the hemolytic plaque assay, and also reported a depletion of spleen cells.

The purpose of this paper is to present a position concerning the immunologic methodologies used in these studies and to try to relate them to potential clinical and social problems in real life. As a basis for discussion I have provided Figure 1, which is a simple diagram of mechanisms operating in the immune system, and Figure 2, which is a schematic illustrating

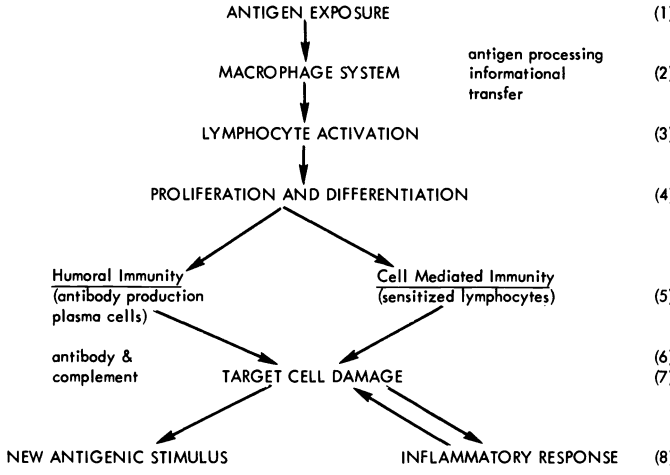

Figure 1

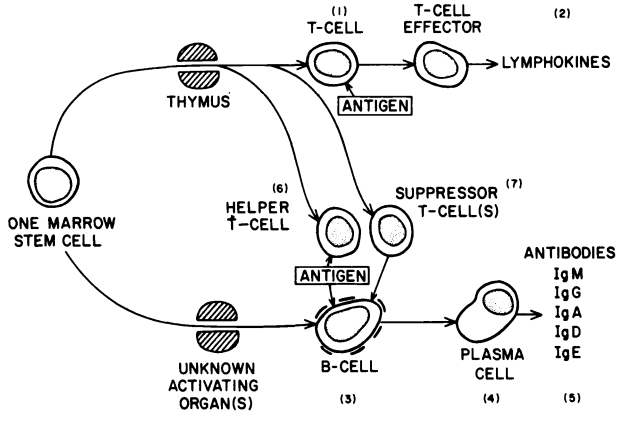

Figure 2

the separate but cooperative functions of T- and B-lymphocytes. The numbers on the diagrams are for references made in the text.

The following immunologic assays will be discussed: (1) skin sensitization to DNCB, (2) lymphocyte reactivity to mitogens and allogeneic cells, (3) rosette-forming cells, (4) skin allograft rejection, and (5) immunoglobulin response to antigen.

2,4 Dinitrochlorobenzene (DNCB)

DNCB is a simple chemical which is a strong allergen. Ninety-six to ninety-nine percent of the normal population can be sensitized. The cutaneous reaction to DNCB requires a functional T-lymphocyte system (Fig. 1-1) and the ability to elicit an inflammatory reaction (Fig. 1-8). The activation of the T-lymphocytes (cell mediated hypersensitivity reaction) results in production of lymphokines (Fig. 2-2), i.e. chemotoxic factors, etc., which elicit the inflammatory reaction. When this assay is employed, the inflammatory process should be tested by the use of an inflammatory agent such as craton oil. The ability to respond to this percutaneous application is called contact skin sensitivity; the ensuing inflammation is an allergic contact dermatitis. DNCB is a persistent antigen which remains for years. Delayed type skin responses to simple chemicals seemed initially to offer important opportunities to clarify the specificities of T-lymphocyte reactions, just as haptens demonstrated the specificities of antibody molecules. However, this reaction depends on the ability of the sensitizer and eliciting agent to form a covalent bond with skin molecules (proteins or lipids), and the complexity of the derived molecules obscure possible specificity requirements of this and other cell mediated responses. The specificity of B-delayed type reaction to simple chemicals, in this case DNCB, is of little consequence in relation to its diagnostic potential for immunosuppression.

This procedure has been valuable in predicting the prognosis of cancer patients (Eilber and Morton, 1970). Patients who have a positive DNCB response, i.e. an intact cell mediated immune response with an accompanying inflammatory reaction, have a better prognosis than the patient who does not respond. Adler (1970) suggests that the reason for this inability to respond to DNCB may not reflect immunodeficience on the part of the patient but rather that he is undergoing an all-encompassing immune response, and because of the massive commitment of lymphocytes to tumor antigen cannot react to the sensitizing agent. This seems to be a definition problem.

Quantitation of the immune response is one of the disadvantages of this assay. Although the degree of inflammatory response can be rated +2, +3, +4, etc., it is not known how this relates to the number of functioning lymphocytes. It may be that only 50 percent of the lymphocytes need be functioning in order to elicit the maximum cellular immune response. Although it may be a good prognosticator for cancer patients whose antigenic tumor load is or will be very large, it may not indicate more subtle problems with the lymphocytes which theoretically can predispose to problems.

Lymphocyte Reactivity to Mitogens and Allogeneic Cells

This assay has become very popular for testing T-cell responsiveness in humans and T- and B-cell responsiveness in mice (Fig. 2-2, 2-3). In the case of mitogens, PHA is a T-cell specific mitogen for mice only. Pokeweed mitogen is a T- and B-cell mitogen for man. I view these mitogens as I would any specific stimulus in a biological system. The response in this case is blastogenesis, which will occur also with other antigens. Blastogenesis is a turning on of the lymphocyte as seen in marked increases in DNA, RNA, and protein synthesis. The cell enlarges, there is an increase in endoplasmic reticulum, and the nucleus to cytoplasm ratio decreases. For those who think in terms of the cell cycle, these agents move the lymphocytes from the *G1* phase to *S* phase. When allogeneic cells are employed as the stimulus (mixed lymphocyte culture), a T-cell response is generated. Some studies suggest that this stimulus is a close correlate to skin allograft survival. Because of this correlation and other studies, it is believed that the stimulus associated with allogeneic cells is the HLA antigen. The problem with this conclusion is that platelets which have strong HLA antigens do not stimulate lymphocytes to respond. Thus, we really do not know the source of the stimulus, although this does not detract from its usefulness in studying immune responsiveness. The unit of measure in these assays is usually the uptake of 3H-thymidine into the lymphocytes, or incorporation of 3H-thymidine into the acid fraction (DNA) of the lymphocytes. A major misinterpretation of this data is that a decrease in 3H-thymidine incorporation is synonymous with a decrease in DNA synthesis, which is not necessarily the case. 3H-thymidine is incorporated into DNA by the salvage pathway. Certainly DNA synthesis can proceed by the *de novo* pathway. However, in many of the early studies it was shown that a decreased incorporation was accompanied by a decrease in the morphological changes. This does not preclude that other biochemical, functional, or morphological procedures should not be used when this assay is employed in new situations.

A recent study by Faguet (1974) on human lymphocyte stimulation by PHA demonstrated the necessity for using a range of PHA concentration. He studied 35 normal individuals on a monthly basis and found a wide variation in their responsiveness, which could lead to the conclusion that all the subjects may be immunosuppressed at any given time. Since the only response that did not change was the minimum stimulatory concentration, he believed that this might be the best measure of lymphocyte responsiveness. As this assay gains popularity, we see more drugs that suppress this response. Examples are amphotericin B and clotrimazole, two antifungal agents (Tarnvik & Ansen, 1974).

I believe the jury is still out on the definitive interpretation of the PHA lymphocyte stimulation assay as it relates to immune response. I do not mean that it should not be used, but rather that it should be part of the data base to better describe the subjects' immunologic status.

Rosette-Forming Cells

B-lymphocytes have receptors on their surfaces which bind immunoglobulin and complement. Antibody-coated sheep red blood cells (SRBC) will attach to these lymphocytes forming rosettes. Under appropriate conditions, but still disputed, T-lymphocytes will bind SRBC which are not pretreated with anti-SRBC antibody. Using this technique, the number of T- and B-lymphocytes can be quantitated. In order to measure functional activity, i.e. ability to form rosettes, the two populations of lymphocytes must be separated and then exposed to SRBC or antibody-coated SRBC. From this perspective, rosette formation is able to quantify B-lymphocytes more precisely than T-lymphocytes.

Skin Allograft Rejections

Skin transferred from a donor to a genetically different recipient of the same species is rejected because of histocompatibility differences. This is a T-cell function. The disadvantages of this assay are that it must be confined to animals, and the end point is difficult to quantify; the advantage is that like the hemolytic plaque assay it measures the functionality of the T-lymphocyte.

Hemolytic Plaque Assay

This assay primarily measures B-cell function but requires T-cells either as helper cells (Fig. 2-6) or as amplifiers (Moller, 1970). By necessity, it is an assay used primarily in animals. Animals, usually mice, are given the antigen, usually foreign erthrocytes, and the number of splenic, lymph node or blood lymphocytes which produced specific immunoglobulin against the foreign antigen are determined. This is visually observed as a

hemolytic plaque in agar. The advantage of this procedure is that the lymphocytes are tested for their ability to perform the function intended. Hemagglutination antibody or hemolysin antibody are similarly quantitated from the serum of the challenged animals.

There are many other immunologic procedures that should be employed in the evaluation of the immune response as it relates to marijuana use, and there is little doubt that in the coming years this area will be thoroughly investigated. At present, the results provide a minimal data base from which to judge the immunologic hazards of marijuana use; more research is needed before conclusions can be drawn.

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Chapter 6

Effects of Marijuana Smoking and Natural Cannabinoids on the Replication of Human Lymphocytes and the Formation of Hypodiploid Cells

Gabriel G. Nahas

In this paper I will describe the interaction of marijuana products on the T-lymphocytes of man.

It was first shown by Nahas et al. '1974b) that PHA-stimulated T-lymphocytes sampled from marijuana smokers have a decreased ability to incorporate 3-H thymidine (Table 1).

^{3}H-Thymidine Uptake of T Lymphocytes in Marihuana Smokers Compared with Normal and Immune Suppressed Subjects

	PHA				MLC			
Subjects	No. tested	CPM	SE	*p*	No. tested	CPM	SE	*p*
Normal controls	81	23,250	1,878	—	81	26,400	1,789	—
Marihuana smokers	51	13,779	1,195	<0.0005	34	15,679	2,867	<0.005
Cancer patients								
primary tumors	16	17,501	480	<0.0005	16	14,894	3,067	<0.0005
regional spread	23	13,345	2,533	<0.0005	23	15,816	1,970	<0.0005
distant spread	21	10,516	2,594	<0.0005	21	8,968	2,053	<0.0005
Transplant patients	—	—	—	—	24	12,307	1,712	<0.0005
Uremic patients	—	—	—	—	26	12,001	1,360	<0.0005

Table 1.

These T-lymphocytes also have a decreased ability to form rosettes (Gupta et al., 1974) when challenged with uncoated sheep red blood cells (Table 2).

Table 2. After Gupta etal (2)

Enumeration of T and B Lymphocytes in 23 Controls and 23 Marihuana Smokers.

Group	T Lymphocytes (%)*	B Lymphocytes (%)†
Control	26.6±3.8	11.6±2.0
Smokers‡	21.4±7.0	11.6±4.7
F-distribution significance	<0.005	NS§
Student's t-test significance	p<0.005	NS

*Mean ± SD by "active" rosette test.
†Mean ± SD (complement receptor).
‡Absolute lymphocyte count 2159 ± 1037.
§Not significant.

Tobacco smokers (Suciu-Foca et al., 1974; Whitehead et al., 1974) do not present similar signs of impairment of cellular mediated immunity (Tables 3 & 4). The studies of Morishima (Morishima et al., in press) indicate

Table 3. After Whitehead et al (3)

COMPARISON OF P.H.A.-INDUCED LYMPHOCYTE STIMULATION BEFORE AND AFTER SMOKING

Group	Time	Mean d.p.m. for group			
		Control	0·3 µg./ml. P.H.A.	0·8 µg./ml. P.H.A.	4·0 µg./ml. P.H.A.
Smokers	Before	932	7333	32,792	41,790
	45 min.	849	5339	32,360	40,705
	2 hr. 30 min.	1118	7936	37,830	47,028
Non-smokers	Before	591	10,659	38,939	34,714
	45 min.	709	7190	43,329	36,563
	2 hr. 30 min.	1000	19,018	53,949	51,913

that these lymphocytes sampled from marijuana smokers present an increase in numbered hypodiploid cells or "micronuclei" (cells in metaphase with less than 30 chromosomes) (Table 5).

In experiments *in vitro* performed to investigate the mechanism of the action of marijuana products on lymphocyte replication, we made three observations (Nahas et al., 1974a). One, that all natural cannabinoids contained in marijuana smoke and their metabolites inhibited 3-H thymidine incorporation in cultured lymphocytes (Figs. 1 & 2). These cannabinoids

Table 4. After Suciu Foca et al (4)

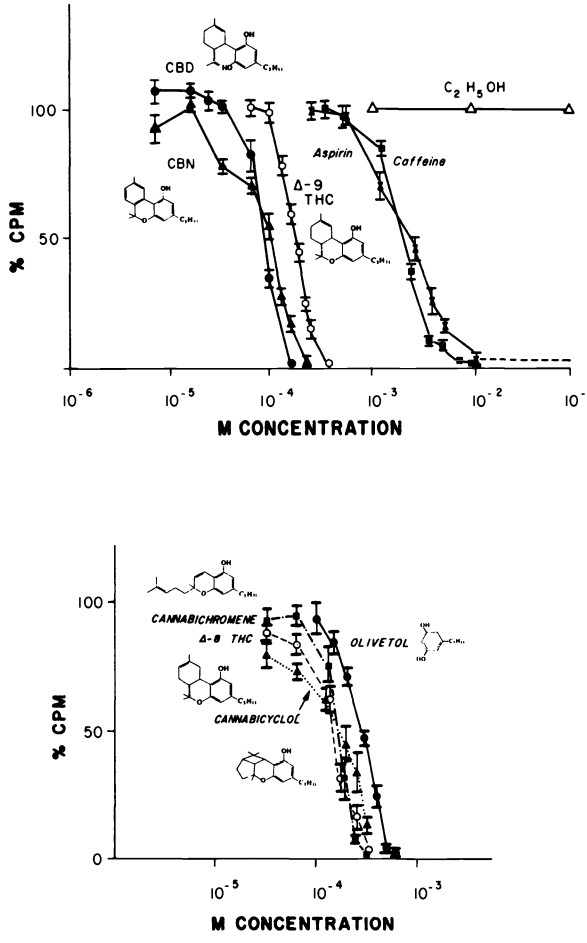
COMPARISON OF E-ROSETTE FORMATION, M.L.C., AND P.H.A. REACTIVITY OF PERIPHERAL LYMPHOCYTES IN SMOKERS AND NON-SMOKERS

Subjects	Age ±S.D. (range)	%E-rosette formation ±S.D.	M.L.C.R. (c.p.m. × 10^3 ±S.D.)	P.H.A.R.
Average of smokers	31±6 (22-46)	56±8	44±16	45±12
Average of non-smokers	33±6 (22-46)	58±10	38±13	46±10

Table 5.

NUMBER OF CHROMOSOMES PER NUCLEUS	PERCENTAGE OF METAPHASE WITH VARYING NUMBER OF CHROMOSOMES (%) 1 - 4	5 - 10	11 - 20	21 - 30	>30	MITOTIC INDEX (%)	NUMBER OF CELLS EXAMINED
	3.17	3.17	3.17	4.76	85.71	7.10	887
PAIRED CONTROLS	5.17	3.02	2.26	2.64	86.88	14.76	5,370
n = 5	0.00	1.00	0.00	1.00	98.00	9.09	1,100
	13.88	8.88	6.35	10.57	60.32	5.58	10,591
	1.49	2.86	2.58	1.63	91.41	2.96	12,385
MEAN	4.74	3.79	2.87	4.12	84.46	7.90	
			10.78				
	7.57	8.33	9.84	21.96	52.27	3.79	3,214
HEAVY MARIHUANA SMOKERS (>3 cgtts/wk)	2.11	7.74	7.74	25.35	57.04	4.81	2,950
n = 5	5.44	4.26	1.47	5.29	83.52	11.83	5,747
	35.76	13.74	7.34	7.47	35.70	7.30	10,914
	2.90	2.32	3.12	1.37	90.27	5.55	12,388
MEAN	10.76	7.28	5.90	12.29	63.76	6.66	
			25.47				

Table 5: Percentage of metaphase, with varying number of chromosomes, and mitotic index in heavy marijuana smokers compared with paired controls. The controls and the smokers are listed in order of the corresponding pairs.



Figures 1 and 2 : Inhibitory effects of the cannabinoids, delta-9- and delta-8-tetrahydrocannabinol, cannabinol (CBN), cannabidiol (CBD), Cannabichromene and cannabicyclol on PHA induced lymphocyte transformation as measured by 3-H thymidine incorporation after three days of culture. This effect is compared to that of aspirin, caffeine, ethyl alcohol and olivetol. All experiments were done in triplicate cultures. Inhibition of lymphocyte transformation was calculated in reference to the CPM of the control culture. The dashed line represents percent of thymidine uptake of unstimulated cells (Fig. 2).

also induced *in vitro* a high incidence of hypodiploid cells (Table 6). Two, that olivetol produces a similar inhibition of macromolecular synthesis and an increased incidence of micronuclei. All cannabinoids and their metabolites present the C-ring of olivetol. Three, that cannabinoids inhibited the uptake of the other precursors, leucine and uridine, required for the synthesis of protein and RNA (Figs. 3 & 4). Similar observations were reported by Blevin and Regan (in press) in normal and abnormal cell lines.

None of the marijuana smokers we studied presented any apparent clinical manifestation of immunological incompetence, a finding documented by Silverstein (Silverstein & Lessin, 1974), who showed that the DCNB test was accompanied by a positive reaction in the marijuana smokers he studied. This test, however, is indicative of a condition of anergy, with a global profound depression of cellular mediated immunity. Such a state of anergy was not present in the apparently healthy population of marijuana smokers we studied.

It is only by systematic epidemiological *longitudinal* studies, patterned after those performed on tobacco smokers for decades, that the clinical significance of the present observations could be established.

A number of drugs used for therapeutic purpose over long periods have been associated with an increased occurrence of auto immune disease as described by Tan (1974) (Table 7). Some of these drugs, such as diphenylhydantoin (Dilantin), which has also been associated with an increased incidence of lymphoma (Anthony, 1974), are characterized,like the cannabinoids by a very high fat solubility and an affinity for cell membranes.

Any weakening of immune defenses would become more apparent in a time of major epidemic and stress, or of social and political upheaval, which have so far spared the North American continent. The projections of Robert Heilbroner in his recent *Inquiry into the Human Prospect* (1974) raise the possibility that such a stable and protective environment, which has been taken for granted by many young Americans, might not endure forever. Without espousing Heilbroner's forecast that our society may be annihilated, the scientist might gain perspective if he heeded Heilbroner's warning and adopted sober solutions to the problems confronting society. A policy which would discourage marijuana use as a social recreational drug, a position both medically and socially justified, would be one such solution. The time for unlimited experimentation is running out. Throughout history, the social acceptance of marijuana in a society has appeared to set citizens on a one-way course; in the past, there has been no way to turn back.

NUMBER OF CHROMOSOMES PER NUCLEUS	PERCENTAGE OF METAPHASE WITH VARYING NUMBER OF CHROMOSOMES (%) 1 - 4	5 - 10	11 - 20	21 - 30	>30	MITOTIC INDEX (%)	TOTAL NUMBER OF CELLS EXAMINED
CONTROL	6.77	5.64	4.51	5.64	77.44	5.05	5,266
			15.79				
Δ^9 - THC 6.4×10^{-6} M	8.23	6.50	5.63	12.77	66.88	4.42	5,231
			24.90				
CONTROL	2.73	6.10	2.09	4.46	84.46	5.63	9,747
			12.65				
OLIVETOL (1.5×10^{-4} M)	31.03	25.16	10.85	7.23	25.72	5.68	10,944
			43.24				

Table 6.

Table 6: Percentage metaphases, with varying number of chromosomes, and mitotic index in cells exposed to delta-9-THC or olivetol compared with paired controls.

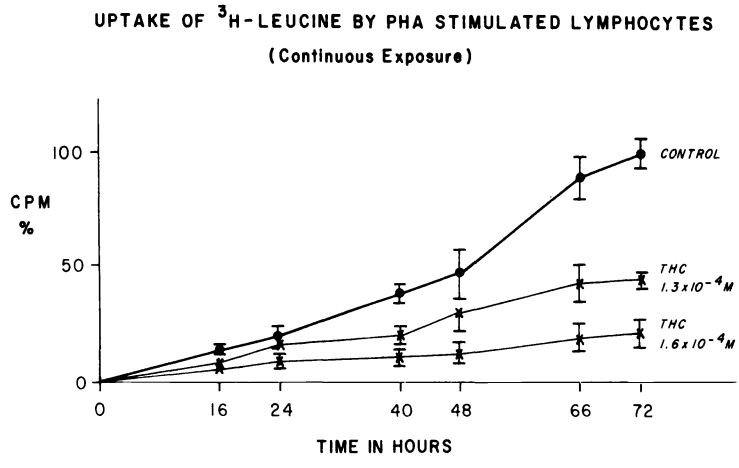


Figure 3: Inhibitory effect of delta-9-THC on 3-H leucine uptake. Note that this inhibition is observed in the early hours after onset of culture.

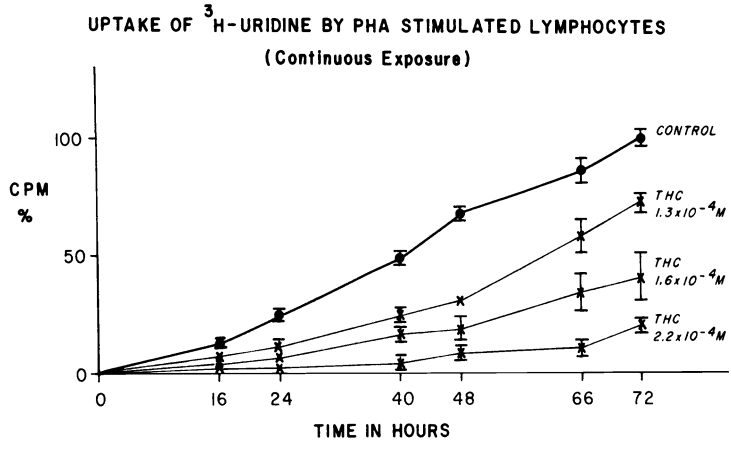


Figure 4: Inhibitory effect of delta-9-THC on 3-H uridine uptake. Note early inhibition.

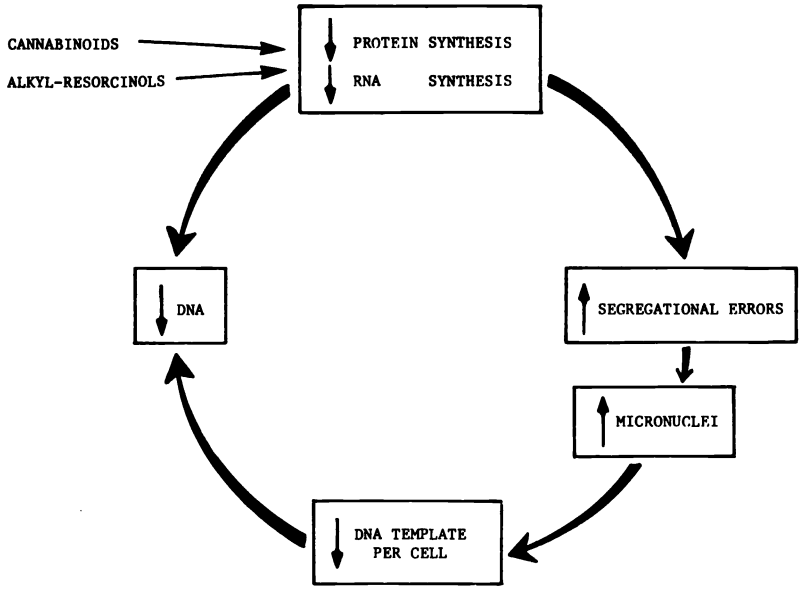


Figure 5: Suggested outline of the possible mechanism of the inhibitory effect of cannabinoids and alkyl resorcinols on DNA formation.

Table 7. After Tan (11)

Drugs implicated in autoimmune disease	
Hydralazine	Chlorpromazine
Procainamide	Trimethadione
Isoniazid	α-Methyldopa
Sulfonamides	Nitrofurantoin
Diphenylhydantoin	

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Chapter 7

Marijuana and Immunity: A Discussion

Dr. Hollister: Dr. Harris, would you like to comment on work you and your colleagues are doing in the area of marijuana and immunity?

Dr. Harris: Our interest in marijuana and immunity developed from the report that there was some specificity so far as T-cell function suppression by marijuana was concerned. It was our feeling that T-cell suppression represented, or might represent, some potential therapeutic utility, especially since as the range of drugs go, the cannabinoids, important chemical constituents of marijuana, are a pretty safe group of drugs. They have an enormous therapeutic/lethal ratio. Our conversation has been on safety factors, public health concerns that might arise from misuse or overuse of marijuana. But to anyone who has been familiar with the development of drugs over a long time, the very wide therapeutic ratio the cannabinoids have is also striking. Therefore, if there was a specific effect on T-cells, you might have an ideal immunosuppressant agent for use in transplant procedures. That was what got us started on this particular line of investigation. However, marijuana turns out not to be as specific for the T-cells as was originally thought; it also interferes with the B-cell function.

In the study of marijuana one is immediately confronted with obstacles to direct, definitive investigations. First is the complex nature of this particular drug. Marijuana's public health problems are related to the inhalation or ingestion of some crude plant materials in forms very difficult to reproduce in the laboratory. We, as scientists, always try to refine the system so that we look at specific compounds or specific components, because then our results can be more precisely analyzed. It is difficult to interpret results from studies where animals or tissues are exposed to smoke, because you don't know all of what is in the smoke. Therefore, most of the work that is done in the laboratory is done with pure compounds which may not have relevance to the overall public health drug abuse situation that is being so widely discussed.

The second obstacle is derived from the nature of this particular class of compounds, the cannabinoids. Cannabinoids are extremely difficult to handle and to obtain reproducible results. I have never come across a group of substances so unreliable and unreproducible from experiment to experiment, and from laboratory to laboratory. We thought in the beginning that we could solve the problem by getting away from the crude plant material and using very pure compounds. But even with the pure compounds, our difficulties continue. Anyone who has worked with marijuana realizes that there are a host of problems asociated just with the physical handling of it, introducing it into the system, and getting reproducible results. In every marijuana system that I have worked with, there is at least one inverted point on the dose response curve. I don't know what the answer to this unreliability is, but it is there.

The last matter I would like to address concerns the safety of the cannabinoids. Our experience with their safety in laboratory animals has been associated essentially with the purified products and not with the crude plant material that is used on the street. From all I have seen of chronic administration over long periods of time in animals, it is my feeling that relative to its pharmacological potency, the drug has a very high safety ratio.

Dr. Hollister: Dr. Kabat, will you comment on these findings.

Dr. Kabat: A fundamental principle of statistics states that a difference is a difference only if it makes a difference. If we use this criterion to examine some of the findings on marijuana, we are really not at all sure what is going on. For example, if you look at the T-lymphocyte experiment, you find that controls have 26.6 percent T-lymphocytes plus or minus 3.8, the experimental group 21.4. This is a rather small difference. Most of these highly regulated systems have a substantial margin of safety so that it is only when you get to a very acute depletion of one particular type of cell that you have a clinically significant effect.

Now the other problem about these data is that they are obtained by a particular type of T-lymphocyte test. However, there are many different tests, most of which are set at different levels of sensitivity. In some tests T-lymphocytes make up 85 percent of the total lymphocytes in blood. The need for standardization of tests and of sensitivity levels in this general type of study is apparent.

It seems to me that there are two major tasks to be done. One is to get an experimental model which can be studied in the laboratory. The second, once you develop such a model, is to set up the experimental design in such a way that you test simultaneously in the same animals as many different immunological parameters as possible. In other words, it means very little to take one test population and sensitize them to dinitrochlorobenzene and find that the control group shows no difference, and then use different tests

to study another population, even if you obtain significant results in the second series of investigations. Because these tests all have their own levels of sensitivity, you must have an internally planned experiment delineating the relationship of these various parameters. Of course, you always have to bear in mind the fact that after you get the data, one question remains: What does this really mean in terms of marijuana smoking?

There are data indicating that people get antibodies from smoking marijuana, but this finding is difficult to interpret because marijuana is a crude plant and you can get antibodies from breaking up almost any kind of leaves and parenterally introducing them into an animal. Thus one has a complicated situation in which many of the constituents of marijuana, if one is using crude marijuana, may be producing effects which seem to be or may actually be completely unrelated to the psychoactive constituents. This also has to be considered in the experimental design.

My feeling is that attention should be directed to designing experiments in which the materials, especially those which have a long survival value, can be independently evaluated by different laboratories. Although I don't think you could send lymphocytes around the country, you could send serum or chromosome preparations. Another useful advance would be to set up an experiment in which one had a good animal model for evaluation. At this point, the difficult problem of applicability of animal data to humans becomes crucial.

With regard to human investigations I want to reiterate the plea for double-blind experimentation. There is simply no question that any results are suspect without double-blind conditions, in which the people who set up the experiments and who are seeing the patients are completely different from the individuals who are doing the test evaluation. Double-blind experimentation is absolutely crucial in an area of investigation such as drug use, which is fraught with bias. With double-blind designs, you should also have a cooperative arrangement whereby experienced people in different laboratories are conducting their tests on the same material so that a set of data can be precisely defined. There is an advantage in drug abuse research in that the same population can often be used by investigators in both genetics and immunology; when different measurements are being made simultaneously on the same population the chances of obtaining important results are increased. And also, if you find that under certain conditions there are changes which are not seen under others, you may be able to identify causal mechanisms. If one uses just one measure on a particular set of data, it may have absolutely no relation to the overall picture of cell-mediated immunity, genetic effects, or whatever else one may be investigating.

Dr. Hollister: Has anyone demonstrated to what degree phytohemagglutinin (PHA)-stimulation is depressed when an active immunosuppressant agent is used? For instance, how do T-cells work in transplant patients who receive immunosuppressant agents and who have an increased prevalence of malignancy? Is T-cell blast formation down to 10 percent, 5 percent, 40 percent, or what?

Dr. Nahas: In patients receiving immunosuppressants, T-cell blast formation is depressed to about 40 or 50 percent of normal. In cancer the depression is related to the severity of the disease and probably ranges from 40 to 60 percent.

Dr. Hollister: I am wondering about those who develop cancer while receiving immunosuppressants.

Dr. Munson: I'm not sure those clinical studies have been done, but I can speak of comparable animal research. We have a dichotomy in our animal models. If we look at E. coli lipopolysaccharide stimulation of the B-lymphocytes, we can reduce the reactivity about 70 to 80 percent with very high doses of delta-9-THC. But the antibody response in these animals to a specific antigen is not reduced that much; it takes even higher doses to suppress the antibody response. This is what I was referring to earlier; I am not sure about this particular correlation, especially with B-cells. There is a little better relationship with the T-cells. Our T-cell studies with delta-9-THC have produced up to 70 percent inhibition of blastogenesis as well as prolongation of skin graft survival.

Dr. Hollister: Dr. Kabat pointed out the great reserve in most immune systems.

Dr. Kabat: There is another complicating factor in getting an answer of this type. We are just beginning to find out how many different kinds of T-cells there are. Suppose only 20 percent of the T-cells are sensitive to your procedure; you are up against a ceiling of 20 percent to begin with. If there were methods of separating those cells and working with only that population, then the range might go from one hundred percent to zero percent or from one hundred percent to ten percent. This is a problem. Of course, also related is the fact that not all of these cells will be in the same phase of the cell cycle. A cell may have to be in a certain stage to be triggered. Even if all the cells are sensitive, you are not likely to get the total population responding at any one time.

Dr. Harris: I would like to reemphasize the reserve in the system. If you inject ten to the eleventh leukemic cells into an animal, it's just about at the point where the animal dies; and if you have an agent that kills 90 percent of those cells, how many cells have you left? Ten to the tenth. I am sure that's the situation that pertains to the lymphocytes. We might reduce them by 70 percent, but we still have an enormous reserve capacity that would

protect against disease. I say this because we have had animals medicated with high doses of THC for long periods of time and they just don't get sick. The animals show very little in the way of overt pathology. If you had substantially reduced their host defense mechanisms, you would expect to see some consequences. But we are just not seeing those consequences.

I am not denying that there are potentially both public health and toxicological importance in the findings we are making, but as Dr. Kabat said, we have to think about the overall picutre. There are specificities in this which I think are important. For instance, we have to find out exactly which population of lymphocytes is effected by the THC. These are important issues.

I would also like to provide Dr. Nahas with some additional information about his hypothesis that THC is interacting at the membrane level and preventing thymidine from crossing the membrane. When we tested this hypothesis, THC didn't appear to affect the passage of thymidine across the membrane into the cytoplasm (Carchman, et al., in press). So at some point after thymidine gets into the cytoplasm, but before it gets into the DNA, the incorporation is affected. THC does not just act as a filter to block the passage of thymidine.

Dr. Mendelson: Dr. Nahas, you presented your data in terms of counts per minute. If you reported specific activity, for example, would you come up with a different finding? Is it possible, for example, that there are changes in the precursors in the cytoplasm? I know that this is a problem in some chemical work, but I don't know if anyone has the answer.

Dr. Kabat: I would also like to see the actual counts.

Dr. Mendelson: I am not only thinking of the actual counts, but also of specific activity.

Dr. Nahas: We have both actual counts and specific activity; we also have some data describing counts per minute (see Nahas, Chapter 6). But to establish a large number of samples in order to be significant you have to conduct a large number of experiments. To express this in terms of dose-response, you have to use a hundred percent counts per minute. You are pooling between five and six different experiments (which all have a parallel control), and there is a great variability from one experiment to another. So the only way of really making a clearly defined dose-response curve is to express it in percent of the parallel control culture.

Dr. Mendelson: But this is counts per minute and not specific activity. In other words, you have measured radioactivity, the incorporation of the tritiated compound, or the disappearance of radioactivity, but you have not reported specific activity?

Dr. Nahas: Yes, we have measured radioactivity after washing the cells in trichloracetic acid and methanol.

Dr. Mendelson: It isn't counts per minute in this specific activity.
Dr. Harris: Nobody has measured the pool sizes yet. We are in the process of doing that in an isolated tissue culture system. It may be that what is happening is an alteration of the various pool sizes. Does THC prevent the precursor from being incorporated into DNA? THC doesn't seem to prevent the precursor from getting across the cell membrane.
Dr. Rose: It is in DNA.
Dr. Harris: As I said before, we don't know what the pool sizes are. They might have altered a great deal. For instance, we measured the rate of transport of the tritiated thymidine into the cell, but maybe the rate of metabolism or egress of the thymidine is increased. There are many factors which can affect turnover and thus alter pool size.
Dr. Munson: I believe this technique measures thymidine inside and outside the cell. Thus it is measuring only transport, and not how much thymidine triphophate or the precursors of the nucleotides are being incorporated.
Dr. Harris: These processes get more complex every six or eight months when someone discovers another pathway in the system. But the fact of the matter is that we have enough data from a variety of laboratories and a variety of dosage levels of THC and other related cannabinoids under a variety of conditions with different concentrations of precursors to be able to say that in some way THC in certain cell systems is interfering with the incorporation of thymidine into DNA. We can also state that THC is interfering with incorporation of uridine into RNA and, perhaps somewhat earlier in the process, interfering with the incorporation of amino acids into protein.
Dr. Kabat: How about doing actual blast transformation measurements? How do these compare with your thymidine incorporation data? Do they run parallel?
Dr. Munson: Are you talking about morphologic changes?
Dr. Kabat: Yes, do you find that the cells which have the thymidine are actually the blast cells?
Dr. Nahas: Yes.
Dr. Hollister: One aspect of this phenomenon that hasn't been mentioned is the potential reversability of THC-impaired thymidine incorporation. There is evidence that the impairment can be fairly rapidly reversed. Would someone like to comment on that?
Dr. Nahas: We have shown that it is almost entirely reversible. If you incubate lymphocytes with THC, without stimulating with PHA for 24 hours, and then if the lymphocytes are washed twice with culture medium and stimulated with PHA, after three days the incorporation of thymidine is similar to that of the control.

Dr. Munson: Are you referring to where you add the cannabinoid *in vitro.*
Dr. Nahas: Yes.
Dr. Hollister: I believe Peterson demonstrated that if you stop for a rather short period the phenomenon is reversible (Peterson et al., 1974).
Dr. Munson: Yes. That wasn't with lymphocytes as I recall, but with granulocytes.
Dr. Pollin: How about phagocytosis?
Dr. Harris: We could never show an interference with that particular system. THC doesn't affect the phagocytes, at least not in the liver.
Dr. Rose: Do I understand correctly that people are saying there is indeed a pharmacological effect observed in various laboratories that THC inhibits the incorporation of thymidine in DNA, but that earlier work reporting an abnormality in the lymphocytes circulating in smokers compared to nonsmokers has not been replicated.
Dr. Nahas: Silverstein (Silverstein and Lessin, 1974) reported that chronic marijuana users had a positive dinitrochlorobenzene (DNCB) test, but he did not report on mixed lymphocyte cultures. Apparently there isn't much contradiction so far in the literature; maybe it will come out later.
Dr. Cole: How can something prolong the life of skin grafts and yet cure cancer?
Dr. Harris: We never claimed that we are curing cancer. All we say is that in animals with certain solid tumors, the tumors grow slower and the animals live longer if they are medicated with THC. With delta-9-THC there is some differential between the biochemical events of normal cells and those events in tumor cells. In other words, you can interfere with the uptake of thymidine into DNA of tumor cells at concentrations of delta-9-THC considerably lower than the levels which are necessary to interfere with the incorporation of thymidine into normal bone marrow cells Surprisingly enough, delta-8-THC does not show that differential, though it shows some anti-tumor activity.
Dr. Cole: Do you think it is a direct effect on the tumor rather than on the T-cells?
Dr. Harris: Yes. But the same mechanisms might be involved.
Dr. Dornbush: On another topic Dr. Munson, I wish to take issue with one of the statements you made at the beginning of your talk — about the usefulness of the Costa Rican study in producing the type of data you might want. To my knowledge, the Costa Rican study is using samples very similar to those used in the Jamaica study and in Greece. The Greek sample was much like a migrant farm worker sample. I was wondering; would you use a migrant farm worker sample to assess incidence of disease in white, middle-class Americans?

Dr. Munson: The Costa Rican study is the one with which I am most familiar. The utility of that particular study is its measurement of a whole group of different parameters that would be responsive to host defense suppression. If they obtained those measurements over a number of years, and indeed there was a suppression of host defenses in one way or another, disease would show up, no matter what population was being studied. We are all continuously subjected to insults, both pathogenic and carcinogenic ones. I think host defense suppression would show up, but it would take a long time.

Dr. Harris: The problem is that since the Costa Rican population is subjected to so much insult at all times, their immune systems are probably working at maximum. Therefore, the ideal study population is perhaps the white, middle-class or upper-middle-class American population, which has not been subjected to so many insults. They might be more susceptible. I think Dr. Dornbush has a valid criticism.

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Chapter 8

Background Paper On Testosterone and Marijuana

Robert M. Rose

Testosterone (17-beta-hydroxy-4-androstene-3-one) is a potent, naturally secreted steroid. In the "average" 70 kg man, approximately 7 mg per day is produced, primarily by direct secretion from the testis. In females, about one-twentieth of this amount is produced, primarily via conversion from precursors secreted by the adrenal cortex.

The normal range of plasma testosterone in the adult male has been the subject of some controversy. Most authors give the range from 400 to 1200 ng/100ml; however, there are numerous reports in the literature of values as low as 275 or 300 in presumably normal males, as well as values as high as 1400 to 1500 in males without any detectable abnormality. Consequently, the variability of normal range is open for discussion; but it is very large.

Most of the testosterone in plasma is carried bound to a globulin, referred to as sex steroid binding globulin. This is keyed to the 17-beta-hydroxy structure, and thus binds other steroids with the same configuration, such as estradiol. Approximately 96 to 98 percent of all testosterone in plasma is reported to be in the bound state, leaving only 2 to 4 percent free. It is presumably this free fraction that is available for interaction with various target tissues. The percent of bound testosterone is higher in women, infants, and children, as well as in adult men who are in the sixth and seventh decades of life.

Testosterone levels are reported to be either identical in newborn males and females, or slightly higher in the male. Males do not significantly differ from females until the age of puberty when there is a very rapid, dramatic rise in plasma testosterone (Lee et al., 1974). Then there is often a two- to threefold rise observed over as short a period as 18 months. A gradual decline in plasma testosterone levels occurs as a function of age: from age 60 to 70, a decline of about 25 percent from levels at age 20 to 40 has

been reported; at age 80 to 90, the reduction is to 60 percent of levels at age 20 to 40 (Vermeulen et al., 1972).

A significant diurnal variation in plasma testosterone is now well documented (Rose et al., 1972a). An approximately 40 percent fall in plasma testosterone levels has been observed during the day, which is much less than the 92 percent fall observed in plasma cortisol. Similar to cortisol, testosterone has been observed to be secreted episodically, although the magnitude and duration of the episodic bursts are not as large as those of plasma cortisol. Individuals differ as to the variation they exhibit on a daily basis. Some individuals whose plasma is drawn at the same time of day, early in the morning to minimize diurnal effects, show relatively little variation over time, i.e., coefficients of variation of 5 to 8 percent. Other individuals show greater variability, with coefficients of variation of 20 to 25 percent over time (Parks et al., 1974). Consequently, variability is a methodological problem which must be attended to in longitudinal studies.

Testosterone was initially assumed to be under the control of luteinizing hormone (LH) secretion by the pituitary, often referred to in males as interstitial cell stimulating hormone (ICSH). However, there are inconsistencies in this model, which is presumably similar to that of ACTH-cortisol axis. In support of the model, LH levels rise dramatically after castration in both males and females; LH increases during puberty in males in association with increased testosterone secretion; and the administration of exogenous testosterone will suppress LH secretion. However, in both monkeys and man, LH fails to demonstrate the diurnal variation of testosterone levels; and during periods of stress, when testosterone levels are clearly diminished, there is often no significant drop in LH levels. Similarly, various drugs known to inhibit testosterone secretion, presumably by hypothalamic action, fail to suppress LH.

Measurement of Testosterone

Over the past five to eight years competitive protein binding techniques or radioimmunoassay techniques have been increasingly used to assay most steroids circulating in plasma. In principle, these involve the displacement of labeled testosterone from a protein or antibody by the testosterone in the plasma that is being studied. The more of the radio-labeled steroid that is displaced, the greater is the concentration of testosterone in the unknown plasma sample. These methods are generally very sensitive, but have varying degrees of specificity.

Initially, most assays employed pregnancy plasma obtained from women in the third trimester which had an increased concentration of sex steroid binding globulin. Many laboratories employed varying degrees of chromatographic separation of plasma testosterone from other steroids

which cross-react with the sex steroid binding globulin (i.e., 17-beta-hydroxy steroids). With the advent of more specific antibodies directed against a testosterone-bovine serum albumin conjugate, the need has diminished for more elaborate chromatographic steps to separate out cross-reacting steroids. However, most antibodies are currently directed against a conjugate of the steroid at the 3 position. Although the resulting antibody is much more specific than sex steroid binding globulin, there usually remains a varying degree of cross-reactivity with dihydrotestosterone (e.g. from 30 to 100 percent).

A major difficulty with sex steroid binding globulin in the measurement of plasma testosterone in males is that it measures not only testosterone but also dihydrotestosterone, as well as various androstane- and androstenediols, and thus could overestimate low values of plasma testosterone by as much as 30 to 50 percent (Murphy, 1971).

Variables Influencing Testosterone Secretion

There are relatively few stimuli known to provoke increased testosterone secretion under normal circumstances. The major naturally occurring stimuli is, of course, the onset of puberty in males. In monkeys, it has been shown that access to sexually receptive females (Rose et al., 1972b), as well as victorious outcome in agonistic or aggressive encounters (Rose et al., 1975), provokes increased testosterone secretion. In man, it is unclear whether or not anticipation of sexual behavior, or even engaging in sexual behavior, provokes an increase in plasma testosterone (Rose et al., 1974).

The fact that stress inhibits testosterone secretion has been well established. This was first observed in response to general surgery, and one recent paper documented a very early LH decrease, which did not persist, while testosterone remained low for many hours (Carstensen et al., 1973). We have also shown, along with other investigators, that testosterone is inhibited in response to psychological stress (Rose et al., 1969), and that specifically it has been documented to decrease in individuals participating in Officer Candidate School (Kreuz et al., 1972), and in monkeys following defeat (Rose, 1972b).

Effects of Drugs on Testosterone

It has been established for some time that phenothiazines block LH secretion in the rat (de Wied, 1967); and indeed chlorpromazine administration is a standard technique for studying brain neuroendocrine mechanisms in lower animals. However, until recent research by Beaumont and colleagues, it has not been known whether in man the phenothiazines induce a parallel decrease in LH secretion or have an effect on testosterone secretion (Beaumont et al., 1974). In their study, individuals being treated

with a variety of different phenothiazines, including thioridizine, trifluoperazine, and chlorpromazine, had their plasma testosterone levels measured during three conditions: when receiving the original phenothiazine treatment, when the phenothiazine was replaced by placebo, and when phenothiazines were readministered after the placebo condition. There was a clear-cut increase in plasma testosterone levels when the phenothiazines were discontinued and the patients were given placebo; most patients showed a drop in testosterone when phenothiazine therapy was reinstated. Individuals who tended to have higher levels while initially receiving phenothiazine showed less of a rise when given placebo. However, it should be noted that it took approximately 4 to 6 months after reinstitution of phenothiazine therapy before plasma testosterone levels fell back to the original phenothiazine treatment levels which pertained before the placebo condition.

In certain animals opiates are known to suppress testosterone production. Morphine pellets containing 75 mg of morphine base administered to male rats have been reported to cause a profound drop in plasma testosterone levels as well as a significant decrease in the weight of seminal vesicles and prostate (Cicero et al., 1974). In man, there are conflicting reports regarding the effects of opiates on plasma testosterone. Azizi et al., (1973) found a significant decrease in serum testosterone levels in male heroin and methadone addicts. Employing a very specific radioimmunoassay technique, they did not find a significant decrease in LH levels, but there was a tendency for LH levels to be lower than controls. Cushman (1973) failed to replicate these findings. In his study there was no statistically significant difference between the experimental and the control group; however, 21 percent of the addicts had levels below 300 mg/100 ml, and four of these had levels less than 200 mg/100 ml. Cushman's techniques, as well as those of Azizi, could be criticized for lack of multiple samples; also in Cushman's study, controls for diurnal variation were possibly inadequate since samples were apparently drawn during different times of the day. In addition, the technique employed by Cushman involved competitive protein binding with a relatively inadequate cleanup step which included only one thin-layer chromatographic separation. Jack Mendelson and Roger Meyer have communicated some preliminary results, indicating that during treatment involving self-administration of heroin, there is a significant decrease in plasma testosterone levels.

Turning from these drugs to marijuana, there are a few preliminary studies regarding the effects on testosterone metabolism of administering marijuana or specific active marijuana components. Ling et al., (1973) reported no significant effect of daily administration of delta-9-THC (4 or 16 mg/kg for four days) on rat accessory sex organs and testes. However,

this technique is not sensitive enough to detect a relatively small 20 to 40 percent decrease in testosterone secretion by the testis. For example, in the report on the decrease in testosterone levels of the animals administered morphine, there was no significant difference in the testis weight of the experimental and placebo groups. Kolodny and associates (1974) reported significantly decreased testosterone levels in individuals who were frequent marijuana users: there were significantly lower levels in individuals who smoked 10 or more marijuana cigarettes per week compared to those who smoked 5 to 9 marijuana cigarettes per week. Both groups were significantly lower than controls. Although urine screening was not done, there were no self-reports of excessive alcohol intake, and presumably the individuals were not on medication. Kolodny and colleagues employed a specific radioimmunoassay technique and control for diurnal variation by limiting the sample collection from 7:30 to 9:00 a.m., but they drew only two samples, approximately one month apart. Two samples are probably not sufficient to give a good representation of an individual's usual testosterone level. Depending on individual variability, three to five samples are usually required to establish the individual's mean. Nevertheless, this criticism would not necessarily explain why the frequent marijuana users would have a lower mean value than the controls, even if only two samples were employed, unless possibly there was greater variability in the marijuana users, with a greater amplitude of swings. We cannot answer this question.

Mendelson and collaborators (1974) were unable to replicate the results of Kolodny. They did not find significantly lower testosterone levels in individuals who had a history of frequent marijuana use. Furthermore, they were unable to show a decrement in testosterone levels associated with marijuana smoking in a controlled laboratory setting. Blood samples were drawn daily for 31 days, always in the morning to control for diurnal variation. The method of testosterone assay they used was somewhat less specific than that employed by Kolodny. Presumably the major nonspecific substance contributing to the plasma testosterone level would be dihydrotestosterone. Mendelson and colleagues reported 30 percent cross-reactivity, which could possibly enhance the values by about 30 ng/100 ml, assuming a concentration of dihydrotestosterone as large as 100 ng/100 ml. It is difficult to conceive, however, how this relative nonspecificity would obscure any drop in testosterone associated with smoking marijuana, since each individual acted as his own control. One would have to posit that the marijuana shifts the percent conversion of testosterone to dihydrotestosterone, but there is no evidence to support this.

General Comments

Since both the Kolodny and Mendelson reports are very recent, no

replication of either has yet appeared in print. Therefore, it is difficult to conclude definitively whether or not marijuana depresses testosterone levels. It is possible that marijuana may have both an acute and a chronic effect; Dr. Kolodny has data to support the notion of an acute effect, which may last for less than an hour or so. If this is so, it is possible that the one-a-day blood sample drawn by the Mendelon group would be insufficient to pick up any acute but transient drop associated with smoking marijuana in a controlled, experimental setting. However, it does not explain why they failed to show lower levels in the group of individuals who reported chronic use of marijuana.

At the end of the Kolodny paper there is discussion about the possible hazards associated with marijuana use. I believe some comment on these is relevant. There is mention of the possible hazard to the developing fetus of a mother smoking marijuana, and thus inhibiting the male fetus's secretion of testosterone.

A great deal of animal research indicates that appropriate levels of testosterone during crucial periods of brain development are essential for normal appearance in adulthood of those behaviors associated with male sexual and aggressive behavior. This has been documented in various rodents as well as in rhesus monkeys. Whether this is so in humans is not clear, but the data of Money and Ehrhardt (1972), working with girls with adrenogenital syndrome, suggest that excess testosterone present during fetal development may affect future behavior. There is no animal experimentation available that directly deals with effects of reducing but not abolishing testosterone in the developing fetus. All the studies so far have employed techniques for essentially eliminating all testosterone and then observing effects. Investigators have also studied the physiological and behavioral effects of administering small amounts of testosterone to the developing female fetus. It can be shown that with very small amounts of testosterone a female rat will develop neonatal sterilization, fail to ovulate in adulthood, and show increased male mounting behavior. From these studies one might conclude that there need not be a great deal of testosterone present to adequately masculinize the male fetus. However, direct data on this question are not yet available.

The other question relates to the relative apathy, decreased sexual libido or drive in chronic marijuana smokers observed by Kolodny. One of the major difficulties in psychoendocrine research in humans has been the inability to replicate the relationship between testosterone and sexual drive or aggressiveness in man as compared to the relationship observed in animals. Male monkeys who are castrated show a great variability in their decline in sexual functions, some animals showing a significant drop in their rate of mounting in as short a time as four or five weeks, while other

animals take as long as two years to show a significant decrement. It has been shown in animals that the amount of past experience is a major intervening variable in the relationship between lower testosterone levels and sexual activity. Namely, animals who have greater sexual experience have a much slower decline following castration than those animals who are more naive.

We are really not clear as to what role testosterone plays in assertiveness or aggressiveness in nonhuman primates or man. For example, there is great variability in plasma testosterone levels in males with Klinefelter's syndrome, even though their average level is significantly lower than normal. Among these individuals, there are many who have a history of criminal behavior, but there are no data to suggest that individuals who have higher levels of testosterone are more aggressive than those who have lower levels.

In summary, after the age of puberty testosterone in man may act more in a permissive way than in actually regulating sexual or aggressive behavior. Consequently, it would be necessary to have a great decrement in levels before a significant reduction of sexual or aggressive behavior is evoked. Nevertheless, these areas are currently the subject of important investigations in psychoendocrinology.

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Chapter 9

Research Issues in the Study of Marijuana and Male Reproductive Physiology in Humans

Robert C. Kolodny

The study of androgens and their correlations with a wide variety of pharmacologic agents, pathophysiologic states, and various forms of behavior has become a popular investigative focus. Relatively recent methodologic developments, such as the advent of radioimmunoassay techniques, have allowed realistic testing of many previously-posed hypotheses in these and other areas to provide a substantial growth of information concerning the role of androgens in health and disease. Despite this, there are numerous problems presently confounding this field, so that our understanding is often incomplete.

Before detailing the specific problems involved in research to determine the effects of marijuana upon the hypothalamic-pituitary-testicular axis in man, it may be helpful to outline briefly some facts about androgen production and metabolism. In a normal adult male, testosterone is produced primarily by the Leydig cells of the testes, with less than 5 percent ordinarily contributed by the adrenal cortex. The average testosterone production rate for normal adult males is 6 to 8 mg/day. Circulating testosterone is bound to a sex-steroid binding globulin which also binds other steroids with a 17-beta hydroxy configuration; dihydrotestosterone, which is present in men in concentrations approximately one-tenth that of testosterone, is bound three times more strongly to this protein (Murphy, 1971). Dihydrotestosterone, delta-4-androstenedione, and androsterone are other androgens besides testosterone that may exert biologic effects; the extratesticular production of these androgens is considerably greater than for testosterone. The exact nature of biologic responses to these different androgens is not at present well understood, but is not necessarily equivalent; for example, dihydrotestosterone administration does not

restore male mating behavior to castrated male rats, whereas testosterone does.

Testosterone and its metabolites are excreted almost entirely in the urine. However, the use of 17-ketosteroid excretion as an indication of androgen status is inaccurate, since less than one-third of the 17-ketosteroid total is testicular in origin. In addition, there are non-androgenic components of the urinary 17-ketosteroids, so that this measurement is now obsolete as a means of evaluating androgen levels. Currently, the majority of investigators have also discarded more specific measurement of urinary testosterone in favor of directly assaying circulating hormone levels. This change has occurred largely because of the convenience of sampling, the elimination of the problem of incomplete sample collection, and the theoretical advantages of direct assessment of plasma levels as closer approximations of tissue concentrations of the hormone. However, it should be recognized that urinary testosterone measurements on carefully collected 24-hour samples may provide a more accurate indication of the daily production rate than blood measurements. Testosterone production is directly controlled by the action of luteinizing hormone, which is produced by the anterior pituitary gland. There is mounting evidence that follicle-stimulating hormone, also produced in the anterior pituitary gland, may exert an indirect action on testicular steroidogenesis, although its primary importance is in regulating spermatogenesis (Paulsen, 1974). A classic negative-feedback mechanism appears to operate in the hypothalamic-pituitary-Leydig cell axis, with low circulating testosterone levels resulting in increased luteinizing hormone output from the pituitary under the stimulation of hypothalamic gonadotropin-releasing factor. Conversely, high circulating testosterone levels lead to decreases in hypothalamic and pituitary activity, with subsequent drops in gonadotropin production and release. The regulatory mechanisms for spermatogenesis are not as well understood and fall beyond the scope of this paper. Clinically, steroidogenesis and spermatogenesis may be viewed as separate but partially interdependent systems. Frequently, men may have disrupted spermatogenesis with normal testosterone production; less often, men with partial androgen deficiencies may have relatively normal spermatogenesis. Total testicular androgen deficiency is not compatible with completely normal spermatogenesis.

Perhaps obvious, but nevertheless necessary to a discussion of research methodologies concerning androgens, is consideration of the accuracy of the assay system used to measure testosterone and other related hormones. The recent availability of radioimmunoassay procedures for the measurement of testosterone, dihydrotestosterone, and androstenedione has led to a marked improvement in precision compared to earlier techniques, with the added advantage of requiring the use of relatively small

volumes of blood for these hormonal determinations. It is important to note that radioimmunoassays are not without limitations, however, which often derive from practical immunologic principles. For example, attention needs to be directed to the specificity of the anti-testosterone antibody utilized in the assay system. When such an antibody cross-reacts strongly with other androgens, the measured result is not testosterone alone but testosterone plus other related hormones. In some situations, particularly in a clinical context, this information may be adequate. However, in research design when the effects of the experimental variable(s) on non-testosterone androgens are not known, it would appear potentially erroneous to draw conclusions from such information. For example, if plasma testosterone was depressed in response to an experimental variable while plasma dihydrotestosterone was increased under the same conditions, an assay system with significant antibody cross-reaction between testosterone and dihydrotestosterone might show no significant change in measured plasma "testosterone." Obviously, such an incorrect conclusion would be highly misleading. This problem can be avoided by utilizing separation procedures such as column or paper chromatography prior to the radioimmunoassay itself so that the cross-reactivity between testosterone and other hormones is reduced to less than one percent. Unfortunately, these separation procedures are usually tedious and therefore effectively limit assay size under ordinary working conditions.

The problems mentioned in the previous two paragraphs make it difficult to interpret the recently reported data of Mendelson and his colleagues (1974). Their well-designed studies of the effects of daily marijuana smoking over a three-week period on plasma testosterone concentrations in young healthy men utilized a radioimmunoassay system with a 30.5 percent cross-reactivity between testosterone and dihydrotestosterone, without a prior steroid separation. Furthermore, shortly after the publication of their report, the supplier of their antibodies and methods, Micromedic Diagnostics, Inc., wrote to its customers stating that deliveries of these materials were being suspended because the results of their radioimmunoassay "may in certain situations be higher thn the endogenous level of testosterone," for unknown reasons (Carter, 1974).

Earlier in 1974, the Reproductive Biology Research Foundation had tested this testosterone assay system using the materials and specific instructions of Micromedic Diagnostics, Inc., only to find that this assay overestimated by 30 to 55 percent the results obtained by a competitive protein binding method (Mayes & Nugent, 1968) and a radioimmunoassay method (Kolodny et al., in press). It is unfortunate that this problem occurred, because the design of Mendelson et al.'s study was in other respects good.

Although a more detailed discussion of androgen assay methods is beyond the scope of this paper, it is relevant to point out that there is often a great difference between one laboratory and another in the actual values reported for a testosterone assay. The experienced endocrinologist is certainly familiar with this problem in other hormone measurements as well, but it does serve to emphasize the necessity for careful assessment of each laboratory's normal male and female range and how these figures were derived.

Because there are a number of factors known to alter circulating testosterone levels, including diurnal variation (Resko & Eik-Nes, 1966; Nieschlag & Ismail, 1969), thyroid disease (Olivo et al., 1970), hepatic disease (Kent et al., 1973), renal disease (Chen et al., 1970), adrenal disease (Saez et al., 1971), a variety of drugs (Azizi et al., 1973; Kent et al., 1973a; Mendelson & Mello, 1974), surgery (Aona et al., 1972), exercise (Sutton et al., 1973), and psychological stress (Kreuz et al., 1972), it is optimal to standardize research protocols in a fashion aimed at eliminating or minimizing these variables in terms of each particular study. Certainly the studies of androgens and marijuana in humans have attempted to control these factors.

With an acceptable assay method and studies designed to eliminate the major variables known to alter testosterone levels, certain types of data may be examined to describe the relationship between marijuana, its various components, and the hypothalamic-pituitary-testicular axis. Theoretically, and usually practically, the chronic endocrine effects of a drug are more readily demonstrable than the acute effects. Our research team therefore began with a study of the chronic effects of marijuana. This early work (Kolodny et al., 1974b) was carried out with non-hospitalized volunteers who smoked their own marijuana, presenting the obvious problem of unknown drug purity and potency. In addition, a separate age-matched control group of men who had never smoked marijuana was used, which posed problems of different types: there might have been other differences between the two groups that, independent of marijuana use, might alter androgen status; and the reliability of the drug use histories was not certain. It was certainly apparent from the outset that this was not as ideal as a design using each subject as his own control, but it was a starting point. The reliability of drug use histories might be clarified by stating that none of the subjects accepted in this study had knowledge of the fact that it was a study of marijuana use, nor did they know what other criteria were being used. Of the original group of thirty men who were using marijuana at least four times a week for a minimum of six months and who met all study criteria (including no other drug use in the past six months and alcohol intake of less than 14 ounces per week), ten began to use other drugs before the

conclusion of the study. These ten men reported their drug use without reservation; their study participation continued, but they were not included as part of the report since in fact they did not meet all the study criteria. This evidence indicates that there was no obvious reason for subjects to be deceptive in reporting their drug use patterns.

When the laboratory results were uncoded and analyzed at the conclusion of the investigation, it was quite clear that *in this relatively small group of men* the chronic marijuana users had lower testosterone levels than men who did not use marijuana. Further, abstention from marijuana use produced prompt increases in plasma testosterone in a number of subjects (Kolodny et al., 1974b). We therefore decided to continue our studies in this area, working under more controlled conditions. The next studies, done in collaboration with the UCLA Marihuana Research Project, permitted us to work with drugs of known purity and potency in a situation where each subject served as his own control. Subjects were hospitalized for 94 days on a special research ward where extraordinary efforts were made to insure that extraneous drugs were not available. Subjects were requested not to use marijuana for the week prior to entering the study (cooperation on this point was variable), and the first eleven days of confinement were without marijuana use. Studies of the effect of acute marijuana smoking (using either one or three standardized marijuana cigarettes) on plasma testosterone over a three-hour period were performed with blood samples taken in a manner similar to obtaining a glucose tolerance test. These acute studies were done on each individual at the beginning of his marijuana smoking period and after he had been smoking on a daily basis for approximately eight weeks. A depression of plasma testosterone within three hours in response to smoking a single marijuana cigarette was seen in these subjects (Kolodny et al., in press). Weekly blood samples were also obtained during this entire 94-day study, which demonstrated significantly lower testosterone levels after 5 weeks of daily marijuana smoking than during the initial period of abstention from marijuana use (Kolodny et al., unpublished).

It is particularly necessary to keep in mind that research in marijuana is not totally separate from other medical research. First, careful attention must be given not only to the protocol of a study but to its data analysis, which is really an integral part of research design. For example, when investigation is made of a hormonal effect of a drug, the hormone in question can respond in one of three ways: it can increase, it can decrease, or it can remain the same. Thus, statistical tests used to analyze such data should employ two-tailed probability tables, when appropriate, rather than one-tailed tables. As another example, to demonstrate correlations between hormones or between a hormone and varying drug doses, it may be necessary

to utilize logarithmic transformations to satisfy the assumption of homogeneity of variance, rather than analyzing all data on a linear scale.

Second, measurement of a single hormone, such as testosterone, provides far less information than simultaneous measurement of the entire regulatory axis, to the extent that is possible. Blood levels of luteinizing hormone and follicle-stimulating hormone are easily determined. Unfortunately, tests to measure hypothalamic gonadotropin releasing factor(s) are still in development.

Third, parallels in one endocrine system may provide a model for effects seen in another system. For example, a single dose of prednisone will briefly alter the hypothalamic-pituitary-adrenal axis, but it usually requires more than two or three weeks of daily administration for this system to become truly suppressed. Even while daily prednisone administration continues, exogenous adrenocorticotropin stimulation produces a marked increase in adrenocortical function within two or three days. In addition to the marijuana-testosterone interaction appearing temporally similar, stimulation with human chorionic gonadotropin promptly increases testosterone output, showing that the integrity of the Leydig cell population is not grossly impaired (Kolodny et al., 1974b).

More sophisticated means of hormonal assessment are available to further clarify the effects of marijuana on testosterone. Use of synthetic gonadotropin releasing factor and clomiphene citrate may be of assistance in more accurately describing hypothalamic and pituitary effects of marijuana. Delineation of 24-hour testosterone production rates and metabolic breakdown patterns during marijuana use may also provide important information. The measurement of other androgens in response to marijuana and the discovery of which chemical components of marijuana are biologically active in exerting endocrine effects would also provide better understanding of this controversy.

Animal studies, though of course not freely generalizable to the human model, have documented decreased testosterone in rats exposed to marijuana smoke (Rosenkrantz and Braude, in press), disruption of spermatogenesis, and Leydig cell regression in mice during daily administration of high doses of cannabis extract (Dixit et al., 1974), depressed luteinizing hormone levels and depleted pituitary follicle stimulating hormone reserve in rats given delta-9-THC (Collu et al., 1974), and deterioration of sexual performance in male rats given high doses of delta-9-THC (Merari et al., 1973). The similarity of these findings to the currently available evidence of marijuana's effects on testosterone in the human are striking.

What does the evidence of lowered testosterone in men mean? Here the discussion departs from scientific fact and takes a strictly speculative direction.

The decreases in circulating testosterone levels that have been observed to date in association with chronic, intensive marijuana use have generally not resulted in subnormal testosterone concentrations (Kolodny et al., 1974b; Kolodny et al., in press). For otherwise healthy individuals there is presumably a large "safety zone" in terms of necessary levels of testosterone before actual evidence of a hormone deficiency might occur. Thus it would be reasonable to assume that specific biologic consequences of a sustained depression in circulating testosterone would be seen mainly in men with subnormal hormone levels. Such biologic consequences in the adult male might include changes in any of the peripheral actions of testosterone, such as impaired nitrogen incorporation into protein with resultant diminished muscle mass, altered calcium and phosphorous retention in bone, impaired erythropoiesis, diminished libido, decreased ejaculatory volume, or impotence (Paulsen, 1974). When testosterone production decreases markedly or even ceases in the adult male, secondary sex characteristics generally regress very slowly, since the adrenal androgen contribution is often adequate for many years to maintain these characteristics once they have developed (Paulsen, 1974). Thus, it would not be expected that changes in secondary sex characteristics would commonly be observed.

A different type of effect might be seen in the man with mild oligospermia, where a decrease in testosterone or a partial suppression of follicle-stimulating hormone levels might cause further disruption of spermatogenesis. It is possible that this might result in infertility, but this area requires careful study that has not as yet been done. A major problem in the investigation of drug effects on spermatogenesis is the fact that it takes approximately 64 days for a single generation of sperm cells to fully mature; at any time there are numerous generations of sperm cells at varying stages of the maturation process within the testes (Steinberger & Steinberger, 1972). Therefore it may require periods of six months or longer to evaluate directly drug effects on spermatogenesis, and additional time of similar length may be required to evaluate the effects of drug withdrawal. There are also ethical problems in such studies that require consideration, particularly in regard to performing testicular biopsies.

The possible biologic consequences of suppression of the hypothalamic-pituitary-testicular axis in pre-pubertal and pubertal males are of great significance. Delays or limitations of normal skeletal growth patterns might be a result of chronic testosterone depression by marijuana, since the so-called pubertal growth spurt is known to be primarily dependent on increasing androgen production (Blizzard et al., 1974). The development of male secondary sex characteristics would also be delayed by chronic suppression of the hypothalamic-pituitary-testicular axis, with potentially adverse psychosocial and sexual maturation of individuals so

affected. Instead of conjecture, this subject needs careful and prompt investigation, since it is well known that junior high school and even elementary school students are using marijuana. Animal studies of the effects of marijuana administered chronically to pre-pubertal males might provide clarification of this important issue.

More speculative than the preceding discussion is the possibility that frequent intensive marijuana use during critical stages of pregnancy might result in disruption of normal sexual differentiation patterns of the male embryo (Kolodny et al., 1974b). The concept formulated by Jost that male phenotypic sexual differentiation is dependent upon adequate androgen stimulation (Jost, 1972) is firmly supported by a wide variety of experimental evidence (Money & Ehrhardt, 1972). Siiteri and Wilson (1974) have clearly documented that by 8 to 10 weeks of human intrauterine development, testosterone synthesis is demonstrable within the embryonic testis. This time period corresponds to the critical time of male differentiation of the urogenital tract (Money & Ehrhardt, 1972; Siiteri & Wilson, 1974). These researchers also concluded that it is likely that the effective testicular androgen synthesized by the testis at the time of onset of male sexual differentiation is testosterone itself (Siiteri & Wilson, 1974). Since delta-9-THC is known to cross the placental barrier (Idanpaan-Heikkila et al., 1969; Harbison & Mantilla-Plata, 1972), the developing genetic male embryo may be at risk for androgen suppression, with subsequent defects of phenotypic development. Admittedly, high maternal intake of marijuana might be required to produce such effects, but there is also a possibility that testicular or hypothalamic tissue might be more sensitive to drug effects during this time than during adulthood. Until this area is better understood, it would appear to be judicious for pregnant women to avoid the use of marijuana or hashish oil.

No controlled studies of the sexual effects of marijuana use have been reported to date. Claims and counter-claims have been vociferously pronounced naming marijuana as a sexual enhancer, if not an aphrodisiac, and purporting that marijuana use has dire sexual consequences. To understand the issue adequately, we must realize that two separate components — biologic and behavioral — are interacting in what we recognize as sexual. Certainly, in parallel to the man with borderline fertility where depression of the hypothalamic-pituitary-testicular axis may cause greater difficulty in conception, a man with marginal sexual functioning may experience decreased libido and/or potency problems if his testosterone levels are significantly lowered as a result of marijuana use. It would appear that such a problem would be relatively infrequent, however, since men with subnormal testosterone levels may have no erective difficulty. Both libido and sexual functioning have major learned components that usually supersede

biologic factors; but understanding the interaction between these two forces is far from complete at this time.

It is also important to note the role of set and setting in a sexual situation as well as in any instance of drug use. This is a familiar area to many and will not be discussed here except to say that the expectations an individual has, the partner he or she is with, the general mood, the level of anxiety, previous sexual experience, and numerous other factors introduce a large number of variables. In addition, acute marijuana use may produce perceptual changes in time and tactile sensations that may be interpreted by the user as enhancing, detracting, or not altering the sexual experience. When the sedative effects of high doses of marijuana are predominating, it is quite possible that acute and transient episodes of sexual dysfunction might occur.

Behavioral consequences of depressed testosterone may be more marked than the biologic; in particular, studies demonstrating a strong correlation between testosterone and aggressive behavior (Persky et al., 1971; Rose et al., 1971) might be interpreted to explain reports of greater passivity or lack of motivation in chronic, frequent marijuana users. This hypothesis requires careful testing by both biologic and behavioral disciplines: ideally, it would involve a prospective study design in which those who evaluated behavior patterns were unaware of drug use in the test and control groups. The problem of matching the test and control group subjects would be understandably difficult, but nevertheless important if it could be closely done.

In conclusion, it is important to recognize that the issues raised in this discussion require further study. Recent reports of striking variability in plasma testosterone levels in adult men indicate a need for more careful evaluation of single daily testosterone measurements in an individual, even when they are obtained on multiple occasions (Smith et al., 1974). Although our own data are strikingly consistent with data obtained from animal studies and closely parallel other well-documented endocrine models, it appears necessary to broaden the scope of such studies to include other age groups and larger numbers of subjects, and to study populations here and abroad that have had truly chronic and frequent experience with marijuana.

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Chapter 10

Effects of Marijuana on Plasma Testosterone

Jack H. Mendelson, John Kuehnle,
James Ellingboe, and Thomas F. Babor

During the past decade the use of marijuana appears to have superceded alcohol as the preferred recreational drug of young adults. A folklore has developed about the effects of marijuana on sexual behavior and function which emphasizes, in part, some of the traditional conflicts between youth and the older generation. Marijuana, the drug of youth, purportedly stimulates sexual desire and facilitates performance, in contrast to the well-known inhibiting actions of ethanol. Anecdotal reports of heightened sexuality exist for both male and female marijuana users — a phenomenon which may reflect the abhorrence of male chauvinism by liberal young adults.

Adverse consequences of marijuana use on sexuality have also been reported. Harmon and Aliapoulious (1972) cited case reports of three males who developed gynecomastia, that seemed to be related to heavy dosage, long-term marijuana use. This finding prompted Kolodny and his associates to investigate plasma testosterone levels in 20 adult males who used marijuana frequently. The results of the studies indicated that plasma testosterone levels were significantly lower in marijuana users than in young adults of the same age who had not used the drug (Kolodny et al., 1974).

The data obtained by Kolodny and his colleagues have much broader implications than just the consequence of marijuana effects on testosterone levels per se and possible concomitant alterations in male sexual function and behavior. The effects of testosterone on crucial organ target cells, including those of the central nervous system, may have important regulatory determinants on sexual growth and differentiation. Thus, any possible deleterious effect of marijuana on androgen function should be carefully investigated with the most rigorous and accurate methods currently available.

Research Design

There are a number of important variables that may effect androgen homeostasis in humans. First, it is well known that plasma testosterone levels may vary widely in healthy adult males (West et al., 1973). As might be expected, there is a diurnal variation in testosterone secretion, but it is important to point out that diurnal cycle activity can only account for approximately 20 percent of the variance in normal adult plasma testosterone levels (De Lacerda et al., 1973). Since heterogeneity in variance is common, one or two determinations of plasma testosterone levels in males segregated into groups (e.g. marijuana users vs. non-users) may generate spurious data. Assessment of possible drug-induced change of plasma testosterone levels in males requires a research design wherein each subject serves as his own control and multiple plasma samples are analyzed.

Second, since a variety of drugs including ethanol (Mendelson and Mello, 1974) and opiates (Mendelson et al., 1975) has been reported to affect plasma testosterone levels, it is important to determine the effects of any specific drug (e.g. marijuana) on plasma testosterone levels under conditions where multiple drug use is not possible. This may be especially important since many young adults who use large amounts of one drug may frequently be multiple drug users. One of the most commonly used drugs by all members of society is ethyl alcohol, and the significance of ethyl alcohol use on plasma testosterone levels in individuals who also may use marijuana will be discussed in greater detail later in this chapter.

Finally, any assessment of the effects of a drug on androgen function should carefully take into account dose and dose-time factors. The concentration of the active-psychoactive principle in marijuana preparations may vary widely. Ideally, plasma concentrations of a drug should be determined simultaneously with measurement of any hormone measured in blood. In studies of the effects of alcohol on endocrine function this is easily accomplished, since blood ethanol levels may be determined with a high degree of precision and accuracy. In marijuana research this is more difficult, since high concentrations of delta-9-THC may be present in a variety of body tissue pools which are not in equilibrium with levels of the drug in blood. Thus, in studies with marijuana it is important to determine as accurately as possible the dose of marijuana administered or self-administered as well as specific time latencies between ingested dose and measurement of plasma hormones.

Plasma Testosterone Radioimmunoassay

The testosterone assay employed in our laboratory is a double antibody radioimmunoassay modified from the procedure used for protein hormones by Niswender et al. (1969) and based upon antibodies and methods

developed by ImChem, a division of Colorado State University Research Foundation, Fort Collins, Colorado, Further commercial development and sales of these products has subsequently been assumed by Micromedic Diagnostics, Inc., 1800 E. Lincoln Avenue, Fort Collins, Colorado.

The anti-testosterone serum available from Micromedic Diagnostics is distinguished from antisera sold by other suppliers in that it is obtained from rabbits immunized with a testosterone antigen coupled at the 11-position rather than the 3-position. A detailed description of the specificities of these antisera have been reported by Bosch et al. (1974). Combined with the selectivity achieved by extracting testosterone from plasma using heptane-benzene (2:1), the specificities of the anti-testosterone rabbit serum and the sheep anti-rabbit gamma globulin permit reliable radioimmunoassay of testosterone without chromatographic purification.

Because no chromatographic separations are involved, the actual "testosterone" concentrations reported using this procedure include a small contribution from cross-reacting substances extracted under the same conditions as testosterone. Dihydrotestosterone, the major cross-reacting steroid (30.5 percent cross-reactivity at 50 percent binding, as determined by the vendor), has been reported at about one-tenth the plasma concentration of testosterone in men (Ito and Horton, 1970). The error due to measurement of dihydrotestosterone along with testosterone therefore results in plasma concentrations about 3 percent higher than the true testosterone levels, assuming that the proportion of dihydrotestosterone to testosterone remains relatively constant. When considered with respect to inter- and intra-assay variations inherent in radioimmunoassay techniques, and the normal fluctuations in plasma testosterone levels, the slight increment due to dihydrotestosterone is insignificant.

Although we have described the procedures used in our laboratory in detail elsewhere (Mendelson et al., 1974), a complete description taken from our previous report is reprinted here for the convenience of the reader.

> The assay is performed as follows: Initially, 100 microliter plasma is extracted with 3 ml of heptane-benzene (2:1); a 300 microliter aliquot of the extract is then evaporated at 60°C *in vacuo*. Controls establish a reproducible recovery of 95 percent when corrected for volume differences. The following solutions are added to the dried extract: 500 microliter buffer (0.14 M sodium chloride, 0.01 M sodium phosphate, pH 7.0, with 1:10,000 thimerosal and 0.1 percent gelatin); 200 microliter anti-testosterone serum (diluted to produce 50 percent binding of the labeled steroid and adjusted to 1:400 serum concentration with normal rabbit serum with use of the above-mentioned buffer,

minus gelatin, plus 0.05 M EDTA); and 100 microliter of 0.15 microCi per milliliter of tritium-labeled testosterone in the same buffer used for the second solution. The tubes are then shaken for 20 minutes and incubated for at least 4 hours at 4°C. After incubation, 200 microliter of sheep anti-rabbit gamma globulin (appropriately diluted with the same buffer used for the second and third solutions described above) is added, and the tubes are shaken for two minutes and incubated for 12 hours at 4°C. The tubes are centrifuged for 30 minutes at 800×g at 4°C; then 500 microliter of the supernatant is mixed with 4.0 ml of Bray's solution (400 g of naphthalene, 28 g of PPO, 1.2 g of POPOP and 3.8 liters of dioxane) before counting in a Packard Tri-Carb Liquid Scintillation Spectrometer.

Testosterone-1,2,6,7-H[3] (312.5 mCi per milligram) was purchased from New England Nuclear, Boston, Massachusetts. Glass-distilled water and high purity glass-distilled solvents (Burdick and Jackson Laboratories, Inc., Muskegon, Michigan) were used throughout the assay. Disposable glass tubes were pre-washed and rinsed well with distilled water. Samples and controls were assayed in duplicate; standards, blanks and total-count tubes were analyzed in triplicate.

Plasma testosterone intra-assay precision was 9.0 percent (coefficient of variation) for samples assayed in the range of 400 to 600 ng per 100 ml and 6.2 percent for those in the range of 900 to 1100 ng per 100 ml. The interassay precision was 8.4 percent for controls assayed with a mean value of 458 ng per 100 ml and 6.1 percent for controls with a mean value of 983 ng per 100 ml. The latter control was obtained from Dr. Robert Rose's laboratory at Boston University Medical Center, where a mean value of 900 ng per 100 ml (-8.4 percent difference) had been determined by a different radioimmunoassay procedure involving chromatographic separation of testosterone from dihydrotestosterone and other cross-reacting or interfering substances.

In a letter circulated to purchasers dated November 18, 1974, Micromedic Diagnostics, Inc. called attention to the possibility that results from the Micromedic Diagnostics Testosterone Radioimmunoassay Kit, Product Code D-0101, may in certain situations be higher than the endogeneous level of testosterone. On the basis of conversations with Dr. Painter (Fort Collins, Colo.), it seems clear that there were two problems. First, the original ImChem methodology used by Micromedics called for the preparation of standards by diluting methanol solutions of testosterone with assay

buffers followed by a direct assay on the solution containing traces of methanol. The presence of the methanol has not, according to Dr. Painter, affected other radioimmunoassay kits sold by Micromedics. It does, however, displace the testosterone standard inhibition curve, leading to erroneously high testosterone plasma levels in samples containing no methanol. The Micromedics testosterone RIA kit, containing standards with methanol, has subsequently been withdrawn from the market; anti-testosterone serum is still commercially available. In our laboratory, standards have been treated identically to samples, being extracted from an aqueous solution. *This problem, therefore, did not apply to our procedure.*

An additional uncertainty in using the Micromedic's kit was that higher than normal plasma testosterone levels were reported for human females. This slight increase over normal proved to be due to a cross-reacting substance which has been separated from testosterone, but not characterized. *This difficulty is peculiar to female testosterone determinations* (Hiller et al., 1973) *and therefore not of particular concern in our studies with male subjects.*

Because the plasma testosterone concentrations for subjects studied in our laboratory were on the high side of normal values taken from the literature (300 to 1200ng/100 ml), we have been especially concerned about factors which could lead to erroneously high results. It should be pointed out that anything that decreases the concentration of testosterone in the standards below the concentrations attributed to them in plotting standard curves and making calculations would cause an apparent increase in the values of testosterone reported for samples. Such errors could occur because the testosterone purchased was not pure, not anhydrous, not weighed properly, or because minute concentrations present in the dilute standard solutions absorb to the glass walls of the vessels used, or because the standard solutions are allowed to deteriorate. It is for these reasons that, in our laboratory, testosterone is stored in a dessicator, that merthiolate is used as a preservative, and that the testosterone standard solution is made up in a silanized vessel and gelatin is present to prevent absorption onto the glass.

As with most analytical data, discrepancies may occur when results obtained in one laboratory are compared with those from another. Not only are there large differences among "normal" subjects and specific "pools" of subjects, as would be expected, but analytical results on the *same* plasma samples vary markedly from laboratory to laboratory. When two bovine controls were analyzed by Micromedic Diagnostics, using their radioimmunoassay procedures, results of 850 and 20 ng per 100 ml were obtained for high and low controls respectively. Analysis of these same control samples by six major, highly reputed, commercial laboratories yielded results ranging from 551 to 1000 ng per 100 ml for the high control and 10 to 20 ng

per 100 ml for the low control. It is therefore apparent that both repeatability and reproducibility need to be improved for testosterone assays.

Marijuana Use and Plasma Testosterone Levels Studied Under Controlled Research Ward Conditions

Data obtained in our laboratory on plasma testosterone levels before, during, and after chronic marijuana smoking have been reported in the *New England Journal of Medicine* (Mendelson et al., 1974). For the convenience of the reader, the Abstract of our report is reproduced in its entirety.

> To test the relation between chronic marihuana use and testosterone levels, we studied 27 men, 21 to 26 years of age. Plasma testosterone was measured daily before, during and after a 21-day period of marihuana use. The mean pre-use testosterone level of 12 casual users (who smoked an average of 54 marihuana cigarettes during the 21-day use period) was 988 + or - 93 ng per 100 ml (+ or - S.E.M.), and that of 15 heavy users, who smoked an average of 119 cigarettes was 1115 + or - 69 ng per 100 ml. No statistically significant changes in plasma testosterone levels were observed during and after the smoking period as compared with the pre-smoking baseline levels.
>
> These data do not corroborate an association between chronic marihuana use and decreased plasma testosterone. (p. 1051)

In our report (Mendelson et al., 1974) we stressed that "marihuana use in the 'heavy users' studied was equivalent to that of the subjects in the Kolodny study who reported using 10 or more 'joints per week.' " It was not possible for Dr. Kolodny and his associates to determine accurately the amount of marijuana leaf in cigarettes smoked by their subjects nor the concentration of the psychoactive principle. The marijuana cigarettes used in our reserach were obtained from the National Institute of Mental Health. Each cigarette was machine rolled to ensure dose equivalence and uniform burning and "draw" characteristics. The content analysis of the 1 gram marijuana cigarettes using Soxhlet and Modified Lerner extraction procedures was as follows: cannabidiol, .18 percent + or - .04, delta-8-THC, .002 percent + or - .002 percent, delta-9-THC, 2.06 percent + or - .08 percent, cannabinol, .08 percent + or -.12 percent.

Our research was carried out on a secure research ward. Subjects did not use drugs other than marijuana during the course of the study, and their state of nutrition and general health was excellent throughout the 31-day period of the investigation. Blood samples for testosterone assay were drawn from each subject at the same time of day (between 8:30 and 9 a.m.)

throughout the study, and samples were processed and frozen in a routine manner shortly after blood collection. All frozen samples were stored at - 40°C until analysis for testosterone was carried out.

The data which we obtained in these studies is reproduced from our article in the *New England Journal of Medicine* (Mendelson et al., 1974).

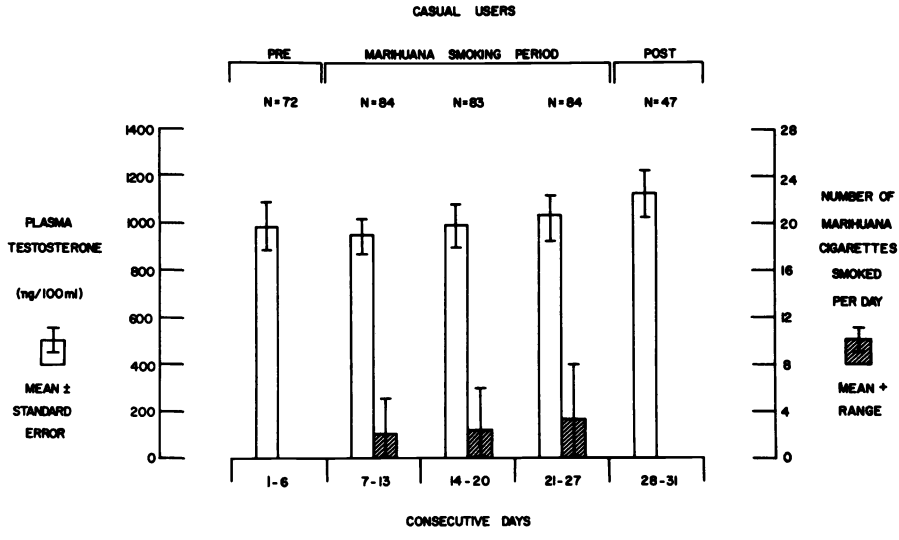

Figure 1: Plasma testosterone values for 12 "casual users" before, during, and after a 21-day marijuana-smoking period.

These data indicate that no significant alterations in testosterone levels occurred as a consequence of marijuana use. There were no dose or dose-time related alterations in plasma testosterone levels with respect to marijuana consumption.

Discussion

Perhaps the most important question concerning possible effects of marijuana on plasma testosterone levels is not whether changes occur but the magnitude and significance of such changes. Stated another way, although statistically significant increments or decrements in endocrine levels may be observed following drug administration, is statistical significance equivalent to biological significance? The episodic variance in plasma testosterone levels in normal, healthy adults may be quite large. Such variance has not been linked to parallel changes in biological function or behavior. Therefore, if drug use does produce an increase or decrease in a

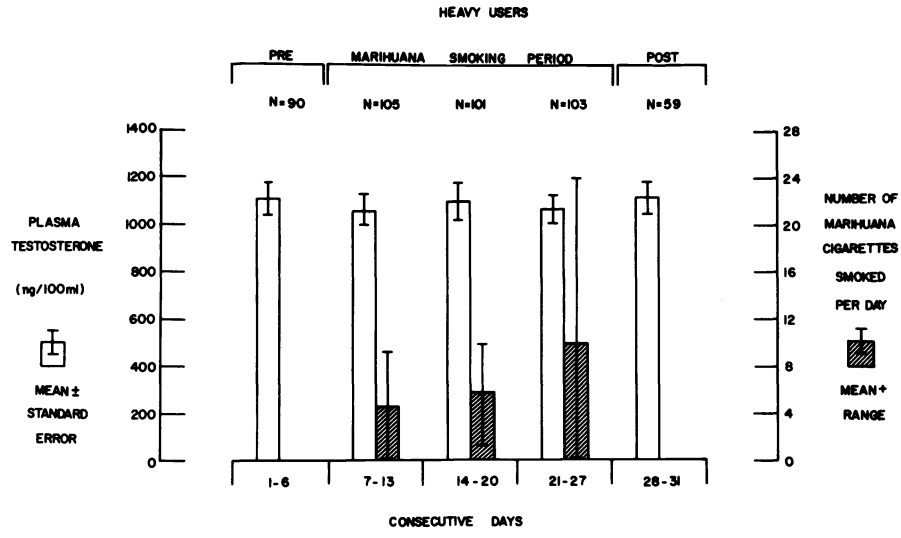

Figure 2: Plasma testosterone values for 15 "heavy users" before, during, and after a 21-day marijuana-smoking period.

Reprinted, by permission from the New England Journal of Medicine, 291: 1053, 1974.

hormone level, but the level is still within the normal range, is it possible to infer that these alterations are biologically meaningful.

It is well known that biological responses following any drug administration are not only dose and time related but may be influenced by factors of individual tolerance. Thus, it is possible that discrepancies in findings of alterations of plasma testosterone levels, or the lack thereof, from different laboratories may be more closely related to intra-subject variables than differences in analytic methods. The precision of biochemical techniques for testosterone assay are generally quite high when comparisons are made from laboratory to laboratory. But the precision of research design vis-a-vis subject selection and control of intra-subject variability may vary enormously.

In studies carried out in our laboratory (Mendelson et al., 1974), plasma testosterone samples were obtained early in the morning, usually 6 to 8 hours following subjects' smoking their last marijuana cigarette during the preceding 24 hours. It is therefore possible that any marijuana-induced decrements in plasma testosterone levels may not have been observed as a consequence of the duration of elapsed time between consumption of the last cigarette and obtaining plasma samples. It is even conceivable that a re-

bound effect could have occurred, namely an increment in plasma testosterone levels following a possible initial decrement.

Recent data reported by Schaefer and his associates (Schaefer et al., 1975) indicates that this is probably not the case. These investigators found no significant changes in plasma testosterone levels 90 minutes after administration of a 20 mg dose of THC. However, 90 minutes may not represent the optimal time for obtaining blood samples, since Kolodny and his colleagues (1975) found that decrements in plasma testosterone levels following acute administration of a marijuana cigarette containing 20 mg of THC did not take place at 90 minutes but occurred 120 to 180 minutes after smoking. Considering the episodic secretory rate of testosterone, decrements found following drug administration must be carefully compared with decrements which may normally occur during the short-term testosterone pulse cycle. Therefore, it is important to stress again the importance of differentiating the effects of exogenous variables from normal endogenous variance in assessment of drugs or hormone levels in humans.

The need to ensure that multiple drug use does not occur at the time when correlations are made between plasma testosterone levels and marijuana use has been stressed in a previous section of this chapter. However, it is important to re-emphasize the need for this control, since a commonly used agent, ethyl alcohol, produces significant suppression of plasma testosterone levels in healthy adult males. Ethanol-induced suppression of plasma testosterone levels may occur when blood ethanol levels are relatively low (between 50 to 75 mg/100 m.) (Mendelson and Ellingboe, in press). Thus, it is possible that some degree of suppression of plasma testosterone may occur during the conditions of normal social drinking.

It is also possible that a variety of other pharmacological agents may alter testosterone levels. During studies in which plasma testosterone levels were correlated with aggressive behavior and social dominance in man, Ehrenkranz and his associates (Ehrenkranz et al., 1974) found lowest plasma testosterone levels in two individuals who took a mixture of phenothiazine and barbiturate medication up to three days prior to blood sampling for testosterone assay. Thus, when decrements in plasma testosterone levels are observed within a series of values obtained for a single subject, or when comparisons of testosterone levels are made between groups of subjects, confounding effects associated with multiple drug use may lead to erroneous interpretation of data. It would appear especially important to entertain this consideration when alterations in testosterone levels may achieve statistical significance but still lie within the range of normal adult male values.

Acknowledgement

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Chapter 11

Marijuana and Testosterone: A Discussion

Dr. Hollister: I would like to ask one question that has to do with dihydrotestosterone (DHT). Is that not an active metabolite? Is it *the* active metabolite?

Dr. Rose: DHT is a necessary hormone, but not a sufficient one.

Dr. Hollister: Thyroxine has to be converted to triiodothyronine to become active. In the case of thyroxine, evidence now indicates that this process of conversion may occasionally be impaired. What is unclear to me is whether testosterone may also require a degree of conversion for full activity.

Dr. Rose: Your question cannot be precisely answered because adequate studies have not been completed. However, it has been established that DHT alone doesn't do the job.

Dr. Kolodny: Testosterone has a separate biological effect from DHT. For instance, in the male rat, if you castrate and give replacement doses of DHT, it does not restore sexual function. You must replace with testosterone to get sexual behavior.

Dr. Rose: There used to be a suspicion that individuals with XYY chromosome configurations had increased testosterone levels as measured in the urine; but more recent work by Price, who measured the plasma levels in the same individuals studied with urinary assays indicated no increased level (Price and Van Der Molen, 1970). The XYY individuals are taller, but their levels of plasma testosterone are not significantly different (Rose, in press).

I would like to respond to something you said, Dr. Kolodny, about the possible effects of marijuana on testosterone-sensitive processes in the developing fetus. The fact that a certain level of testosterone must be present in the developing fetus for the regression or stimulation of the Wolffian system so that the structural development is appropriately sexed has been well documented. There is also recent evidence to suggest that testosterone may alter future behavior, as well as bring about altered hypothalamic func-

tion with regard to cyclic LH release. My question to you is, how much testosterone is really necessary to bring about this altered potential? How much testosterone do you have to remove before a male doesn't look like a male or later on doesn't behave like a male? There have been no direct studies done on this, but indirect evidence suggests that it doesn't take much testosterone to induce male characteristics. For instance, male children from alcoholic mothers or from mothers taking chlorpromazine or opiates do not seem to have scrotal abnormalities. I don't know if there is any systematic data, but it seems as though you might have to suppress testosterone almost completely before the male fetus would not look like a male fetus. Do you have data that indicate otherwise?

Dr. Kolodny: I don't know the answer to that, since I am not familiar with any studies that document anatomic defects in a dose-response curve with regard to lowered testosterone during pregnancy. There are many animal studies, reviewed concisely and authoritatively by Money and Ehrhardt (1972) that show that when androgen is suppressed prenatally by use of chemical substances such as cyproterone acetate or estrogens, the genetic male fetus may develop the external genital appearance of a female or may have malformation of the penis or scrotum. Of course, the timing of the androgen suppression is of at least as much importance as the degree of suppression. The functional hypothalamic changes are not yet known, but in terms of human behavior that would be a study of much interest. Yalom and co-workers investigated the sons of diabetic women who received exogenous estrogen and progesterone to maintain their pregnancies (Yalom et al., 1973). The hormone doses were not sufficient to abolish testosterone production completely, but certainly they altered the balance between androgens and female hormones in the developing fetus.

Dr. Rose: Are you saying that if the mothers were treated with estrogen the secretion of LH in the male fetus would be shut off? Estrogens are secreted in mothers during normal pregnancy.

Dr. Kolodny: Treatment with estrogens might not totally shut off androgen production but it would change the hormonal balance. There is a possibility that depression of androgen synthesis would occur in such conditions, since adult males receiving estrogen have depression of androgen output (Kent et al., 1973).

To continue, Yalom's study does address the questions you are raising. Boys aged six and sixteen who were exposed *in utero* to estrogen and progesterone were rated lower on variables related to general masculinity, assertiveness, and athletic ability than socioeconomically-matched control subjects of the same age whose mothers were also diabetic but had not been given hormonal therapy during pregnancy. Two of the forty boys exposed

to these hormones *in utero* had hypospadias, whereas the usual incidence of this anomaly in offspring of diabetic women is 1 in 1400 (Kucera, 1971). This is a difficult study to interpret and will perhaps be more significant on future follow-ups that assess gonadal and sexual functioning, but it suggests that we do not know the answers about long-term effects of alterations in hormonal balance.

Dr. Harris: Dr. Rose, aren't you measuring total testosterone, which includes both bound and unbound?

Dr. Rose: That's correct.

Dr. Harris: You therefore have no idea of the relative levels of bound and unbound, and although you are lowering the overall levels, you can assume that there may still be enough unbound to have the activity necessary to carry on normal functions. There have been a number of studies, some from our laboratory, showing that THC is bound very tightly to alpha- and beta-lipoproteins or the globulins. Would this fact play any role in the lowered levels that you have? For instance, if THC was competing for binding sites with the testosterone, you would see somewhat lower testosterone levels.

Dr. Kolodny: *In vitro* studies using pure THC or different metabolites with known concentrations of testosterone have not yet been done. I understand that they are exceedingly difficult because of the glass binding problem. In preliminary *in vivo* studies we have documented no real differences in the percentage of testosterone bound versus unbound in men using very large amounts of marijuana, both on the street and as part of the UCLA controlled marijuana project.

Dr. Harris: So when the total levels are lowered, the unbound is proportionately lower?

Dr. Kolodny: Yes, the percentage is about the same.

Dr. Rose: The reported studies imply the same thing. In a given age group the amount of variability in percentage of bound versus unbound testosterone is much less than the variability in the total amount of testosterone. Therefore, the amount of variance accounted for in terms of binding is relatively small compared to the shift of binding that occurs when one passes from childhood to adulthood (Vermeulen et al., 1971; Rosenfield, 1971).

With regard to specificity, the globulin binding for testosterone is a high infinity, low capacity system that is quite specific. It picks up neither cortisol nor those steroids without a 17-beta-hydroxy group. I don't know if it picks up something as dissimilar from testosterone as THC.

Dr. Mendelson: In our Hong Kong series there were certain young men who initiated heroin use when they were quite young — age 10, 11, 12 — and continued to use heroin daily. When we assayed them at age 15, they

had very low testosterone levels — 50 to 75 nanograms. But in terms of psychosexual development, physical stature, muscle development, this group of young men didn't impress me as being significantly impaired.

Dr. Rose: There are techniques available that make it possible to have blood samples drawn continuously throughout the day. One such technique that we use involves a non-thrombogenic catheter which is hooked up to a pump on your waist. Since it doesn't get in the way, you can go about your daily activities (Rose & Hurst, 1975). It would help to answer the question of the acute, short-term effect of heroin, marijuana, or other psychoactive drugs on testosterone levels because testosterone is secreted episodically. Maybe there is some rebounding that occurs over a 24-hour period. To study this possibility, these techniques easily permit one to put the catheter in, smoke marijuana, and continuously assay blood levels for hours. The procedure is not that uncomfortable; on several occasions I have worn it myself for hours.

Dr. Hollister: Has anyone measured 17-ketosteroids in urine?

Dr. Rose: That is not a good approach for telling you about alterations in testosterone secretion. 17-ketosteroids come from testosterone as well as a variety of other hormones, not all of which are androgens. 17-ketosteroids are a hodge-podge that we had to live with ten years ago.

Dr. Falek: Is the variability *in* an individual as wide as the swing *between* individuals?

Dr. Mendelson: Yes, both the intra-individual variability and inter-individual variability are enormous. If you try to explain the sources of variance, the diurnal cycle accounts for only about 20 percent. Eighty percent of the variance is due to factors outside the diurnal cycle.

Dr. Falek: Can I conclude that there are not necessarily inconsistencies in the marijuana and testosterone relationship? At this point, is there a possibility that all the results are right?

Dr. Kolodny: Yes, for instance, Dr. Mendelson's data are not necessarily inconsistent with ours. He is describing a three-week study of marijuana administration on people who had been asked to discontinue drug use for a couple of weeks before they came into the research ward. In our UCLA study, which is parallel in terms of what the men were doing and the conditions they were living under, the first five weeks of hospitalization with daily marijuana use did not produce a statistically significant drop in testosterone levels. There are so many factors that enter into the equation having to do with what sorts of sexual opportunities the people had on the research ward. Were they looking at *Playboy* magazine? Were they masturbating? Were their girl friends visiting them? How much exercise were they getting? It is known that exercise can raise androgen levels. In the UCLA study we found one or two subjects who started lifting weights and doing

hundreds of push-ups a day. When they began this rigorous exercise their testosterone levels went up by 40 or 50 percent.

Dr. Rose: I wish you would publish those data on how exercise may affect testosterone levels. Dr. John Mason and collaborators (personal communication) have recently found that plasma testosterone rises approximately 20 to 25 percent during moderate exercise, i.e. treadmill exercise requiring 40 percent of maximum oxygen uptake and lasting for three hours.

Dr. Mendelson: I'm still bothered by the lack of concordance between androgen levels and sexual behavior or sexual potency. There are impotent but otherwise normal, healthy males who have normal testosterone levels. So far as I know testosterone has not been of any value in the treatment of impotence.

Dr. Kolodny: Testoterone is helpful when it is a testosterone deficiency that causes the impotence. Giving someone thyroid hormone for his nonspecific complaint when his thyroxin level is normal will not be effective either.

Dr. Rose: People with low-normal levels of testosterone — three, four, five hundred nanograms — used to be treated with testosterone. But such treatment has not been proved effective.

Dr. Nahas: What is the level of alcohol intake needed to lower testosterone levels?

Dr. Mendelson: It varies in individuals. We have seen substantial decrements with blood levels of 100 and 200 milligrams ethanol per hundred ml. These are fairly high levels of alcohol for inexperienced drinkers. In several months we will be able to say what lower doses of ethanol will do.

Dr. Jones: We are measuring testosterone levels in subjects who receive substantial oral doses of THC or crude marijuana extract for up to three weeks. The 210 mg per day doses produced fewer behavioral effects than the doses of alcohol Dr. Mendelson just talked about. None of these subjects rated themselves as high on our laboratory marijuana as they had been outside the hospital after smoking street marijuana. Our proc-dures are quite similar to those used by the Mendelson group in terms of the time of day for sample collection, etc.

In the first twelve out of fourteen subjects we studied, the lowest testosterone level appeared in the first few days after the large doses of THC were begun. Also, the highest values appeared during the withdrawal phases when the subjects had stopped taking THC and were clinically showing something that probably represents abstinence behavior. The testosterone levels changed from about 700 nanograms pre-drug to about 500 nanograms early during the THC phase and went up to 1,000 to 1,200 nanograms during the withdrawal phase. Despite the statistical significance of these findings, the biological significance remains unclear since,

although there were changes, the levels remained within normal clinical limits.

Dr. Hollister: That's always an interesting question. When there are changes in a biological parameter but the values remain within normal limits, what do the changes mean in terms of function? That is our present state with marijuana and testosterone. It is not only an issue of whether there is change, but if so, whether it is of any importance. I should like to ask Dr. Kolodny just one last question which has to do with the acute administration.

Two years ago we studied three subjects before and after intravenous infusion of 6 mg of THC and found absolutely no change in serum testosterone level over the three-hour period. What is your experience with acute administration?

Dr. Kolodny: We have not used any route other than smoking. The subjects smoked either one or three consecutive cigarettes that contained 9 milligrams delta-9-THC in the form of natural marijuana. Within a three-hour time period there was a 30 to 35 percent decrease in testosterone levels and also a significant decrease in LH levels.

One last comment about the frequency of sampling. When we take only one blood sample of testosterone per day, it is as though we were trying to rate human behavior on the basis of taking one or two Polaroid snapshots a day. This is why assessment of 24-hour production rates are potentially more accurate, if what we are trying to see is whether there may be real biological effects. The episodic release of testosterone is certainly well documented. We are doing the best we can and asking people to be patient until we know more about it.

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Chapter 12

Marijuana and the Central Nervous System

Rhea L. Dornbush

In the past decade there has been a vast increase in the knowledge of marijuana's effects on the central nervous system. However, despite the fact that the effects of the acute administration and short periods of daily administration have been well defined, much less is known about the long-term effects of heavy marijuana use. Of the studies done thus far on long-term use, most are methodologically deficient and have only limited applicability to middle-class users in the United States and other developed countries.

While the acute and daily administration investigations have been laboratory studies permitting experimental control and employing sophisticated, rigorous methodology, there are still many inconsistencies in the data. These inconsistencies generally disappear when variables such as the following are appropriately considered.

(1) Dose. Simple central nervous system functions are not affected by low doses of marijuana but may be slightly affected by high doses; in contrast, complex functions are often affected by low doses and more so by high doses. Each study, however, has different criteria for what constitutes low and high dosage levels. For example, Dornbush et al. (1971) used 7.5 mg and 22.5 mg delta-9-THC in marijuana leaf as low and high doses; Klonoff et al. (1973) used 4.9 mg and 9.1 mg respectively. Also, it is difficult to assess the amount of the drug actually consumed, especially when marijuana is smoked. It has been estimated that only 50 percent of the measured dose is delivered to the subject when the mode of administration is inhalation (Manno et al., 1970).

(2) Type of marijuana preparation. Effects, particularly mood changes, are somewhat different for marijuana, hashish, and delta-9-THC. The different combinations of marijuana constituents (e.g. delta-9-THC, delta-8-THC, cannabinol, and cannabidiol) in these preparations may influence the marijuana experience (Karniol et al., 1974). There is the possibility that the

effects of delta-9-THC can be blocked or lessened in interaction with cannabidiol (Karniol et al., 1974).

(3) Route of administration. The most popular method of consumption is via inhalation, but marijuana has also been administered orally and intravenously. Intravenous administration permits the greatest control over the amount absorbed, but generally is used only when the substance injected is pure delta-9-THC. The delayed and longer lasting effects are obtained orally, but these may be heavily dependent on the solvent used as a vehicle of administration.

(4) Set and setting. Many daily administration subjects are hospitalized for the entire duration of the study. Such a setting may not produce the most favorable attitudes for testing, especially in the later stages of the experimental period when irritability, and tedium may affect many test results.

(5) Type of task. Estimations of time sense are indicative of problems associated with task presentation. Anecdotally, marijuana users consistently indicate that time passes very slowly, but this has been difficult to demonstrate reliably with experimental procedures. There are three popular methods of measuring time sense: (a) reproduction—the subjects are asked to reproduce a previously indicated time period, e.g. press this buzzer for the same time period as the buzz you just heard; (b) production—the subject is asked to produce a specified period of time, e.g. tell me when you think thirty seconds have elapsed; (c) estimation—the subject is asked to estimate the length of a given time period, e.g. for how many seconds was the bell ringing? These different methods of measuring time sense will result in different data (Bech et al., 1974).

Reproduction is by far the easiest method, merely requiring the subject to count off during the interval to be reproduced. Regardless of what his counting schedule is, he simply has to repeat it. Thus, reproduction is rarely sensitive to marijuana effects (Dornbush et al., 1971; Hosko et al., 1973). To be consistent with anecdotal reports of time passing slowly during marijuana intoxication, production methods should result in underjudgments, e.g., the subject intoxicated with marijuana should state that thirty seconds have elapsed when only twenty seconds have passed. In fact, Karniol et al. (1974) and Tinklenberg et al. (1972) obtained this result, but Jones and Stone (1970) did not. Using estimation methods during marijuana intoxication should result in overjudgments, e.g. the subject should estimate that one minute has elapsed when actually the interval was thirty seconds. Clark et al. (1970) and Jones and Stone (1970), obtained overestimations, but Weil et al. (1968) did not. One reason for the apparent inconsistencies on time sense tasks is that results vary considerably depending on the interval used, and marijuana studies have used intervals ranging from one second to

five minutes (Dornbush et al., 1971; Tinklenberg et al., 1972; Dornbush & Kokkevi, in press). In addition, time sense is different if the intervals are filled, i.e. if the subject is required to perform an additional task during the interval, or empty, i.e. if the subject is merely passive. Furthermore, the results are influenced by whether the instructions given emphasize subjective or objective time (Bech et al., 1974).

(6) Methods of evaluation. The precise methods used to evaluate results obtained in marijuana experiments differ widely among various investigations and may lead to different conclusions. For example, the effects of marijuana on the electroencephalogram (EEG) have been difficult to interpret, in part because there are different methods of recording and analyzing. Most EEG studies of marijuana effects depend on the visual analysis of the EEG, but this technique is often inadequate for measuring the subtle dose-related effects of marijuana. Recently developed and applied computer analysis of the EEG may facilitate evaluations since they are more consistent and less dependent on rater differences (Fink et al., in press).

(7) Organismic differences. Marijuana effects on the central nervous system depend upon the duration and frequency of prior marijuana use, age, education, and time of last drug consumption. The effects of marijuana also depend upon the personality characteristics of the user. For example, daily administration subjects who are willing to participate in prolonged experiments as hospitalized patients may not be the most appropriate subjects from whom generalizations can be drawn.

When differences in research methodologies are evaluated and accounted for, the experimental investigations of acute and daily administration indicate that marijuana does have consistent effects on central nervous system (CNS) functioning. Dose-related patterns of response occur in psychomotor, cognitive, perceptual, and physiological functions. Generally, simple functions such as simple reaction time and memory span are not obviously affected by marijuana. But more complex functions, for instance, complex reaction times, tasks which require coordination of information with simultaneous mental operations, and memory tasks requiring a delay of recall, are affected. EEG data obtained from computer analysis indicates a slight decrease in frequency, increase in alpha, decrease in beta, a peak of effects early in the post-smoking period, and continual change of the EEG pattern during the course of measurement (Rodin et al., 1970; Volavka et al., 1971, 1973). In relatively short-term marijuana smokers, say of one to five years duration, these effects appear to be transient and reversible.

Do these changes ever become permanent? Does continual use of marijuana result in CNS dysfunction and permanent brain damage? To evaluate these questions we must rely on methodologically less precise and perhaps inappropriate studies, those on long-term smokers, generally con-

sidered to be ten years or more. The most obvious limitations in these studies are the samples selected and the adequacy of the testing battery.

Samples

Until recently the long-term studies have typically been based on surveys or clinical observations. The samples have been multi-drug users, prisoners, or psychiatric patients (Mayor's Report, 1944; Williams et al., 1946). For example, Williams et al. (1946) initiated studies with pyrahexyl compound (judged qualitatively similar to marijuana) and marijuana. The subjects were prisoners; the potency of marijuana was not assayed, and there was no control group. In these people, early effects of exhilaration and euphoria were replaced after several days by lassitude and indifference. It is doubtful whether one should attribute the presence of lassitude and indifference in these subjects to marijuana use alone without an appropriate control group, especially when consideration is given to the conditions of the experiment, which included confinement, a limited selection of ward mates, and no diversion or activity.

Soueif's study of Egyptian marijuana users employed over 800 prisoners, 60 percent of whom were illiterate (1971). When compared with the control group, the work capacity of the users was significantly impaired in quality and quantity; they were slower learners than the controls and did significantly worse than controls on objective tests of mental performance. What is typically ignored in considering these data is the fact that a significant number of marijuana users also used other substances, especially opium. For example, there was a positive correlation between duration of hashish and opium use. Obviously, marijuana cannot be considered the sole causative factor in performance differences between the two groups.

The Campbell et al. study (1971) on the deleterious effects of marijuana consumption on brain tissue further highlights the methodological deficiencies and investigator bias in studies being used to evaluate CNS changes. By the use of pneumoencephalograms, Campbell demonstrated the existence of cerebral atrophy, i.e. the shrinking of brain tissues in certain marijuana smokers. His sample size consisted of ten young adults who were culled from thousands of patients in metropolitan London precisely because they showed evidence of cerebral dysfunction and symptoms of senility. This sampling procedure is limited from several perspectives and limits the interpretations that can be made of this study. Since juvenile onset of cerebral atrophy does affect a number of young people who are not drug involved, one would expect some subjects in the London population to show atrophy regardless of whether or not they smoked marijuana. In addition, many of the subjects were either multiple drug users or were known to have histories of brain damage. One subject had epileptic attacks,

three had histories of head injuries, and one had been continuously treated with sedatives from the age of two. At least six had initiated their drug use with amphetamines and then began smoking marijuana which they used concurrently with amphetamines. Two used alcohol excessively. Once again, methodological limitations, especially the lack of adequate controls, preclude attributing the cerebral atrophy to marijuana or any other single cause.

Kolansky and Moore (1972) reported a specific pathological organic response in the CNS to marijuana. This view, which was a clinical impression not supported by objective measures or laboratory data, was based on a sample of 13 marijuana-using subjects who were identified over a six year period. A number of methodological considerations limit interpretations from this study: the stated bias of the interviewers that marijuana induced CNS damage must have affected their interview and diagnostic techniques; the conviction that they knew what they were looking for before they found it; and the non-blind assessment of subjects and the non-standardized interview techniques which are particularly relevant methodological limitations when two investigators are evaluating different patients and then grouping their findings. Although Kolansky and Moore emphasize the organic implications of marijuana use, without appropriate neurophysiological and neuropsychological examinations there is simply no way to substantiate their conviction.

Our problem with selecting populations of people who are referred for treatment to psychiatrists is that one cannot tell which came first or what caused what. One possible interpretation of cases where marijuana appears to have been the cause of mental disturbances, as might be the case in Kolansky and Moore's patients, even discounting their methodological deficiencies, is that these individuals may be allergic to marijuana. They react adversely to it just as some individuals do to penicillin or food or any other drug. Indeed, there are times when an individual's vulnerability to any substance is greater than at other times.

Several of Kolansky and Moore's patients smoked very heavily without the experience being part of a group experience or cultural practice. They came to depend on marijuana, very much as the solitary drinker does on alcohol. This pattern of solitary marijuana use contrasts with more typical marijuana use which is a group and social phenomenon. For long-term, heavy hashish users in Greece, for example, daily consumption was a group occurrence and traditions surrounding smoking were so strong that they had to be simulated in the laboratory. This might suggest that the "lone" or solitary daily use of marijuana is itself deviant and suggests underlying problems. It is difficult to accept the notion that these syndromes of such severity documented by Kolansky and Moore could have occurred after

only three years of smoking marijuana unless there was some specific vulnerability coexisting at the time of initiation.

Because American populations of very heavy, very long-term users who limited their drug use to marijuana are not available, studies on chronic long-term users have been undertaken on user populations in other countries. Two studies, one in Jamaica (Rubin & Comitas, 1972), and one in Greece (Fink & Dornbush, 1972) have been completed. What is unique about these chronic long-term marijuana users is that their drug use has been restricted to marijuana preparations. There is virtually no use of other substances, with the exception of tobacco and occasional social use of alcohol.

In Jamaica, 30 chronic marijuana users, confirmed as daily users for not less than seven years, were compared with 30 non-users. Subjects reported that they smoked between 1 and 24 marijuana cigarettes a day with an average of 7. The samples contained a mean delta-9-THC content of 2.96 percent. In Greece, 40 users who had smoked hashish for over 10 years were compared with 40 non-user controls. At the time of observation, subjects reported that they smoked an average of 3.1 gm of hashish a day. Analysis of samples indicated a THC content of 4 to 5 percent. Assessments included tests of physiological, sensory, cognitive, and perceptual performance as well as medical and psychiatric measurements. In both samples these extensive assessments indicated no abnormalities of mood, thought, or behavior.

A limitation in cross-cultural studies lies in the populations' comparability to populations in the United States or other countries where extensive marijuana use is a relatively recent phenomenon. For example, in the United States the user is drawn from the most achievement oriented social classes and use reaches a peak in the college years; marijuana is a drug used primarily by those under the age of thirty (Hochman & Brill, 1973). In the studies of foreign countries where marijuana has been consumed for decades, the user comes from the lowest, least productive strata, and is usually well over thirty. Thus there are social, economic, and individual differences between the two types of populations being compared. In foreign populations where marijuana use has been indigenous, surrounded by tradition, folklore, and a set of expectations, Bowman and Pihl (1973) suggest that many findings may be cultural artifacts rather than drug effects. They point to user expectation, citing the Jamaican habit of taking marijuana to enhance memory and thinking, whereas in the United States short-term laboratory studies suggest that memory is one of the functions most impaired by marijuana intoxication.

The retrospective nature of long-term studies adds further difficulties to evaluation of outcome, for there is no baseline pre-marijuana use assess-

ment. Since information obtained cannot be compared with pre-drug-use levels of performance, we never know if any observed "effects" were present before marijuana use, are the result of, or are aggravated by such use. With retrospective studies it is also difficult to assess accurately the frequency, quantities, and strengths of marijuana used in the past. The type and quality of marijuana varies from country to country; in some countries, particularly those in the Middle East, it may be mixed with tobacco, opium, or other substances which might significantly affect the user (Le Dain Commission, 1972).

Testing Battery

Cultural, social, economic, and individual differences aside, the question of the adequacy of testing CNS dysfunction arises. There are two major approaches to testing CNS functioning: the neurological and the psychological. While these measures have been fairly well defined in clinical practice (Freedman et al., 1972; Arieti, 1974), they have not yet been systematically applied to marijuana studies. Confidence in specifying CNS changes increases with an increasing number of similar judgments derived from different investigative procedures. A reasonable approach might consist of a battery designed to test a variety of brain functions. Yet many investigators pursue a single test instrument, or a haphazard or incomplete sampling of subtests from different instruments (Jones & Stone, 1970; Rodin et al., 1970; Grant et al., 1973). In addition, there is no uniformity in this selection from study to study.

For example, Bowman and Pihl (1973), in their study of chronic marijuana use in Jamaica, used three tests of sensory, perceptual, and motor functioning including the Halstead Category Test and the Pins Test-Lincoln-Oseretsky; three tests of concept formation, abstracting ability, and cognitive style, including the Kohs Block Design, Wisconsin Card-Sorting, and Embedded Figures Test; and four tests of memory, including paired associates, memory span, and Knox Cubes. Jones and Stone (1970) used rod and frame, time judgment, digit symbol substitution, and EEG. Grant et al. (1973) used the Halstead Category Tests, Tactual Performance Test, Reitan's Trail-Making, Raven's Progressive Matrices, and the GDSA. Rodin et al. (1970) used EEG, Bender-Gestalt, and tests of the vibratory sense.

A single test or even sample of tests may not be sufficiently sensitive to brain-behavior impairment or sufficiently reflective of the wide range of brain functions. Frequently researchers give greater credence to the neurological aspects of brain function, taking the attitude that neurology is a "hard" science and psychology a "soft" science. For this reason many studies have emphasized EEG effects (Volavka et al., 1971, 1973),even

though normal EEG tracings are frequently found to be associated with diseased brain tissue. Furthermore, since the EEG taps only the outer shell and a small portion of the under and medial surface of the cerebrum, only approximately one-third of the brain is available to electroencephalography. EEG tracings are also apparently influenced by factors other than pathology, such as mood, vigilance, age, sex, metabolic states, levels of consciousness, and sensory excitation. Thought disturbances and other psychopathology have been related to EEG characteristics. Depending on the problem being evaluated, EEG effects might best be interpreted in association with corollary procedures such as the neuropsychological examination.

It is necessary to recognize the limitations of neuropsychological test batteries, particularly when generalizing from one country to another. Batteries of tests, like the EEG, are influenced by many variables other than brain damage: genetic factors, cultural deprivation, educational disabilities, the presence of emotional conditions, and the normal aging process. Thus, test scores may depart from normative standards for reasons other than brain damage (Freedman et al., 1972; Arieti, 1974).

Because of the lack of availability of standardized mental function tests for the Greeks and Jamaicans, tests standardized for, or appropriate to United States populations were used. In Greece, for instance, the Wechsler Adult Intelligence Scale was translated into Greek. Even though the Jamaicans spoke an English dialect, the tests used may not have been completely comprehensible or appropriate to the Jamaican frame of reference.

The difficulties in interpreting even an adequate neurophysiological and neuropsychological examination are many. When insufficient measures of cerebral function are employed in combination with population limitations, the difficulties in interpretation are considerable.

One of the more extensive tests of marijuana effects on the central nervous system with regard to the number of functions tested was performed by Klonoff and associates (1973). Although their subjects were only short-term occasional marijuana users, their study might serve as a model for future investigations of long-term users because of the expansive nature of their test battery and the integration of data from both neuropsychological and neurophysiological tests. The neuropsychological measures consisted of thirteen tests with multiple measures obtained from some of the tests. The neurophysiological battery, administered to the same subjects, included the scalp EEG, visual and auditory evoked potentials, the contingent negative variation (CNV), and other tests.

The correlation of the findings from the neuropsychological and neurophysiological test batteries led these authors to suggest that marijuana has a "qualitative bimodal effect over time with an initial brief 'rush' lasting approximately 30 to 35 minutes and a subsequent longer period of quieter

action." They relate these bimodal effects to the time frame of biotransformation of delta-9-THC to 11-OH-THC, suggesting that delta-9-THC may be an activator or stimulant and its metabolites, such as 11-OH-THC, a depressant to the CNS. They conclude that "marijuana induces changes in brain function which can be profound but which are associated with only relatively minor measurable changes using standard electrophysiological recording techniques such as the EEG. This fact alone strongly implicates subcortical, medial, or basal brain structures as being primarily responsible for the experimental and performance changes induced."

Conclusions

Where does this leave us? Is there central nervous system damage associated with marijuana use? Marijuana does alter, at least transiently, cerebral functioning in short-term users. Does this alteration in cerebral functioning become permanent when marijuana consumption continues? Acknowledging deficiencies in samples and testing procedures, and evaluating data in terms of these limitations, when performance is behaviorally measured there is no present evidence of cumulative detrimental effects of marijuana which interfere with the functioning of the individual. However, the limited nature of the present evidence underscores the need for more systematic research.

In future studies of marijuana we must exercise care in experimental designs, research strategies, samples, and most important, in our interpretations. Even when designs, strategies, and samples may be appropriate, interpretations may be flights of fancy. We might now, since we know the limitations of retrospective studies, support prospective studies of already existing short-term users: identify them, obtain baseline data, and follow them yearly, with major evaluations every five years.

The most important point to be made is that data on marijuana use in central nervous system functioning must be put into perspective. What populations are we evaluating; what are their characteristics, health, social and cultural status; how do they relate to the populations at greatest risk for intensive marijuana use? Until now marijuana effects have usually been classified according to the type of function tested. It may be worthwhile to reevaluate the data so as to classify outcome in terms of the samples used: e.g. middle-class Americans with limited other drug use vs. people with multidrug use, adolescents vs. young adults. A clearer picture of marijuana effects might emerge, and we may get further insight into the type of populations in which marijuana might be particularly hazardous.

As a final note, perspective might be better achieved if multidrug comparison studies were undertaken in which there was a direct comparison of marijuana and alcohol, marijuana and amphetamines, and marijuana and

diazepam. Discussing marijuana effects without comparing them to other substances already in use is comparable to assessing any compound without a control group: there is no standard by which to interpret the extent and magnitude of the effect.

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Chapter 13

Effects of Marijuana on the Mind

Reese T. Jones

Marijuana has effects on the brain which are presumably reflected in the workings of the mind. Unless one believes in some sort of mind-body dualism or that brain function has nothing to do with thinking, feeling, or behaving, marijuana *must* have effects on the mind. The reason most people smoke marijuana is to change the way they think and feel. Much anecdotal and experimental evidence indicates that a person's behavior is altered when intoxicated by marijuana. So the question should not be "are there effects" but rather how long do the effects last: for hours, for days, for months, or forever? Are the effects more likely to persist if one is a frequent rather than occasional user? Are specific areas of the brain (or specific aspects of behavior) more affected than others? And finally, how are the effects evident in cognition or other aspects of behavior?

To begin to answer some of these questions it would be useful to know at what level of brain organization to look. Such a decision is complicated since marijuana effects have been reported at various levels (at cellular, subcellular, and membrane levels, synaptic, biochemical, metabolic, electrophysiologic, and behavioral levels). I suspect that both society and the marijuana smoker are far more concerned about drug effects on behavior, thinking, and performance than drug effects on the synapse or on septal spiking. However, many of us don't quite trust measures of behavior or thinking. They don't seem as real, or at least as scientific, as do brain-related squiggles on a polygraph. So more as a matter of faith than of fact, we hope that studies of brain chemistry and electrophysiology will help us predict marijuana effects on behavior, even at times forgetting that it is the behavioral correlates of the events we are really interested in.

Since I earn my living as a scientist, I think such research activity is fine. However, when using scientific research data to change laws or otherwise shape human behavior, certain things need to be kept in mind. For example, there is often less than perfect correlation between synaptic events or electrophysiologic events and behavior. Sometimes scientists talk as if they know more about the relationship between changes in the brain and

subsequent behavior than is warranted. This seems to be particularly true when talking about the interaction between drug use, brain function, and behavior (and sometimes even more true when discussing the specific drug marijuana). If the reader thinks I am perhaps too pessimistic or unduly critical of certain tentative conclusions made by recent researchers and others about the effects of marijuana on the brain, I would urge you to consider the state of affairs concerning alcohol and brain function. Alcohol is a drug that is in many ways far more simple to study than marijuana. Alcohol has been studied for a long time, yet most recent reviews of alcohol effects on brain function contain statements like "strikingly little experimental work has been devoted to the neurological complications of alcoholism" (Wallgren & Barry, 1971). Reasons for this include uncritical acceptance of clinical impressions not scientifically validated and appreciable methodological difficulties (Freund, 1973). Some marijuana researchers might profit from considering the experiences of alcohol researchers and thus be more cautious about over-interpretation of their data. Although alcohol and marijuana have many differences, there are probably some shared characteristics. For example, any chronic effects from marijuana use probably will be modified by genetic, environmental, nutritional, and other individual, nonpharmacologic factors. Not everyone who uses alcohol develops alcohol-related disorders. And even some very frequent users of alcohol have no demonstrable brain pathology. Yet when looking for marijuana-related disorders, research strategies are often used that would be appropriate only if pathology in marijuana users was very frequent, common, and probably not dose related (Stefanis et al., in press'. Other papers in this book discuss the issue of optimal research strategy, so I won't dwell on that.

A major problem in interpreting some of the recent research results concerning marijuana effects on the brain is related to the relatively inadequate measures of brain function and pathology we are forced to use. The electroencephalogram as commonly used by researchers is of limited reliability and validity in picking up subtle drug-induced, non-localized brain changes. Other techniques that might provide more precise indications of brain pathology, such as brain biopsy, pneumoencephalography, or implanted electrode techniques, cannot be used with "normal" drug users. Reading clinical EEG's is still more of an art than a science, and unless care is exercised, bias can enter into interpretation. The more objective and "scientific" methods of quantitative EEG analysis (for example, power spectra analysis or evoked potential techniques) are largely unvalidated as to their clinical applicability. Particularly when using such quantitative techniques, care is needed when interpreting "significant" (statistically speaking) differences between users and non-users. Because of the nature

of these techniques it is relatively easy for the persistent investigator to come up with a "significant" drug effect. Each EEG spectral plot or evoked potential can provide the diligent investigator with literally hundreds of possible measures if various combinations of amplitudes and latencies of evoked potential components or frequency spectra are examined. Thus by chance alone it is possible to find some differences between populations of marijuana users and non-users. As is the case with many of the marijuana-related changes discussed in this book, it is important to distinguish between statistically significant differences and practical or biologically significant differences. For example, a 0.2 Hz frequency shift in the alpha frequency of the EEG after smoking marijuana might be of some scientific interest in terms of theory building, but the practical or behavioral import of such a frequency shift is unknown. Other EEG changes are of similar utility as predictors of behavior or clinical change; they validly indicate neither drug safety nor potential harm.

Despite their many limitations, clinical EEG's, when abnormal on repeated examination, do have some utility as predictors of brain pathology. A review of such data from experimental EEG studies involving marijuana users is mostly reassuring and a little puzzling. Most reports describe no abnormal EEG's in users (see Klonoff and Low, 1974; or Fink et al., in press, for good reviews). Data on EEG characteristics of almost 200 subjects are included in the various published reports. The reports generally describe acute experimentally administered marijuana effects on the EEG. But only one of the dozen or so better controlled studies describe *any* abnormal EEG's. That is a puzzle because most EEG textbooks say that an incidence of 10 percent abnormal EEG's is not unusual in typical unselected young adult populations. One study found 6.6 percent of their "normal" young adult, volunteer, marijuana using subject population (N=76) had abnormal EEG's (Klonoff & Low, 1974). They thought such an incidence was to be expected in a normal sample and that it probably wasn't related to marijuana use. One other study reported an amazingly high incidence of abnormal EEG's (90 percent and 73 percent) in groups of marijuana users (Campbell, 1971). Such a high incidence of EEG abnormality would be surprising even when the most marked organic pathology was present. Campbell's interpretation of his findings has been soundly criticized by other researchers (Klonoff and Low, 1974). Thus the relative absence of reports of abnormal EEG's in populations of marijuana users could indicate that marijuana has a normalizing effect on the EEG, that volunteer subjects are not representative of the general population, that marijuana researchers cannot properly interpret EEG records, or that they are not looking for evidence of pathology. Probably all of the above are partially true.

Scalp EEG's do not always reflect abnormal activity in deep brain structures. Thus, recording techniques using implanted electrodes are sometimes useful for diagnostic purposes. Such studies, of course, are rarely possible in normal humans. However, a patient with epilepsy and an array of implanted subcortical electrodes was given marijuana cigarettes to smoke by Heath (Heath, 1972). As the patient became euphoric after smoking, focal high amplitude slow wave activity appeared in his septal region. In past studies with schizophrenics and others, Heath has tried to establish a relationship between septal region activity and pleasure responses. Thus the finding is an intellectually appealing one. A drug produces pleasure and septal region electrical activity changes. Of course, there is a question of whether this is necessarily to be considered a bad effect. Also, in terms of the theory, one might wonder why other pleasure-producing drugs given this epileptic patient did not produce similar changes. Alcohol, amphetamine, and tobacco, all which are taken by people to make themselves feel good, produced no such EEG changes.

In a subsequent study where monkeys were forced to inhale measured amounts of marijuana smoke over long periods of time, similar but not identical EEG changes developed (Heath, 1973). The diffuse non-cortical EEG changes persisted after the smoking ceased. The findings in the monkeys are worrisome, but unfortunately adequate control animals were not tested. Also, the theoretical link between a drug that produces pleasure, resulting in septal activity followed by habituation, adaptation, or exhaustion of the septal response, which in turn is followed by amotivation, lethargy, etc., is just a little tenuous and not yet proved. One might ask if with this model, bananas should not produce septal spiking in primates followed by long-lasting behavioral changes after chronic high dose use? The findings in both man and monkey should be followed up with properly controlled studies. However, it is premature to use the findings to prove that marijuana is harmful, no matter how elegant the theory. It is still only theory.

A host of investigators have reported on acute marijuana effects on waking, resting, and sleep EEG and on sensory evoked potentials (AER) (for a review, see Fink et al., in press). Although not always consistent, generally enhanced and slowed alpha activity, unchanged or decreased beta activity, and decreased mean frequencies are reported. Although by definition these findings represent brain effects, none of the changes are necessarily indicative of any abnormal function. The sleep EEG changes induced by marijuana are even more dramatic, but the relation between the behavioral effects of psychoactive drugs and their effects on sleep has yet to be proved (Feinberg et al., 1975). The AEP changes associated with acute marijuana intoxication are less consistent and have been interpreted as

representing evidence for or being correlates of various changes in attention, arousal, etc. Again, the theories are sometimes elegant and have an intellectual appeal, but they are nevertheless only theories.

A study describing dilated cerebral ventricles in marijuana users as measured by pneumoencephalograms is the only evidence in humans of actual tissue damage associated with marijuana use (Campbell et al., 1971). The sample was small and the measures difficult to determine accurately. Because of the hazards and discomforts of pneumoencephalography it will oe a difficult study to replicate in humans. As is usually the case in similar studies of alcoholics, other drugs and other variables complicate the interpretation of such changes, assuming they are real. But even if it is a combination of marijuana plus LSD plus amphetamine that leads to changes in pneumoencephalograms, it would be of interest to know. The application of computerized X-ray tomography makes further large-scale surveys of ventricular alterations feasible. Assuming that dilated brain ventricles are a rare consequence of marijuana use (dilation is rare after chronic alcohol use), a very large sample of users and controls must be studied.

Longitudinal, ideally prospective studies are needed to properly assess the importance of CNS changes in marijuana users. They are also needed to determine the significance of genetic, immunologic, hormonal, and other marijuana-related changes. Such studies would be of long duration, expensive, and would raise many issues concerned with the rights and protection of experimental subjects. Even then, would publication of the results make much difference in drug-seeking behavior? I think not, even if a clear-cut indictment describing the dangers of marijuana resulted. Consider the data and the results with tobacco smoking. With marijuana the data are not likely to be as clear and consistent. Consider alcohol. After many years of research there is still much disagreement over whether the etiologic agent is alcohol or malnutrition, genetic predisposition, other drug use, etc. (Freund, 1973). Alcohol has been implicated in cases of brain atrophy, with evidence as good as that presented for marijuana. Alcohol has been said to be associated with fetal abnormalities. It can produce profound hormonal changes, cardiovascular changes, and death. Such information has only little, if any, impact on drinking behavior. There is at this time little reason to think that the results of long-term marijuana studies will be any less controversial or have any greater impact on drug use if other powerful forces support marijuana use.

In such situations where scientific data are to be used to shape social, political, and legal decisions, it behooves us all to be even more clear and honest when interpreting and explaining the results of our studies to nonspecialists.

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Chapter 14

Marijuana and Brain Dysfunction: Selected Research Issues

Homer B. C. Reed, Jr.

I will begin by classifying some of the basic problems that arise in looking at the general issues of CNS dysfunction as these relate to marijuana use. Three sets of methodological problems can immediately be identified. One consists of the basic considerations relative to the design of the study: whether it is to be a retrospective or a prospective study; whether one is studying acute effects or long-term effects. A second has to do with instrumentation: these have to do with identifying the measures best suited for examining CNS damage or CNS changes. A third has to do with data analysis.

In terms of design considerations, the obvious question one must ask in the beginning is: What is it we are trying to find out? And conversely, what is it we already know or what is it we think we know?

With regard to CNS damage and marijuana—but let me not use the word "damage," rather the phrase "CNS changes"—it is obvious that there are some kinds of CNS changes in acute intoxication. It is also obvious that these changes, at least in light users, are reversible. To put this another way, marijuana is not a drug that has immediate and dramatic effects on brain functions. Compare, for example, marijuana to strychnine, or to barbiturates. I think it is quite clear that marijuana is a drug that has much milder effects, and the short-term consequences for CNS functions are probably innocuous.

What remains is the important question of identifying long-term consequences. Are there irreversible CNS changes that are seen in association with chronic use of marijuana? This question must be answered in a prospective study for all of the reasons previously detailed. A particularly good model for such research was recently reported, which consisted basically of a prospective study onto which was added a retrospective study, because at the last minute the authors became interested in the prob-

lems of drug abuse. This study, done by Lloyd Johnston (1974), entailed a longitudinal investigation of 2,200 high school students which began when they were in the ninth grade and extended until they were a year past graduation. In his last data collection period he sampled drug attitudes and drug abuse. It is an example of the kind of study which, if the author had been sensitive earlier to the need to know something about drug intake and drug use habits, would have been a useful model to follow. We will need a sample of that kind, followed over many years, to answer certain questions about the effects of marijuana use. But even with the incomplete drug use component, the study produces a number of interesting findings. For instance, there is a pronounced association between drug use and criminal behavior. The association is apparent, however, before drug use begins. There is a pronounced tendency for multiple drug users to have poorer grades than non-drug users, but again it is a tendency that is present before drug use begins. The study is a good example of epidemiological research and explodes a lot of current myths regarding the effects of drug use on a variety of behaviors.

Again, let me emphasize that investigating the long-term effects of marijuana use is going to require a prospective study of enormous magnitude. First we must determine who it is we wish to study. Most of the marijuana research has been done with college-age populations, but we might solve a lot of problems if we studied high school age youngsters, beginning before their drug use becomes frequent and continuing for several years.

The second class of problems I wish to discuss are those related to instrumentation. Here the basic questions to be asked are: What kind of error is one willing to accept, or, What kind of error is one afraid of making? In looking at CNS dysfunction or damage, I think one must choose between procedures which are rarely in error from the standpoint of the number of false negatives and procedures in which the number of false negatives may be quite large, but which will, correspondingly, reveal fewer false positives. For example, if one studies reflex changes, the number of false positives is almost zero. One can be quite sure that given certain kinds of reflex changes one is working with verifiable CNS pathology. Unfortunately, the absence of such changes tells one nothing about the organic condition of the brain. This applies to most of the instruments commonly used in the investigation of brain damage. If one uses an EEG, for example, there are certain EEG criteria that anybody would accept as being indicative of structural changes in the brain. But the absence of such EEG changes is no evidence for the absence of pathology. People die every day with perfectly normal EEGs; to use an even more dramatic example, one can watch the EEG return to normal as the patient continues to grow worse. The association between many

of the EEG measures used to indicate CNS changes and the clinical condition of the patient is approximately zero.

The other consideration in instrumentation has to do with the generalizability of the investigative findings. To the extent that one relies on measures which have a direct validity, such as neurosurgical procedures, and to a lesser degree on radiologic contrast procedures, one diminishes the likelihood of being able to generalize the findings to social situations in which one might be interested. I would submit, as Dr. Jones was arguing, that the reason we are interested in brain dysfunction is because of its presumed behavioral correlates. There are all kinds of brain damage which have no known relationship to any sort of deviant behavior. If one wishes, then, to generalize to situations such as school or vocational productivity or interpersonal relationships, one is, I think, obliged to use behavioral indices of brain damage in spite of the inferential difficulties with which you are faced.

The third set of considerations involve data analysis. Most of the standard statistical procedures for analyzing differences between group performances on specific tasks conceal much more information than they reveal. I have frequently done studies in which it can be demonstrated that the individuals in the study are perfectly lawful and that their test protocols are highly interpretable. Group differences, however, may be entirely washed out. The principal reason for this is that the statistical methods used are sensitive to mean differences and not particularly sensitive to intra-individual effects. These effects can be pronounced, they can be lawful, and they can be quite regularly interpreted, but they may not be revealed in standard data analysis procedures.

The most expeditious way we have found to handle this problem in data analysis is to use expert judges, which in turn means that you have diminished replicability of the study. The Halstead Battery of tests is, I think, the most sensitive battery in existence for detecting brain dysfunction, provided it is used by someone who has had a great deal of experience with it. That constraint, however, eliminates many of the people in the country who would like to use the Halstead Battery. They are not proficient in its use, simply because they have not seen a sufficient number of cases. Those who are competent to use it can use it, I think, with a substantial validity, but it is the kind of validity that people do not like to hear about. It is the validity of a clinical judgment which is not reproducible in terms of statistical analysis and which is very difficult to replicate in different parts of the country because the necessary personnel do not exist in any great numbers.

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Chapter 15

Marijuana and the Central Nervous System: A Discussion

Dr. Cole: I have a question about central nervous system (CNS) function as reflected in driving performance. It seems to me there must be more automobile accidents involving drivers using marijuana. Does anyone have data on this?

Dr. Jones: I'm told that it is not uncommon in California to find marijuana in the car after accidents involving young people. But since no practical blood or urine assays of cannabinoid levels are yet available, the importance of such findings is unclear.

Dr. Nahas: What is your evidence, Dr. Cole, that there are few auto accidents related to marijuana?

Dr. Cole: My evidence is all indirect. Since in Boston the police hate marijuana users, it seems to me that accidents related to marijuana would have been publicized, but they haven't been.

Dr. Harris: There may be some validity to negative associations. Some years ago there was a study which went over arrest records; only a few incidents of reported moving violations or accident reports involving automobiles were associated with possession of marijuana. (*Marihuana, A Signal of Misunderstanding,* 1972).

Dr. Reed: I think Dr. Klonoff at Vancouver studied this specifically and has shown that marijuana *can* interfere with driving. Whether or not it does in real life is a different question, which I think has not been answered.

Dr. Harris: I have a question for Dr. Reed about the statistical handling of his psychological testing data. It is true that psychological data are often not amenable to parametric statistics, but how about non-parametric statistics in which medians rather than means are being compared? Doesn't that work either?

Dr. Reed: No. The problem is not dealing with a single distribution but with a set of interrelationships on a small end of the distribution.

Dr. Harris: That's what non-parametric statistics are designed to handle. It may be that instead of doing analysis of variance, you could usefully assign

positive-negative values and use non-parametric statistics. For instance, if you have 10 subjects and none of them go in one direction but 9 of them go in another direction, that is of statistical significance.

Dr. Reed: You are talking about the performance of these 10 subjects on a particular measure. The changes in CNS that we study are often intra-subject relationships—in other words, motor speed related to pure set-up speed, verbal abilities related to spacial abilities, or the motor skills of the right side of the body versus those of the left side. In other words, I'm talking about intra-individual changes rather than changes on a single test across subjects.

As long as you are looking at a single test distribution you can use sign tests, but what you interpret in a CNS test battery is not the change of a single test but a different set of relationships. Since there are so many contingencies built into the battery, we have found neither parametric nor non-parametric statistics to be adequate. However, certain CNS changes can be consistently demonstrated in that trained raters will come up with the same conclusions. But then you are accused of being a member of an elitist club which others are not competent to join.

Dr. Rose: There seems to be a philosophic issue here that is of interest to me. Dr. Jones, you said significant behavioral changes are observed or caused by marijuana, and yet you state that we don't know enough about the human brain to be able to detect CNS alterations per se. How significant are the behavioral changes? In experimental animals, events that produce significant behavioral changes are associated with measurable alterations in brain activity. Are you saying that not enough is known about what goes on in the brain to predict changes in behavior, or are you talking about the fact that we don't know what changes there are in brain activity?

Dr. Jones: I am talking about both those limitations. There are modest correlations between changes in the electrical activity in the brain and changes in certain behaviors. The study of such relationships has and should continue to generate a lot of research. But I would be reluctant to make clinical decisions, or social and legal decisions, about the safety of marijuana on the basis of such EEG measures. The validity and reliability of such observations are too doubtful.

Dr. Rose: But in clinical pharmacology some predictions can be made about the relationship between drug effects in experimental animals and effects in humans.

Dr. Jones: Only imperfect predictions; if you want to describe the behavioral effects of a new tranquilizer or other CNS drug, it is best to give it to some people and observe its effects on their behavior. You can do some EEG screening in animals, but you can't be certain of human behavioral effects until you try it on humans.

Dr. Harris: I agree with Dr. Jones. The state of the art of translating from our animal experiments to man is very poor. To give you an analogy which was provided by Dr. Donald Kennedy of Stanford, our present direct methods of studying brain function are like a group of Martians who land on the Astrodome and decide to do an analysis of the football game that is going on. Their experts are analogous to our experts: the EEG people stick surface electrodes on the top of the dome; the depth people stick probes down into the dome; and the biochemists go around collecting samples. When they finish their testing the Martians pool their results, which combined look something like this: an audio recording of "get your hotdogs, cold beer"; a chemical analysis of mustard; and several hundred lights from the scoreboard. From this they try to figure out the game. That's really where we are with our direct measurements of CNS activity.

Dr. Jones: I share Dr. Rose's concern that we not appear too nihilistic when studying the effects of low doses of marijuana. We are trying to look at subtle phenomena, and we are dealing with low doses of a substance compared to the astronomical doses of barbiturates, alcohol, and other drugs investigated in past years.

Dr. Falek: In measuring behavioral changes in marijuana users where considerable inter-individual variability is reported, I wonder why the twin study approach is not used more frequently? Investigators are concerned that since they conduct prospective studies they are unable to compare pre-marijuana behavior with what results from drug use. If you had a retrospective study of identical twins who were discordant for use of marijuana, it seems to me that many methodological problems would be obviated.

Dr. Pollin: After years of studying a series of 25 families with identical twins discordant for schizophrenia, I would say that the twin study approach is a seductive methodology, with significant payoffs, but I would not recommend it here.

Dr. Falek: What you did find, though, Dr. Pollin, was that there were behavioral similarities and differences which were measurable between twin partners and among twin pairs. What I hear the speakers describe this afternoon is their inability to specify any behavioral or CNS differences between users and non-users of marijuana.

Dr. Pollin: There are many reasons why I think twin studies may be a methodological approach that we should think of ten years hence but not now. Today, we are not even certain that there is a marijuana abuse syndrome in the same way that there is an alcoholism syndrome. If there are serious health hazards from prolonged overuse of marijuana we should have more documentation than presently available before we launch sophisticated twin studies. What specific kind of pathological change, if any, do we have? If there are changes, what is their significance, and how

pervasive are they? Once we establish definite changes we may be at the stage where we can test relative genetic, environmental, familial, and cultural factors via a twin study. But it seems to me that at this point twin studies would be premature.

There are numerous populations we haven't yet adequately studied. We know, for instance, that the largest percentage increase in marijuana use in San Mateo County, California, which has been studied for six years, occurs in twelve and thirteen year olds. We know that what might happen to kids who go through the rapid changes of adolescence intoxicated with marijuana much of the time is a serious question, but we don't have the answer yet. Although there are hundreds of thousands of such adolescents, practically all of our studies have been conducted on older, college-age populations. We don't have a single good study on young adolescents. It seems to me that such vulnerable adolescent populations, and others which could be similarly defined, should take priority.

Dr. Hollister: Dr. Pollin is saying, "Here is a ready-made, ready-to-go prospective study with an enormous number of subjects." To find enough identical twins discordant for the use of marijuana would be like looking for an adequate sample of one-eyed alligators.

Dr. Nahas: Professor Soueif, in his study of chronic hashish users in Egypt, found that the alteration of psychomotor performance among hashish users is related to educational status (Soueif, 1971). His studies indicate that the higher the educational status the more the psychomotor performance is impaired; or conversely, at the lower socio-cultural level there is little difference in psychomotor performance between smokers and non-smokers. I wonder to what extent this categorizing of marijuana smokers according to educational background is being considered in our evaluations?

Dr. Jaffe: Drs. McGlothlin, Freedman, and Arnold did a follow-up investigation several years ago in which they studied people who had taken LSD in research or psychotherapy settings. Using this group, they were able to rule out gross deficits prior to drug use (McGlothlin et al., 1969). Compared to controls matched for age and intelligence there appeared to be some decrease in performance on tasks involving non-verbal abstraction and concept formation, even though those who had taken the drug appeared perfectly normal and tested out at the same level on standard I.Q. tests. This suggests that if any similar subtle changes occur with marijuana use, they may occur in functions that are not picked up by the tests we ordinarily use, and that the groups that may be vulnerable—those who require a high level of non-verbal abstract ability to perform their jobs—may not be the groups we are studying.

Dr. Freedman: I agree. With the small brain lesion that produces aphasia, the corresponding disabilities for a farmer whose communication is largely non-verbal and for a professional lecturer would be different. Regarding the study Dr. Jaffe referred to, the essential data that came out of it consisted of slight alterations on a few spatial tasks. The question is, were the changes in spatial abilities of any clinical significance? I interviewed a sample of the people in this study and found many to be eccentric in their histories and beliefs. Then I realized that if I took a base line of the California population at that time, I would find many people interested in what were once called "spooky" things: magical, solipsistic concerns ranging from dominant body image preoccupation to explorations—creative or ruminative—of the nuances of inner experience, and the fads and social systems that reinforce such concerns. From a clinical point of view, the best I could come up with was that people who had marked weight problems as kids seemed highly represented in this select drug-using population. But if you approach the world in a skewed way, it may be that the Halstead-Reitan "norms" are not relevant to you. Pre-testing, of course, is missing. Given the lack of identical "slight changes" in the several studies done, we have at this moment a Scotch verdict.

Dr. Mendelson: There has been a controversy raging for years about whether high doses of ethanol are directly toxic to the central nervous system and cause behavioral disorders which are associated with structural change. This question has never been resolved because people who use very high dosages of ethanol generally tend to behave in a disorderly way and to have a variety of nutritional disorders. My point is that ethanol has been studied for a long time and yet the evidence for direct CNS damage is inconclusive; thus, it is premature to pass judgment on the evidence that exists now, for or against marijuana being capable of producing behavioral changes associated with structural changes in the CNS.

Dr. Harris: In addition, studies with alcohol are easier because alcohol is a relatively simple molecule whose metabolism has been worked out. The problem with THC is that right now 18 metabolites are known and more are being discovered every day. Some of these are active while others are not active and may even be inhibitory.

Dr. Hollister: On the theme of relative toxicity, Dr. Mendelson, can you tell us at what level alcohol damages a neuron?

Dr. Mendelson: Some investigators extrapolate that about 75 mg per 100 ml, which is two cocktails, can damage one hundred to one hundred thousand neurons. But this extrapolation is based upon studies of uptake of nucleic acid precursors, and such studies have inherent problems similar to the studies we have discussed. There are no convincing direct data that any finite amount of ethanol produces a specific degree of neuron damage. In

state hospitals one is constantly confronted with individuals having a past history of very heavy alcohol use, who presumably have a fair degree of cortical atrophy and impairment in various behavioral functions. But they also have had numerous episodes of infectious disease; they have been eating rather poorly; and they have vitamin B deficiencies as shown by peripheral neuropathies. So whether ethanol is a causal mechanism in irreversibly damaging a neuron has not yet been definitively established.

Dr. Harris: I find it hard to believe that every time you drink two cocktails you damage ten to the fifth neurons, especially since there are only ten to the tenth neurons in the brain. A thousand drinks would damage about five to ten percent of the central nervous system.

Dr. Mendelson: Those are not my data, but were generated from studies by Noble and his associates on impaired uptake of labeled precursors into the CNS protein.

Dr. Hollister: As we discussed regarding marijuana, impairment of uptake doesn't mean there would be a permanent change. Speaking of permanent change, one of the issues raised by Campbell's study was the suggestion of marijuana-induced cerebral atrophy. This study was especially frightening because nobody is going to be able to replicate it. A new non-invasive technique, using the EMI Scanner, could possibly settle that issue. If we can verify cerebral atrophy, it would be tremendously important; if the claim of atrophy is fallacious, it would be well to get rid of it.

Dr. Goodwin, you have been silent while we have been talking about alcohol. Would you care to comment on its toxic effect?

Dr. Goodwin: With respect to *in vitro* studies of alcohol and neurons, it requires higher alcohol levels than can be obtained by drinking to alter significantly oxidative processes or respiration of the cell. It takes far higher limits than you could possibly put into yourself to have any effect upon nerve potential. I'm not sure about the amounts that are required to alter sodium or potassium sensitive ATPase, but it seems to me that it is a considerable amount.

Dr. Mendelson: It is high. It is a level that would exist in an individual who was drinking near lethal levels of alcohol. The dosage would be equivalent to about 600 mg per 100 ml.

Dr. Goodwin: Of the several pneumoencephalographic studies of alcohol I am familiar with, only one of them had a control (Haug, 1968). And in that study, unfortunately, there was a considerable age difference between the experimental group and the controls. Because there is naturally some dilatation with age, the results are difficult to interpret. The psychometric studies are a true hodge-podge. The I.Q.'s of alcoholics don't change at all; every study is consistent in that regard. There is some consistency with the Halstead category tests which measure abstracting ability; four studies of

alcoholics have shown some defects (Fitzhugh et al., 1962; Goldstein & Shelly, 1971; Jones & Parsons, 1971; Smith et al., 1973). But these findings were obtained within a two to three week period after the patients had been admitted to the hospital to be detoxified. As with marijuana, the question remains open as to the reversibility of these subtle kinds of changes. Clinically, the impression I have of alcoholics is that after three months of drying out, they appear to be very much intact.

Dr. Hollister: I think that is reassuring to this somewhat biased, pro-alcohol panel. Everyone seemed very discouraged that 75 milligrams per hundred ml might do you in.

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Chapter 16

Psychiatric Consequences of Marijuana Use: The State of the Evidence

Roger E. Meyer

The Fourth Marihuana and Health report of the Department of Health, Education and Welfare (1974) serves as a benchmark of how far we have come in the past seven years in defining the psychopharmacology of marijuana. It has been clear all along that scientific advances would not resolve many aspects of the marijuana controversy. Indeed, scientists have been labeled as pro or con on the basis of their scientific findings and the interpretations that could be applied thereto. One question that persists concerns the psychiatric consequences of different degrees of marijuana use. The literature is a crazy quilt of clinical descriptions occasionally backed by the "implications" of laboratory investigation. The problems in this field are not unlike the problems in the rest of psychiatry: we have difficulty agreeing on syndrome definitions, and the issues of cause and effect are hard to separate from mere association.

Acute Adverse Reactions

The syndromes which are easiest to relate to marijuana use are those which are temporally contiguous to the consumption of marijuana and can be confirmed by experimental laboratory research. Isbell (Isbell et al., 1967) and Hollister (Hollister et al., 1968) demonstrated in the laboratory that, in sufficient doses, tetrahydrocannabinol (THC) produced subjective effects that could not be distinguished from LSD. Talbot and Teague (1969) described twelve cases of acute toxic psychosis in Vietnam soldiers using more potent forms of marijuana. Chopra and Smith (1974) described a group of patients exhibiting symptoms of "an acute toxic psychosis manifested by excitement, confusion, disorientation, delusions, visual hallucinations, depersonalization, emotional instability, and a delirium. The

symptoms were of short duration, varying from a few hours to a few days, following which they quickly reverted to their previous normal state." In the latter sample, potency and dosage schedule of marijuana, as well as younger age, were generally related to the occurrence of the "toxic psychosis." Thirty-eight percent of 150 self-selected student marijuana users in Tart's sample acknowledged that they had seen other people "freak out" on marijuana (Tart, 1970).

Weil (1970) differentiated "panic reactions" from "toxic psychoses" in the general category of acute adverse reactions. He felt that the vast majority of all acute adverse reactions to marijuana were panic reactions in which the users interpreted the physical or psychological effects of the drug to mean that they were dying or losing their minds. He felt that toxic psychoses, in contrast, were "temporary malfunctions of the cerebral cortex due to the presence of toxins in the body; they disappear when the toxins disappear." He classified the clinical manifestations of the toxic psychosis in terms of the following characteristics: disorientation, confusion, auditory and visual hallucinations, and a prostrate appearance. In general he felt that these symptoms resembled the delirium of high fever.

In essence, the evidence favoring the existence of acute adverse reactions under marijuana comes from the following sources:

I. Case finding and identification
   A. The adverse reactions that come to treatment (Talbot and Teague, Weil, Chopra and Smith)
   B. Surveys of known samples of marijuana users (Tart)

II. Acute administration of marijuana to human subjects in the laboratory; the subject's responses suggest the likelihood of adverse reactions under non-laboratory conditions (Isbell, Hollister)

The evidence seems firm. Those like Weil and David Smith (Smith, 1968) who argue that toxic reactions are relatively uncommon favor the explanation that set, setting, and personality factors are responsible for most acute adverse reactions. If the majority of acute adverse reactions occur among relatively naive users in unfamiliar settings, then Weil's interpretations of the data would seem to be correct. It is my impression that casual users who first experience (unexpectedly) the potential hallucinogenic effects of marijuana may also experience a panic reaction.

The crucial factor, I feel, in describing the toxic psychosis concerns errors in judgment. Frosch (Frosch et al, 1965) differentiated toxic reactions to LSD on this basis; the same rule should apply to toxic marijuana reactions. The presence of paranoid thinking and hallucinations in a person who has taken a hallucinogenic drug is not specifically a toxic reaction.

Fleeting paranoid thoughts and hallucinations may be frequent concommitants of the marijuana experience, particularly with more potent preparations. Deficits in judgment and the presence of confusion and/or delirium define a toxic reaction (e.g. Talbot & Teague, 1969), while panic reactions consist of overwhelming anxiety, a fear of going crazy or dying and/or a sense of helplessness and loss of control in response to drug-induced symptoms. In more psychoanalytic terms, the observing ego is intact and can be influenced by outside reassurance in panic states. In toxic states, there are serious deficits in observing ego functions. Toxic reactions should also be dose-related, while panic reactions may occur at any dose which is unfamiliar to the user. Factors relating to set, setting, and/or personality which may lead the user to respond to the pharmacological effects of the drug with severe anxiety are generally responsible for acute panic reactions.

Apart from the subject of syndrome definition, the subject of frequency and relative risk becomes even more difficult to resolve. Case reports of adverse reactions cannot clarify the frequency of occurrence among the large population of persons who have experimented with or used marijuana on a casual or a regular basis.

Laboratory data from the 1940's (Mayor's Committee on Marihuana, 1944; Williams et al., 1946) suggested a high frequency of acute adverse reactions in a research setting, but these occurred among research subjects who were prisoners (and therefore not "normal subjects in a normal setting"). Mendelson, Meyer, and Rossi (1972) found a single acute adverse reaction in one casual user (among a population of 10 heavy and 10 casual users) on the first day of marijuana smoking. Nine of the ten casual users had gathered in the day room to light up early on the first smoking day. They had specifically excluded the tenth member of the group who had held himself aloof during the first five-day non-smoking period. When this individual discovered that the others had started without him he dressed himself in a suit and appeared in the day room, identifying himself as a member of the research staff. This caused one of the nine smokers to panic and to fear that he was losing his mind. He was reassured by the staff psychiatrist over a one to two hour period. He chose to continue in the experiment and experienced no further adverse reactions although he continued to smoke marijuana. These data from the initial report by our group (Mendelson et al., 1972) and the follow-up report by Mendelson et al. (1974) suggest that the frequency rate of panic states with marijuana may not be insignificant. Such reactions appear to be due principally to factors of set, setting, and personality. The laboratory data also suggest the extreme fluidity of the mental state, which may be significantly influenced by authoritative reassurance.

Tart's survey (1970) of marijuana users suggests a method of approaching the definition of frequency. The two limitations in his results involve his failure to validate by actual observation the reported marijuana experiences of his respondents, and the failure of 600 of his 750 potential respondents to complete the survey. Surveys of larger samples of marijuana users, plus additional laboratory studies of repeated marijuana administration in in-patient settings, should help to clarify the risk of acute adverse reactions. They will likely confirm that the risk can be significantly reduced by attention to set and setting.

Flashbacks

The second general type of psychiatric consequence of marijuana use is the "flashback." Two types of flashbacks are described. Smith (1968) and Weil (1970) report the occurrence of flashbacks among marijuana smokers who have also used hallucinogenic drugs such as LSD. Keeler et al. (1968) have attributed flashbacks to effects of marijuana per se, presumably in the absence of significant hallucinogen use. Smith (1968) feels that if such "marijuana only" flashbacks occur they are extremely uncommon among the population of marijuana users he has known in the San Francisco area. Again, as with acute adverse reactions, the risk of flashbacks cannot be defined by clinical reports. Surveys of marijuana users, with attention to histories of other drug use (particularly hallucinogens), will be necessary to define the relative frequency of flashbacks. These data might also be obtained by follow-up studies of research subjects who have participated in acute and longer-term marijuana experiments.

Prolonged Reactions

It is in the area of prolonged reactions that the greatest controversy about the psychiatric consequences of marijuana use resides. The syndromes which have been attributed to marijuana include the following:

I. Psychotic Reactions
   A. Triggering of a schizophrenic reaction
   B. Cannabis psychosis
II. Non-Psychotic Reactions
   A. Character change and alterations in life style
   B. Neurotic levels of anxiety and depression
   C. An amotivational syndrome
   D. Heavy use of other drugs

In all of these conditions it has been especially difficult to separate the issues of cause and effect from mere association. Does marijuana trigger acute psychotic reactions in vulnerable people (Smith, 1968; Weil, 1970)? Is

heavy (or any) marijuana use merely a symptom of already severe psychopathology or is marijuana being used as self-medication to prevent more serious psychiatric sequellae (e.g. psychiatric hospitalization) (Grinspoon, 1970)? Is there a specific constellation of psychotic symptoms unique to users of marijuana that may result in chronic hospitalization (Dhunjibhoy, 1930; Chopra & Chopra, 1957; Christozov, 1965)?

Triggering of a Schizophrenic Reaction

Most authors agree that marijuana can precipitate schizophrenic syndromes in vulnerable individuals. Smith (1968) and Weil (1970) felt that the psychosis can be related to the personality structure of the user rather than the pharmacology of the drug. Chopra and Smith (1974) described a group of patients, with histories of personality or psychiatric disorders, whose underlying psychopathology emerged with greater intensity as schizophrenic and paranoid symptoms after acute marijuana intoxication. In general, there seems little disagreement that marijuana can precipitate psychosis in vulnerable individuals. The key question concerns the diagnosis of the premorbid state in the cases of psychosis which have been referred for psychiatric treatment. Recent studies (Lemberger et al., 1970) indicate that intravenous THC persists in plasma for three days, and its metabolites are excreted in urine and feces for more than eight days. This finding confounds the difficulty of separating the toxic and personality variables which may be involved in triggering an acute schizophrenic reaction during marijuana intoxication.

The clinical case material interpreted by the relative advocates of marijuana use tends to emphasize pathological premorbid personalities of psychotic users, without offering adequate case histories. Interpretations of case material made by those who oppose more liberal laws generally emphasize a pathological view of marijuana. The same arguments have been advanced in the past for LSD and the hallucinogenic drugs. One exception was a clinical study by Bowers who compared 12 patients with acute psychotomimetic drug-induced psychotic reactions with 26 patients hospitalized for acute psychotic reactions unrelated to drug abuse (Bowers, 1972a; Bowers, 1972b). He found that his population of drug users had good premorbid histories and gave evidence of decreased central 5HIAA formation. These biochemical changes, which were analogous to data obtained in animals given LSD, persisted during phenothiazine treatment. The data suggest that LSD may serve more than a triggering function in the genesis of certain schizophrenic reactions. On the other hand, it may be, as Bowers notes, that vulnerability to a psychotic reaction under LSD is a discreet ingredient in the structure of psychotic states, separate from the issue of premorbid personality characteristics and social development.

The data for marijuana are less clear. Kolansky and Moore (1971) described 8 psychotic reactions in a population of 39 marijuana smokers between ages 13 and 30. Four of these patients made suicide attempts. The cases were described sketchily, and the authors failed to document their assertion that there was no evidence of a predisposition to mental illness in their patients. They also described a neurological disorder without a neurological examination. Wurmser et al. (1969) described a series of cases which included several psychotic individuals who seemed surprisingly responsive to the psychotherapy that was offered to them.

Throughout these various papers one is struck by the relative paucity of case material, and one wishes for some of the clinical research sophistication that Bowers employed in his study of the psychotomimetic drug-induced psychoses. In his work, acute symptoms were scored by means of the Brief Psychiatric Rating Scale (Overall & Gorham, 1962). In addition, adolescent social adjustment (Gittelman-Klein & Klein, 1969), intelligence (Offord & Cross, 1971), and bizarreness of delusions (Freeman et al., 1965) were rated by means of a three-point scale. Finally, the Stephens-Astrup prognosis form (1963) was scored in order to classify the prognosis of patients based upon premorbid and family history variables.

Beyond the need for more sophisticated clinical case reporting, it would be useful to compare the premorbid histories of marijuana users who became psychotic with those who have continued marijuana use without apparent adverse effect. Marijuana users who become psychotic should also be compared with psychotic individuals of similar age without a history of drug use (see, e.g. Bowers). Finally, the ages 15 to 25 are years of risk for a first episode of schizophrenic psychosis. Utilizing the methods of Robins and Murphy (1967) it should be possible to carry out a follow-up study of individuals who turned 15 in 1965 and determine the prevalence of psychotic episodes among users and non-users of marijuana in 1975. By the same survey it should be possible to ascertain the risk of other presumed psychiatric consequences of marijuana.

Cannabis Psychosis

The concept of a specific marijuana psychosis appears largely in the Eastern literature. McGlothlin (1972) has compared the patterns of smoking marijuana in the East and in the West and feels that the quantity of marijuana smoked in the East far exceeds that which is normally smoked among young people in the United States. Dosage issues apart, the psychiatric literature from North Africa, Egypt, and India is not comparable to the psychiatric literature in the United States. Most of the Eastern literature supports the notion of an acute marijuana psychosis associated with very heavy use and lasting one to six weeks. There is fairly general agreement

that persons suffering from marijuana psychosis do not develop psychotic thoughts or systems which are characteristic of schizophrenia. The Moroccans, in particular, emphasize an excited, confused, manic state which may lead to impulsive acts of violence (Benabud, 1957; Defer and Diehl, 1968). Curiously, there is also some residual amnesia.

The Eastern literature (McGlothlin, 1972) also refers to a long-lasting marijuana psychosis in which the psychotic episodes do not clear in the usual time but persist in residual form or as recurrent episodes over repeated intoxications. The problem of relating marijuana psychosis to Western countries involves differences in smoking patterns in the East and West, the difficulty of translating the psychiatric symptom picture from one body of literature (and culture) into another, and the impossibility of generalizing from cases which come to psychiatric attention to the general marijuana-user population. Premorbid characteristics are not described adequately in the Eastern literature, making it impossible to sort out the specific role of marijuana. However this does not mean that such syndromes do not exist. Interestingly, NIDA-supported studies of marijuana use in Jamaica (*Marihuana and Health*, 1972) and Greece (Freedman & Fink, 1972) failed to document the existence of a specific marijuana psychosis. It would be useful to extend this work to studies in India and Morocco where these syndromes have been described.

Non-Psychotic Prolonged Adverse Reactions

The issues of change in lifestyle, chronic anxiety, depressive symptoms, and an amotivational syndrome are linked theoretically by a few observers to some of the pharmacological properties of marijuana. One question of great importance in this discussion concerns the risk of habituation. Substances such as alcohol, opiates, and barbiturates which result in compulsive patterns of use are associated with changes in lifestyle and focused drug-directed motivation. McGlothlin and West (1968) have described an amotivational syndrome among marijuana users: "Changes include apathy, loss of effectiveness, and diminished capacity or willingness to carry out complex, long-term plans, endure frustration, concentrate for long periods, follow routines, or successfully master new material. Verbal facility is often impaired both in speaking and writing. Such individuals exhibit greater introversion, become totally involved with the present at the expense of future goals, and demonstrate a strong tendency toward regressive, childlike, magical thinking." McGlothlin (1974) has listed four ways in which marijuana may enter into the amotivational syndrome:

> 1) Persons who exhibit these traits may simply be attracted to the use of marihuana... 2) The individual may focus so much

> of his time and energy about cannabis use and associated activities that this largely substitutes for other behavior... 3) The passivity may be causally related to cannabis use through learning... 4) Repeated exposure to cannabis may result in a chronic brain syndrome.

Is Marijuana Use Habituating or Addicting?

Drugs of abuse may be characterized on the basis of whether they are reinforcing in the monkey self-administration paradigm (Schuster & Villareal, 1968; Thompson & Pickens, 1970). Opiates, hypnotic sedative drugs, alcohol, amphetamines, cocaine, and nicotine are reinforcing in this paradigm, and patients presenting with these forms of drug abuse usually enter treatment in order to stop the use of drugs. In contrast, hallucinogenic drugs are not self-administered in this paradigm, and users of hallucinogenic drugs (when they come to a physician) enter treatment for relief of acute or chronic adverse reactions rather than for the behavioral modification of a compulsive habituation.

The question of marijuana reinforcement is crucial to our definition of an amotivational syndrome. More recent studies (Harris et al., 1974; Leite & Carlini, 1974; *Marihuana and Health,* 1974) using various models of self-administration suggest that animals cannot be trained to self-administer THC; THC does not seem to be reinforcing in the self-administration paradigm. Thus it should be possible to convince patients to stop their use of marijuana, and compulsive use of the drug should be relatively rare. Evidence from the Eastern literature (Soueif, 1967; McGlothlin, 1972) suggests that heavy use is not uncommon; and daily use of marijuana in the United States seems to occur with a frequency of approximately 10 percent of the marijuana-user population (*Marihuana, A Signal of Misunderstanding,* 1972). On the other hand, data provided by Wurmser (Wurmser et al., 1969) and by Kolansky and Moore (Kolansky & Moore, 1972; Mendelson et al., 1972) suggest that patients can be persuaded to stop marijuana use, with apparently some remission of pathological symptoms. Thus the psychological dependence associated with marijuana consumption does not appear to be based upon primary reinforcing properties but rather upon some complex interaction between social and psychological variables on the one hand and subjective variables on the other. The situation is more similar to hallucinogenic drug use than to the use of opiates and barbiturates. If lack of motivation is a function of daily intoxication, then it should be possible to treat the amotivational syndrome by getting the person to stop daily use.

The question of physical dependence should also be examined in trying to understand patterns and consequences of marijuana consumption.

Some animal data have suggested the possibility of a withdrawal syndrome secondary to marijuana (Deneau & Kaymakcalan, 1971). These data have not been confirmed. Bernstein et al. (1974) failed to find physiological evidence of withdrawal among marijuana users who were exposed to 21 days of self-determined marijuana smoking on a research ward. Curiously, the smokers gained weight during periods of marijuana consumption and lost weight during the drug-free period, but there were no physiological signs of withdrawal. More recently, Jones and his colleagues (1974) have reported a withdrawal syndrome after oral administration of THC and marijuana extract. This group administers marijuana every four hours around the clock in high doses. Under these conditions, the withdrawal syndrome does occur. It may be that when high blood levels are maintained by a q. 4 hour medication schedule, an abstinence syndrome may occur on withdrawal. Jones' data do not, therefore, contradict Bernstein's findings. Moreover, it is not clear that the abstinence syndrome described by Jones is associated with a subjective sense of craving for marijuana or THC. Some physiological symptoms may occur upon withdrawal of phenothiazines and tricyclic antidepressants, but drug-seeking behavior is not a part of this picture.

The issue with regard to marijuana is still uncertain. Jones' data should cause us to rethink the question of physical dependence and the liability of habituation among daily users. His data are also consistent with data obtained in interviews with Egyptian users of hashish who reported an inability to stop their drug use and a craving for the drug during drug-free periods (Soueif, 1967). At this writing, it appears that the patterns of marijuana use and dosage that have evolved in the United States are not marked by compulsive smoking behavior (as is the case with tobacco) and/or abstinence distress. On this basis we cannot conclude that a syndrome of focused (drug-directed) motivation exists. If an amotivational syndrome occurs, it does not occur on this basis.

Does Marijuana Cause Work Impairment as a Function of Behavioral Toxicity?

Addiction per se does not cause work disability. Methadone-maintained opiate addicts are able to work in a wide variety of jobs (consistent with their basic skills) once they have become tolerant to some of the depressant actions of the drug. Are marijuana users able to work while intoxicated? In our initial study for the National Commission on Marihuana and Drug Abuse, Mendelson, Meyer, and Rossi (1972) found that research subjects could maintain a very high work output for reinforcement points obtained by operating a hand counter while they were smoking marijuana and experiencing the maximum effects of the drug. The reinforcement points could be converted to purchase marijuana cigarettes or banked for

collection as money later. The data were compared with earlier data obtained with alcoholics, who regularly stopped work during periods of intoxication (Mendelson & Mello, 1966). Moreover, alcoholics frequently attempted to terminate participation by overt or covert aggressive behavior when intoxicated. All subjects in the National Commission study expressed a strong desire to complete the research program and rarely attempted to impede the research. Of particular significance was the fact that all subjects maintained interest in and participation in a variety of personal activities such as writing and reading literature, interest in and knowledge of current world events, and participation in both athletic and aesthetic endeavors.

More recently, Mendelson was quoted in *Science* magazine (1974) regarding the results of studies of heavy and casual users completed for the Department of Defense (Mendelson et al., 1974) subsequent to the National Commission inquiry. He has found that casual users showed a strong decrease in daily operant work output with increasing marijuana consumption, while heavy users maintained a stable operant work output (in relationship to the number of cigarettes smoked). Since subjects did much of their smoking during the evening hours and the effects of marijuana may be persistent, marijuana-influenced work output could have been more reflected on the day following marijuana consumption. When the operant points were plotted against the number of cigarettes smoked on the previous day, both heavy and casual users manifested progressively lower work outputs as a function of increasing marijuana use on the previous day. The *Science* article implied that the investigators have concluded that their data justifies a rethinking about the amotivational syndrome. An unpublished manuscript by Greenberg (1974) and the report itself, are appropriately more cautious in their conclusions. The authors note that "motivation" may be as much a function of situational variables as of drug influence. Moreover, there are serious methodologic questions that need to be clarified before assigning a meaning to the observed work decrement. An amotivational syndrome would imply that the marijuana users in Mendelson's experiment had lost interest in work for money. If the work decrement were a function of a drug-induced impairment of performance, this would not be lack of "motivation."

In the Mendelson experiment (1974) subjects were working for reinforcement points that could be converted to marijuana cigarettes or money. It is probable that during the marijuana period they were working mostly for the cigarettes. If this is the case, their work output would be expected to decrease once they had obtained all the cigarettes they desired. Because of the experimental method used, it is impossible to determine whether there was a real decrement of work output for money or whether the work decre-

ment was a function of satisfaction of interest in working for marijuana. Moreover, the authors did not contrast the work decrement observed on a fixed-interval schedule with their previous failure to demonstrate a work decrement on a fixed-ratio schedule (Rossi et al., 1974). Animal studies have indicated that reinforcement schedule behavior is depressed in a dose-related manner by cannabinoids under differential and temporally related schedules but not in ratio schedules (*Marijuana and Health, 1974*; McMillan, in press). Moreover, the effects of marijuana on time estimation might lead to specific impairments of temporal schedules of operant behavior on a pharmacological basis rather than on the basis of altered motivation. It is of particular interest that most animal studies have demonstrated decrements in performance in acute studies which disappear with the development of tolerance (Ferraro & Grisham, 1972; Harris et al., 1972). It is possible that heavy and casual users exceeded their normal intake of marijuana on the ward, resulting in the impairments that were observed.

In his unpublished review, Greenberg noted that Rubin and Comitas (1972) found that there were no reductions in work output among marijuana users in natural settings in Jamaica. The tasks for these marijuana-using Jamaican workers were analogous to the fixed-ratio operant schedule of the National Commission study. The more recent Mendelson study (1974) is consistent with acute laboratory studies in man that suggest drug-related impairments of performance of certain tasks. These tasks involve complex psychomotor functions: goal-directed serial alternation (Melges et al., 1970), complex reaction time (Clark & Nakashima, 1968), time estimation (Meyer et al., 1971), short-term memory (Tinklenberg et al., 1970), and reading comprehension (Clark et al., 1970) will all show impairment. There is a loss of selective attention, immediate recall, and systematic thinking which suggests the likelihood of work-related deficits in natural settings among chronically intoxicated users not tolerant to these symptoms of behavioral toxicity. Again, these are specific deficits in work performance and are not specifically related to motivation. If one found compulsive marijuana-related behavior *and* decrements in work performance, one would have the ingredients for an amotivational syndrome such as one finds among alcoholics. These characteristics have not been clearly established for marijuana; and they have not been demonstrated to be a consequence of marijuana use in the United States.

Apart from the issues of habituation and specific work task impairment, changes in lifestyle and the amotivational syndrome have been attributed to specific pharmacological effects of marijuana and/or other hallucinogenic drugs. On this basis, the amotivational syndrome could also

result from casual or non-daily use: the passivity would be on the basis of learning or a chronic brain syndrome (McGlothlin, 1974).

In studies of acute marijuana administration, Melges et al. (1974) found that oral doses of marijuana extract produced a greater concentration on the present and a shortening of the span of awareness into the future. The greater concentration on the present was associated with a euphoric mood. This change in the temporal span of awareness is presumably related to changes in time estimation under the drugged condition. Marijuana speeds up the subjective (internal) clock so that time appears to be passing more slowly in the external world. At higher doses there is a sense of timelessness. There seems to be no question that acute marijuana intoxication is associated with a passive attitude. The relationship between this passive attitude and an evolving lifestyle is subject to question. It is possible that chronic heavy smokers of marijuana will consistently adopt the passive attitude even while they are not intoxicated. Tucker et al. (1972) compared standardized Rorschach evaluations of the thinking of hospitalized schizophrenic and non-schizophrenic drug abusers with similar hospitalized psychiatric populations of non-drug users. Their results showed a tendency for drug users (regardless of diagnosis) to have more signs of increased intrusion of primitive drive material, and a conceptual boundary disturbance which marked the drug users as different from other patients. These changes were related to length of drug use over time more than to the variety or amount of drug use. Culver and King (1974) compared groups of LSD-mescaline users with marijuana-hashish users and non-drug-using controls among a class of college seniors. Subjects were tested on the Halsted-Reitan battery of neuropsychological tests, the Wechsler adult intelligence scale, a laterality of discrimination test, and three tests of spatial perceptual abilities from the Educational Testing Services manual. The LSD-mescaline users performed within normal limits, but significantly worse than the marijuana users or the non-drug-using controls. The data on marijuana users are consistent with the data obtained by Reed (1974), who employed the Halsted-Reitan battery, a tactual performance test, the Seashore rhythm test, and a finger-tapping test on the 20 heavy and casual user subjects in the studies carried out for the National Commission on Marihuana and Drug Abuse by Mendelson, Meyer, and Rossi (1972). Curiously, although the heavy and the casual users differed significantly in their use of hallucinogenic drugs (the heavy users tended to be heavy hallucinogen users), there were no significant differences between the heavy and casual users on any of the test procedures. These data should be contrasted with the allegations of Kolansky and Moore (1971) who postulate a "specific pathological organic response in the central nervous system to cannabis products" on the basis of their clinical experience with

13 adults between 20 and 41 years of age and 38 "adolescents and young adults." They refer to the findings of Campbell et al. (1971) who claim to have demonstrated cerebral atrophy by air encephalography in 10 individuals who had smoked marijuana for three to eleven years. Kolansky and Moore state that persons with a history of regular marijuana or hashish use (three to ten times per week) will manifest symptoms of apathy and sluggish mental and physical responses. They will also show a loss of interest in personal appearance and a flattening of affect. They state that "the symptoms of mental confusion, slow time sense, difficulty with recent memory, and the incapability of completing thoughts during verbal communication... seem to imply some form of organicity either of an acute biochemical nature or ... structural encepalopathy when found in cases with prolonged heavy marihuana use." The inadequacy of the methods used by Kolansky and Moore coupled with the absence of a comparison group of non-users with the same life style (or users without these symptoms) raises serious questions about their conclusions. Their methods were limited to a mental status and routine psychiatric history examination of 13 patients who were seen as part of a psychiatric consultation practice involving approximately 100 patients per year. They interviewed each patient as well as his family approximately four to ten times "in order to establish the patient's history and mental status." They also observed that "a cessation of the smoking resulted in either total or partial remission of symptoms so that only a minimal supportive follow-up was necessary." They attributed the symptoms to unspecified "biochemical changes" and "biochemical changes with structural changes." Not only do the authors provide no evidence to support such terminology (biochemical and structural changes), they also fail to document the methods that were used in determining "distortion of time sense," confusion, "memory difficulty," and other deficits in the mental process. The authors rely on a simple description of psychiatric syndromes (really, behaviors) in which there is a history of marijuana usage. They describe these syndromes through the initiation, continuation, and termination of marijuana use. The syndromes described in their initial paper are so varied as to defy common definition apart from the associated history of marijuana use.

The effects of marijuana use upon psychosocial adaptation cannot be established by individual case histories. These reports lack controls or comparison groups. It is impossible to isolate the effects that may be directly attributable to the drug, predisposing personality characteristics and/or environmental factors. McGlothlin (1972) suggests that moderate regular users (who do not also use other intoxicants) be compared with non-users who have been matched on the basis of age, education, social class, and other relevant variables. If there are no significant differences in health and

social functioning in the two groups, then it is possible to argue that the effects of cannabis use of specific frequency and dose are negligible. If the marijuana group is different with respect to certain variables, the difference cannot be specifically attributed to marijuana but might suggest a relationship. In establishing a cause-and-effect interaction between tobacco smoking and cancer of the lung (and other serious illnesses), large groups of heavy smokers have been compared with non-smokers and smokers of different intensity. The same sorts of studies need to be applied to this field.

Some preliminary work has been completed. Walters et al. (1972) compared drug users and non-users at Harvard University, and found that drug use (mostly marijuana) was related to differences in "self-concept, values, and attitudes" as suggested by subjective alienation, visits to a psychiatrist, less definite career plans, and sexual activity. All of these characterized the user before he began drug use, and were unrelated to his "official" college life. Halikas et al. (1972) compared 100 regular marijuana users and 50 non-user friends in a psychiatric interview. Half of each group fulfilled criteria for some psychiatric diagnosis. Almost every diagnosed psychiatric illness among the users began before marijuana use.

Brill and Christie (1974) have been carrying out a longitudinal study of a representative sample of 1970 college students in an attempt to sort out the relationship between marijuana use and psychosocial adaptation. No significant difference in grade point average or educational achievement was found between users and non-users, but marijuana users experienced more difficulty in deciding on career goals, and left college to reassess goals somewhat more frequently than non-users. Moreover, a smaller percentage of regular users was seeking advanced or professional degrees as compared with non-users or occasional users. Regular users were more likely to give either a B.A. or "other" as their ultimate educational goal. Of particular interest was the observation that only 6 percent of non-users reported a worsening of their emotional state since their admission to the college. Ten percent of users reported a worsening of their emotional state; 20 percent of those using for seven or more years reported negative changes in their emotional state. This finding suggests that regular marijuana use has a deleterious effect on mental health; or, that persons suffering from significant emotional difficulty in a setting where marijuana is used may use marijuana longer and more intensively than "normal persons."

One of the problems that Brill and his associates encountered was difficulty in finding marijuana users who had not experimented with other drugs. The longitudinal study also suffered from the loss of a significant percentage of the initial sample over a three-year period. Moreover, because Brill did not interview or otherwise examine his subjects, he was merely reporting their own assessment of their psychosocial adaptation. He con-

cluded that "the absence of a general relationship between the degree of marijuana involvement and emergence of poor adaptation in representative studies of using populations here and in Jamaica (McMillan, in press) ... suggests that idiosyncratic factors are likely involved when an association between marijuana use and psychosocial disorder is observed." Because of methodologic procedures, Brill's assessment is not completely justified by his data, although it is very likely that he is correct in his judgment.

Another non-psychotic prolonged adverse reaction that has been attributed to marijuana use in the United States is the association, in heavy users, of marijuana consumption with the use of other drugs. The progression hypothesis, which has been evolved to explain this, is one example of a theoretical construct that has been reified by repetition. In a previous review of the literature (Meyer, 1972), I could find only one published report which attempted to conclude that marijuana led to heroin use among urban addicts. Ball, Chambers, and Ball (1968) found that marijuana use preceded heroin use in this population. Yet, as discussed above, the assessment of risk of any outcome of marijuana smoking can only occur in a population of marijuana users. By looking for a history of marijuana use in a population of heroin addicts, the outcome had already been decided. Indeed, the authors could have chosen other antecedent variables such as education, cigarette smoking, delinquent history, or place of residence and come to the same conclusion. It is a philosophical truism that looking backward always indicates a single path while looking forward suggests an infinite variety of choices. There is no evidence to support a progression hypothesis among marijuana users.

However, there is a strong correlation of polydrug use (and intensity of polydrug use) with frequency and intensity of marijuana use (King, 1969; Goldstein et al., 1970; Goode, 1971; Mirin et al., 1971). We have been unable to find persons who had used marijuana 20 to 30 times/month who had not also used hallucinogenic drugs (Mirin et al., 1971). Blum and associates (1969) described "a general disposition toward psychoactive drug use" which may broadly explain these observations. In another paper I quoted Balter who observed that when a substance is widely used in a given population (e.g. alcohol), the most psychologically disturbed individuals in that population will use the substance most intensively (Meyer, 1972). When a substance is used by a small portion of a population (e.g. heroin), the mere use of the substance suggests significant psychopathology in the individual. In the past ten years marijuana use has moved from deviant to normative behavior among the young. Thus the relationship between marijuana use of different intensities is most likely a function of psychopathology. The relationship between that pathology and the use of marijuana is necessarily complex. In the absence of contradictory evidence,

however, I do not believe that the heavy use of marijuana is a benign experience. This is true of any substance use.

In the context of the tremendous changes in values and lifestyle which have occurred over the past ten years, it may be extremely difficult to sort out a specific etiological role for marijuana. While the data are not definitive, they do suggest that marijuana use is more likely to be one aspect of social change rather than the responsible agent. The persistent case reports suggesting clinical risk of marijuana use need to be put into some broader perspective. A detailed follow-up study using psychiatric interviews and assessments of medical status and social performance need to be carried out utilizing models which have been developed by Robins and Murphy (1967) and by Valliant (1974). It is suggested that high school classes graduating in 1965 be chosen for a follow-up study of medical, social, and psychiatric status. Individuals would be chosen on a random basis, and drug or marijuana use would not be a factor in selecting the sample. Marijuana users of different intensities could be compared with non-users in order to sort out the role of marijuana, if any, in determining ten-year outcome. Alternatively, there could be a follow-up of survey respondents who were questioned about their drug use in 1966, in order to determine how they have fared. The population whose 1966 drug history was known would be selected by one group of investigators and evaluated (i.e. outcome) by another group of investigators. The Vietnam follow-up study was a model for this type of research (Robins, 1974).

Despite negative reports of adverse effects from Jamaica (McMillan, in press) and from Greece (Freedman & Fink, 1972), a well-documented set of appendices prepared by the National Commission on Marihuana and Drug Abuse, and a continuing research effort, the marijuana controversy rages on. The problem of measuring risks of tobacco smoking should reassure us that epidemiological and laboratory investigations can help to clarify relative risk. Clinical case reports may suggest questions but they can never, by themselves, establish a cause and effect relationship. The logical fallacy of drawing sweeping conclusions from individual case reports needs to be better understood by the media and by the public. Scientific and medical journals need to be more circumspect in their presentation of such material, and the reporting physicians should be more modest in their summaries and conclusions.

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Chapter 17

Psychiatric Consequences of Marijuana — Unanswered Questions and the Policy Implications of the Evidence To Date

Jerome H. Jaffe

Dr. Meyer's thorough and scholarly review of the psychiatric consequences of marijuana use is a good companion to the excellent review of the central nervous system consequences presented by Drs. Dornbush, Jones, and Reed.

I would like to address myself to some policy implications based on the findings and the unanswered questions covered in these reviews. As a starting point, I hope we can agree that society will not judge all adverse consequences of marijuana use to be of equal significance. For example, I expect that over the long run, acute adverse psychological reactions to marijuana (provided they are not associated with violent crime and do not occur too commonly) will be less disturbing to society than less frequent but prolonged changes (impairment) in social adjustment, if such can be demonstrated to occur. I would emphasize, however, that at present, with the possible exception of the acute psychotomimetic effects—I would include here, merely for convenience, transient psychotic episodes and panic attacks not requiring hospitalization as well as those which require a few days of hospital care where the causal linkage seems clear—all we have are correlations between marijuana use and a variety of psychological and neurological adverse effects. Causal relationships between most of these effects and marijuana use have not been established, and in many instances the methodology used in reports of adverse effects leads us to doubt that a causal relationship will be established. Yet Drs. Dornbush, Jones, Meyer, and Reed point out that there is, nevertheless, a pervasive worry, at least among researchers and policy makers, that chronic heavy marijuana use

will do permanent damage to capacities we all value greatly: our capacity to reason abstractly and our motivation to compete. It may be that only among the intellectual elite is this concern with the CNS so marked; perhaps among people who work with their backs something that might injure the spinal muscles would get as much attention, but I doubt it. I believe that this concern with intellectual function and motivation reflects a basic value in our society, and that regardless of how our policies toward marijuana evolve, researchers will continue to be expected to provide definitive answers despite the methodological problems.

Dr. Meyer feels that thus far the negative findings on long-term psychological adverse effects are based on firmer ground than the positive findings, especially with respect to such phenomena as the amotivational syndrome. I think we should recognize that the method of recruitment for the groups of subjects usually involved in studies of heavy marijuana is very different from that used to recruit alcoholics for comparable studies. The alcoholics we study usually come to our attention as the result of hospitalization or as referrals from situations where they have sought medical attention. I suspect that the marijuana users are often recruited by advertisements and are therefore still interested in and able to read ads. Such interest in and willingness to be part of an experiment may in turn be a distinguishing feature that may affect the results obtained when tests like the Halstead-Reitan Battery are administered, or when we inquire about levels of social functioning. Yet on the whole I would agree that most studies to date, including the most recent (Bruhn & Maage, 1975), have found no long-term intellectual impairment from drug use, including heavy use of marijuana.

As difficult as it may be to get better information about causal relationships between marijuana use and CNS or psychological impairment, I want to point to another problem that is even more difficult and more complex, with the hope that in a meeting such as this we might be able to plant the seeds for a well-planned, long-term investigation that may provide some of the critical data needed for rational policy.

Marijuana use, light or heavy, does not occur in a vacuum; it occurs in a society where a variety of other psychoactive substances are available. We must ask, therefore, not only how those who experienced long-term adverse consequences from marijuana would have fared if they had not used it but also how they would have fared if they had used some other psychoactive substance such as alcohol or opiates or sedatives? As Dr. Meyer has indicated, several investigators have found that many if not all marijuana users who lost interest in academic achievement or have had psychiatric difficulties in association with marijuana use seem to show these tendencies before their use of marijuana began. However, they did use marijuana.

We should try to imagine what would have happened if, instead of marijuana, they had used alcohol in heavy doses, or opiates, or perhaps tranquilizers recommended by a psychiatrist.

There is still much that we do not know about why and how individuals differ in their susceptibility to drugs. There seems to be some data indicating that the children of alcoholics are at greater risk of developing alcoholism (Goodwin et al., 1973; Goodwin et al., 1974; Goodwin & Guze, 1974). But are such children equally vulnerable to developing a pattern of heavy marijuana use or in showing adverse effects when a drug like marijuana is repeatedly used? Are there some people who are more susceptible to the adverse effects of marijuana just as there appear to be people who are particularly susceptible to the hyperlipemic effects of alcohol (Mendelson & Mello, 1973)? In short, if marijuana poses risks, is the population at risk identical to that which would be affected by alcohol, or does marijuana pose different risks for an entirely different population?

Whatever we may find about the potential adverse effects of marijuana, eventually we must ask not whether it poses risks—even on the basis of present evidence it surely does—but whether it poses more or fewer risks than the likely alternatives, and to what extent the nature of the risks are congruent with our society's values. Anybody who has worked in a hospital emergency room knows that there is a correlation between the acute effects of alcohol and aggressive behavior. I personally have not seen release of aggression with marijuana. Many investigators who have conducted studies of chronic drug use on hospital units have remarked on the frequency of aggressive behavior among drinkers participating in such studies and on its absence among marijuana users in comparable studies (Mendelson et al., 1972). Is this difference due to very different effects of the two drugs, or are we dealing with very different populations? The information on differences in population is not clear. A point of view that was once popular was that marijuana smokers avoided alcohol, either because they don't like its effect or because they feel that alcohol spoils the "marijuana high." There are, indeed, alcoholics who do not seem to like marijuana. But clearly some of this aversion is a cultural bias based on social stereotypes of marijuana users and is neither universal nor has it persisted as larger proportions of young people become experienced with the drug. The literature on drug use clearly shows that among younger drug users the two drugs are often used together and that regular marijuana users may be heavier drinkers than non-users of marijuana (Lloyd Johnson, 1973; *Marihuana and Health,* 1974).

Despite these questions, the observations to date suggest that for some people marijuana use might be better for both user and society than heavy use of alcohol. This is not the first time someone has suggested that some people who use alcohol might be better off using marijuana instead. The

morbidity and mortality from alcoholism is so well known and so high that some investigators have suggested that it might be helpful if confirmed alcoholics could be persuaded to use marijuana, but forego alcohol entirely. At least one such study has been undertaken. Rosenberg (1973) attempted to motivate alcoholics to take disulfiram (Antabuse) by offering them marijuana. Most rejected the idea, but the sampling problems and cultural biases make it difficult to draw inferences. It is possible and even likely that most confirmed alcoholics may not find marijuana an acceptable alternative. However, we do not know how much of this preference for alcohol involves specific or unique effects of alcohol and how much involves learning and conditioning to both alcohol effects and alcohol withdrawal (Ludwig et al., 1974; Ludwig & Wikler, 1974). One line of research suggested is to study people identified as high risks for alcoholism—for example, the children of alcoholics.

Recent observations on the drug use patterns of American service men who served in Vietnam indicate that many of those who used alcohol heavily prior to their Vietnam service sharply reduced their alcohol consumption when they started using the relatively low cost heroin readily available in Vietnam. While a substantial percentage of those who used heroin became physically dependent at some point during the period of Vietnam service, more than 90 percent stopped heroin use upon return to the United States. The major drug use problem for returnees was alcohol (Robins, 1973; Goodwin et al., 1974; Robins, 1974). This observation suggests a considerable flexibility among problem drug users with respect to the drug selected. Since it seems inconceivable at this time that our society will ever consider recreational use of heroin as socially acceptable behavior, there is little value in speculating about whether a given individual would fare better as an opiate dependent than as a heavy user of alcohol or as an alcoholic. However, it is entirely conceivable and even likely that marijuana use will become socially acceptable some time in the next decade, and that its availability will increase as its acceptability increases. Eventually, therefore, our concern about adverse consequences of marijuana will have to be made in terms of comparisons with other drugs which are used with equal moderation or imprudence, as the case may be, rather than as a simple cataloging of the potential dangers of the use of marijuana itself.

Griffith Edwards (1974), in a thoughtful discussion of the issues that need to be examined in considering marijuana legalization, points out that there is no evidence to suggest that legalized marijuana will "drive out" other recreational drugs; he thinks it much more likely that marijuana will be used in addition to other available psychoactive agents, including alcohol and tobacco, and thus we need to know more about how these substances will interact in different segments of the population.

Such questions should also lead us to a concern about the relative reversibility of adverse effects. For example, are the long-term consequences of marijuana use (if any) easier to reverse than the long-term consequences of alcohol use? The methodological difficulties here should not be minimized. We might also ask about the relative degree of difficulty in "treating" those who do become heavy users. If they ask for help, will we be as able (or unable) to help them as we are to help the alcoholic? And what of those who use both drugs?

In short, given the expense of long-term prospective studies, I hope that some thought will be given to selecting our samples in a way that might shed light not only on what chronic marijuana use may do to individuals but also on what impact widespread use may have in a society where alcoholism and other forms of drug use seem to be increasing. Can we design a study that will help us obtain some clue as to whether marijuana might be a safer drug for people prone to developing problems with already available drugs, and whether its increased use by such individuals would produce a net decrease in adverse consequences of drug use for both users and society in general? And, if there are such people, can we dare to hope that they are not outnumbered by others whose use of marijuana would further aggravate problems induced by the use of other drugs?

Dr. Meyer began his review by acknowledging that scientific advances are not likely to resolve social controversies. Part of the problem is that even dispassionate experts cannot always agree on the facts, let alone the significance of the facts, which leaves those with strong biases to interpret the results in whatever manner will support viewpoints and goals that are in most instances non-medical and non-public health oriented. But the other part of the problem is that those of us in the scientific community who disagree with this country's past policies on marijuana ought to concede that we simply don't have enough information to permit us to shape an entirely rational policy. The view that the risks posed by marijuana use does not justify criminal penalties for use or possession is at present based more on our conception of the role of law in a free society than on our capacity to predict the long-term effects of major policy changes. Most advocates of change point to benefits which are largely non-medical, including reduced costs of law enforcement and reduced criminalization of otherwise law-abiding citizens. There is, however, legitimate concern that such changes could have adverse effects in terms of public health. I felt it appropriate to observe that there might also be some offsetting benefits, and I have tried to point out areas where additional studies might help to resolve the questions. For the near future we should undertake such research because it is important to acquire knowledge; and we should engage in mutual criticism so that we do not delude ourselves about what our findings mean. However,

we should not delude ourselves into believing that over the short term the best or most carefully conducted work will influence the direction of the complex social forces that are at present bringing about changes in attitudes and policies. I am less pessimistic about the long run, and I suspect that eventually careful work on relative risks will lead to rational social policies.

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Chapter 18

Marijuana and Psychiatric Problems: A Discussion

Dr. Jonathan Cole: I am reminded of the book *To Kill a Mockingbird,* in which the little girl keeps asking her father, "Should we worry yet?" My general reaction to this conference is that we shouldn't worry yet. The longer we discuss these issues, the more the evidence, though vague and confusing, seems reassuring to me.

I do believe that the issue of statistical versus biological significance is highly relevant when examining the possible psychiatric consequences of long-term marijuana use. Many years ago, before marijuana use was as widespread as it is today, I had worried about the possibility of having problem smokers, "marijuanics," if you will, in the same sense that we have problem drinkers—alcoholics. We may indeed now have some "marijuanics," but it is extremely difficult to determine who they are, what they are like, and whether or not they constitute a significant health problem. They certainly don't seem to turn up on psychiatrists' doorsteps very often. On the other hand, when psychiatric problems do arise in the marijuana-using age group, it has become all too easy to attribute these difficulties to the drug. For instance, I recently saw a young patient who was presented to me as a classic example of the so-called marijuana-induced amotivational syndrome. Upon close questioning, however, I discovered that she hadn't ever used marijuana. If we could give a rigorous operational definition to the "amotivational syndrome," and screen the entire population of eighteen to twenty-five year olds, we might find that the syndrome involves a higher proportion of young people who have never used drugs than of those who do. For such reasons, we need to do some serious, well-designed, epidemiological studies. No one denies that there can be psychiatric consequences for those who use marijuana; the question is, "Are the consequences a serious health problem"?

Perhaps a new "Midtown Manhattan Project" could be conducted, in which we could concentrate on the twenty- to thirty-year age group to deter-

mine how many of them are having serious problems and what their drug-using habits are. This might give us a better idea of the prevalence of the drug-related health hazards that exist today. A survey of mental health facilities' admissions might prove helpful, although there is an inherent difficulty there in that people are not hospitalized for psychiatric problems as frequently today as in the past. In any case, one could determine who turns up in psychiatric facilities and whether any cases are even remotely connected with marijuana use. My informal impression of patients coming into McLean Hospital, where middle-class families tend to send kids in trouble, leaves me with the feeling that marijuana is not a common presenting complaint. In any case, a large-scale epidemiological survey would help to answer the question: Is severe psychopathology or social impairment associated with drug use?

Ideally, a large-scale anterospective study of early adolescents followed for ten to fifteen years should be carried out, in which base-line measures of social adjustment, psychological symptoms, and neuropsychological functioning would be obtained and taken again at intervals. Such a study wouldn't settle the cause and effect issue regarding marijuana and psychosocial impairment, but it would be a beginning. We might also learn more about alcohol use and impairment, a form of substance abuse that seems both more prevalent and more harmful.

One last thought along these lines. I first became aware of the possibility of substituting marijuana for alcohol as a treatment modality at a 1951 meeting of the Southern Medical Association where a clinical report was given stating that alcoholics receiving Synhexl during withdrawal felt better. My initial reaction to this idea was very negative; why substitute a dangerous new addiction for alcoholism? I have subsequently been impressed with the relatively low toxicity of marijuana, however, and the more I hear, the more I think it is a safer drug than alcohol and that perhaps we should try it as a treatment for alcoholics. This suggestion stems from the fact that I've been looking for a good psychiatric use for marijuana, based on the premise that anything that is so widely and so happily self-administered ought to be good for something!

Dr. Mendelson: Marijuana has been tried in the treatment of alcoholics. When Dr. Michael Rosenberg offered a group of alcoholics marijuana, they were shocked and disturbed. Their response was, "Doctor, I'm not a drug addict, I wouldn't touch that stuff." These alcoholics had a specific cultural bias against using marijuana because they feared they would become "junkies."

Dr. Meyer: The alcoholics treated with marijuana also had a higher frequency of adverse reactions than we normally have in our marijuana experiments.

Dr. Jones: At our clinic we don't see the sharp differentiation between alcohol and marijuana users. We have encountered a number of heavy marijuana users who are also probably alcoholics, drinking a couple of six-packs of beer a day along with smoking a considerable amount of marijuana.

Dr. Freedman: In the early 1950's I used to see a group of people reminiscent of the British "remittance men." They were periodically given small amounts of money by their families to keep them in Greenwich Village, with the tacit understanding that they were not to come home again. Their ages ranged from the late teens to a large percentage in their late thirties, and their lifestyles, which usually included extensive use of drugs, were considered avant garde. A few would occasionally come to the Yale Psychiatric Institute for a variety of psychiatric dysfunctions, and we would discuss their views on marijuana and other drugs. A borderline psychotic who was an alcoholic asked me whether I would prefer his continuing alcohol abuse or switching to marijuana. Having been taught the silent method, I just nodded my head and said Um Hmmm, Um Hmmm. But as I watched his drug taking more closely, I saw the following pattern emerge. Intermittently he would interrupt his heavy drinking to start smoking marijuana. He would smoke intensively and gradually become paranoid, which he recognized. He would continue to smoke and to stay paranoid until he decided that it was too upsetting, when he would stop using marijuana for a period and resume his drinking. Gradually his paranoia would subside, but then he would start having problems with his drinking. I am not at all convinced, having seen this pattern of response in a number of people before I had much of a social set about the drug, that marijuana doesn't bring its own problems to the drug-prone person.

Dr. Meyer, regarding the people you refer to as "our problem population," I wonder if it wouldn't be wiser to assume that there is a group of people who will have problems if *any* drugs are available? The kind of compulsive drug use that I have seen was a compulsion to use *any* subjectively active drug with abuse potential. Phenothiazines helped some of these people, but if they left the therapeutic regimen, they stopped taking phenothiazines and began using non-medical drugs. Therefore, although marijuana may not *physiologically* lead to compulsion, people with inadequate defenses who are vulnerable to compulsions may, when they have drugs readily available, feel a compelling need to relieve themselves through the use of drugs. They are "prepared" to learn what we call maladaptive behavior with a wide range of drugs. There need not be a single type of "addictive personality"; this population may instead represent a variety of psychobiological and personality types with a common vulnerability. We need much more research to identify this vulnerability, and we should expect a

range of people for whom it is increasingly *less* difficult to "learn" to utilize drugs to maladaptively reinforce certain needs.

Dr. Nahas: I have a question for Dr. Tinklenberg. I was impressed by his data which indicated that under the acute effects of marijuana there is an impairment of short-term memory function which seems to have some impact upon the learning process. Since, as Dr. Pollin tells us, marijuana is used more and more by thirteen- and fourteen-year-old adolescents, I would like to ask to what extent this impairment of short-term memory might lead to impairment of long-term memory.

Dr. Tinklenberg: Unfortunately, I don't think we or any other group have data that would directly answer your question. Some psychologists include in their conceptualization of the human memory system the two components you describe: short-term or working memory and long-term memory (Atkinson & Shiffren, 1968). If short-term memory is not working well, certain information never reaches the long-term system; in other words, information is never available for long-term storage and hence never accessible for subsequent retrieval. So in a sense, yes, there is no doubt that since marijuana will transiently impair certain aspects of short-term memory function, there will be impairment of learning while the individual is under the influence of the drug (Darley & Tinklenberg, 1974; Tinklenberg & Darley, in press). However, this impairment seems to be relatively transient, at least in most individuals. The more important question, What might be the long-term effects of chronic marijuana use on these processes?,' is simply not answerable by the data we have. Our measures don't record long-term effects. I suspect you would have to use certain components of the Halstead-Reitan Battery, the Cantor Background Interference Test, or similar tests.

Dr. Cole: A noted pharmacologist described to me a subject of his who smokes fifteen marijuana cigarettes a day, drives a taxicab part-time, and still gets straight A's at Wayne State University. If that subject uses that much marijuana while performing complicated tasks, one wonders if some tolerance does not develop to prevent short-term memory impairment.

Dr. Jones: After studying in our laboratory people who extensively smoked marijuana, I find it unbelievable that someone could smoke fifteen reasonably potent marijuana cigarettes a day and still function adequately as a cabdriver and university student. Even our most frequent San Francisco users tend to reduce or discontinue marijuana use if they have complicated brain work to do. For example, one of our subjects was a carpenter who smokes about eight marijuana cigarettes a day if he is doing simple carpentry work. However, if he has to lay out a stairway or a joist and rafter system on the roof, he smokes much less or none that day. In other words,

even some marijuana enthusiasts decide that complicated mental activity is not consistent with marijuana intoxication.

Dr. Dornbush: In response to your question about tolerance, Dr. Cole, in our daily administration studies we found that while short-term memory is very obviously affected during the first few days of use, with continued daily administration, short-term memory performance keeps improving (Dornbush et al., 1972). I think this is a fairly consistent effect. Short-term memory functions are no longer affected after a week to ten days of daily marijuana administration.

Dr. Jones: In our chronic study, even at the substantial doses we are giving, most subjects show no impairment whatsoever on memory tasks such as remembering the last five letters of a series that can be anywhere from six to thirty letters long. The subjects can handle this task with two hundred milligrams of THC per day and show no decrement at all. On the other hand, half an hour before or after doing such a test which showed no decrement, one might see a subject standing in the hall looking perplexed. When asked "Where are you going?", he will answer, "I don't know, I forgot."

Dr. Mendelson: Our findings are similar to Dr. Jones's. When we used a running digit span, which was quite difficult, we did not find any impairment during marijuana intoxication. On the other hand, there have been a number of studies carried out with alcohol addicts, employing a variety of short-term memory tasks, and I think there is a fair amount of disagreement about the relative impairment of short-term memory, particularly in regard to blackout.

Dr. Nahas: This is exactly the type of situation I am referring to—acquisition of new materials that a school child has to acquire daily. I'm constantly being asked how many marijuana cigarettes you can smoke a week and still function adequately. I tell them it is better not to smoke marijuana because it will at least transiently impair your memory and eventually your whole education.

Dr. Cole: My answer has always been, How are you doing now? If a person is getting all A's and smoking a marijuana cigarette a day, I don't get upset. But if he feels in any way that he isn't functioning up to top form, I tell him to stop.

Dr. Mendelson: I would like to make a brief comment on something Dr. Jaffe alluded to. Regardless of the type of dependent or independent variable used in assessments of behavior, there is a difference between intoxication with moderate doses of ethanol and the behaviors which occur with a moderate or high dose of marijuana. In our initial studies, we were investigating alcohol addicts who were recruited from correctional institutions, and it is not fair to compare these subjects with people who respond

to an advertisement in the Boston *Phoenix*. But more recently we have been looking at young adults who are initiating their drinking careers and are the same age and from the same socioeconomic class as our marijuana users. We have studied in a research ward setting almost 50 marijuana users and about 12 young individuals who drink ethanol episodically. These individuals are not drinking enormous amounts, but they are drinking as much as 18 cans of beer on occasion. My point is, the *quality* of intoxicated behavior with ethanol is very different from the *quality* of intoxication with marijuana. We rarely observe hostile, acting-out, disruptive behavior with marijuana. This is not an endorsement for marijuana, but with ethanol, exhibitionistic behavior, covert and overt homosexual acts, and overt aggressive behavior are not uncommon. I don't know whether you have seen this too, in your heavy dosage marijuana experiments, Dr. Jones, but the incidence of adverse reactions, bizarre or antisocial behavior is relatively small in comparison to what we see with alcohol.

Dr. Jones: I too have been impressed by the absence of aggressive behavior in marijuana intoxicated individuals in the laboratory or research ward.

Dr. Tinklenberg: In our studies of California adolescents, extremely aggressive and sexually assaultive behaviors were much more common during ethanol intoxication than during marijuana intoxication, despite the fact that the adolescents generally reported using marijuana almost as frequently as alcohol (Tinklenberg et al., 1974).

Dr. Jaffe: This is why I felt it important to point out that in any study one should try to make an assessment of what would happen if there was a change in bias from one drug to another. Would a different drug compound the problems?

Dr. Freedman: That raises a theoretical question: If only one drug, marijuana or alcohol, was to be permitted, which would be best? I don't know how equivalent levels of intoxication with marijuana and alcohol could be established. It seems that at a small dose of alcohol, the range of behavior that is available to the individual is far wider than the range at the minimally effective levels of marijuana. I have always been curious about how that difference in intoxication is valued, because I think people may preferentially select one way or another of reaching an altered state. Marijuana offers a different experiential state than that usually attributed to alcohol. In other words, what is the difference in the desired end point between a glass of wine and a marijuana cigarette? People are trying to reach different end points. The question is whether and how these end points are socially valuable.

Dr. Jones: Your emphasis on differences between marijuana and ethanol intoxication is not consistent with our subjects' usual behavior. The pattern

for our subjects is often a marijuana cigarette plus a glass of wine rather than either/or.

Dr. Pollin: I would like to make two points. One, all of us who are involved in drug research should take note of the fact that there are medical problems not complicated by the complex non-medical issues surrounding marijuana use which have evoked similar intense controversy and remain as yet unsolved. I am thinking of the controversy regarding the effectiveness of oral hypoglycemic agents in diabetes; the controversy as to whether radical or simple mastectomy is the more effective procedure for breast cancer; and so forth. Investigators involved in those controversies don't have the additional ideological burden which bedevils the marijuana issue. I think we should make a concerted effort to free ourselves from this burden. We should realize that the intrinsic biological complexities of evaluating marijuana effects will take a long time to resolve.

The second point I wish to emphasize is the fact that only recently has there begun to be a substantial research effort on the effects of marijuana. As a result, it seems to me that at this point it is very difficult to know where on the hazard continuum we should place marijuana. The history of reactions to the introduction of coffee is relevant to one end of the continuum. There was initially great concern in Europe about coffee's mind-changing qualities, the likelihood of its causing social disorder and health problems. All kinds of political, legal, and social prohibitions were invoked. I'm sure there was no one definitive experiment, but somehow the collective wisdom of human experience made it clear over time that those concerns were unrealistic.

At the other end of the spectrum, many reports during the past century, some from most reputable medical journals, strongly advised the use of morphine as a treatment for alcoholism. And we are all familiar with the early recommendations that heroin be used to treat opium addiction.

It seems to me that with marijuana we are now somewhere between the excessive concern over coffee, and the Pollyannaish optimism about the therapeutic usefulness of morphine and heroin. Where on the reality continuum we will finally emerge requires more years of research. If we are to clarify the issue we have to give ourselves more time and simultaneously get the message across to those who try to hurry us; this is not a simple issue, and it is going to take more investigation.

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Epilogue

Passions, Pot and Science Policy

Daniel X. Freedman

I think we stand closer to a sane approach toward marijuana issues today than we did several months ago, and I am pleased to see scientists who hold differing views conversing with one another. Our task is simple. We must tell the truth about research: the kind of work it is, poised always on the edge of the unknown, and the passion and commitment that are necessary to this venturesome but disciplined reach into the future.

I don't think scientists have any less passion than non-scientists. The only difference is that for scientists there must be a mandatory final checkpoint — the presentation of our data to the scrutiny of colleagues. We have to decide and to stipulate what errors we are willing to make or not make. Having clarified our methods, we must submit the data so generated to peer scrutiny. Furthermore, precisely because we are limited by methodology, we gain reliable data but lose our right to generalize. Hopefully, we can make new hypotheses and on occasion, with converging data, we may justifiably come to sweeping conclusions.

On this topic we have to come to terms with what we really need to worry about. For myself, I worry about any smoke! I worry about the fire that generates it, its source, purpose, and function. We must always attend to the signals from any quarter that indicate that there *may* be unanticipated consequences of drug use. But in so doing, we have a problem of perspective regarding relative health hazards. Preliminary observations may scientifically compel us to complete a task of testing and evaluation which may take many years; and we will often take risks in deciding to pursue a hunch or a lead. En route to that unknown destination, we cannot always stop to point the way for public policy.

Now if an informed consumer or science writer had attended this conference, he would have been impressed by the tremendous complexity (and rapidly evolving new developments) of the research methodologies being used, and the number of variables that must be kept in mind when con-

sidering the significance of any given finding. He would be aware that there aren't any quick and easy ways of summarizing what any of these tests "mean," and indeed that colloquy on significance is an inescapable part of scientific review. It is what scientific training is all about — to evaluate the meaning of method in order to appreciate the significance of data.

This procedure is not elitist. It is necessary that we query as sharply as possible the methods at issue, their suitability for determining some aspects of biological functioning, and often this judgment cannot finally be made because the field is not yet ripe for it. So we struggle with the latest in immunology to find the relevant parameters and to try to determine the order of certitude which the experts are giving to specific tests. It takes training to have an understanding of the problems that both new *measures* and new *systems* entail, whether in the field of genetics, endocrinology, immunology, biology, or psychopharmacology. We find that our knowledge of relevant components of, for example, the immune system, is still being expanded, so that if we measure a component — and would not argue with the method of measurement — its ultimate significance may still be quite obscure. It would be tragic ever to lose an important scientific "lead," but the mandate to pursue that lead (indeed to test whether or not it truly *is* one) derives from the logic of the problem, not from the intensity of our *wish* that our perspicacity "pay off" for public good. Such passion is more relevant to the risk in pursuing the unknown and may privately sustain the scientist as he goes about his task.

In assessing marijuana research we ought — practically — to think occasionally of the clinical model and of some of the constraints this brings to the physician who must apply basic knowledge to a specific person. In working with diseases and dysfunctions we always have an array of laboratory tests, the *meaning* of which has to be assessed for the individual case. The findings of x-ray, for example, may be confounded at surgery; an array of biochemical tests and diagnoses may be confounded at autopsy. Biochemical tests vary in their elegance, specificity, relevance, and so forth. The clinician must weigh the reports, critically, with the patient and his condition *and* response in mind. And this is key: the clinician, if he is an ethical and scientifically trained physician, is dealing with health *consequences* in an individual, so he has a certain end point that we, in exploring the frontiers of science, are looking for. In this sense laboratory findings eventually have to be "relevant," just as the clinician's *hunch* — if he wishes to generalize — must be reviewed for its reliability and scientific confirmation.

Now it is obvious that in considering the health hazards of marijuana we are not dealing with something of the order of the plague. The effects of marijuana, like alcohol or diseases such as schizophrenia and depression,

are not easily diagnosible by a discernible CNS tissue pathology; no effects of marijuana intoxication can yet be found at autopsy.

This means that we must continue to use different methodologies and to explore different avenues of research in order to understand effects on *function.* Few major leads have emerged to advance our recent knowledge here. The obvious fact is that the drug produces intoxication and yet we are only beginning to understand the details of brain systems that may underlie and "permit" any intoxication from *any* kind of drug. Several of the contributors have said that they thought a particular line of investigation was "urgent." I think we should ask for the criteria that determine that a particular approach should be urgently followed. Is it because the next step would teach us more about immunity as a general biological phenomenon than ever known before? Or that one has encountered a puzzle that, if solved might — *or might not* — tell us what we should do next in order *eventually* to understand either immunity or drug effects? In brief, is the urgency relevant to scientific methods to be developed, or the fact that we are at a point where one last piece of a puzzle — if directly in hand — would definitively guide public health policy?

Unlike Huxley with his soma, scientists can never promise the public a rose garden in terms of the kinds of drugs and medicines we come up with. While there does not appear to me to be any danger to clinically defined immunity with marijuana, we can never promise immunity from some danger! We can only be alert and pursue knowledge where it takes us. Similarly, it is difficult to determine the significance to pubertal development or human sexual function on the basis of hormonal changes of the magnitude being dealt with; but it would be foolish not to ascertain the facts, as well as establish their general meaning to health, sexual function, or development. After all, any effects of marijuana on hormones, immunity, birth defects, etc., that become firmly lodged in our knowledge, would *far* transcend their relevance to marijuana; they might indeed provide a reliable index for the range of available chemicals used in medicine!

This being the case, the real concern today — that is, the heat behind the last five months — is, I think, the use of drugs by children. It is most bizarre that we have now had commissions and squads of scientists searching for therapeutic effects of certain drugs because the children of the middle class took up a particular chemical, as if such a discovery would sanctify the recreational use or compulsive misuse of a chemical. But in a larger sense, the heat and concern in every society is always centered around who shall capture the mind of the child. Few would argue that the healthy development of children and adolescents is enhanced by unsupervised and unregulated intoxications, and none of us can be unconcerned about the fact that all of our recreational drugs *seem* to be used by some to their psy-

chological disadvantage. And we do not know cause or effect in these instances, nor can we predict exactly who might be in trouble if he takes marijuana. But we all can and do share concern about how to guide the young to adulthood.

What we probably should require is some thoughtfully designed study to find out what our public policy options are. There exist people who smoke marijuana with the same ease, discretion, and self-control that a connoisseur of fine wines uses. The epidemiological and clinical data from India, Jamaica, and elsewhere do not show statistics such as have finally been assembled for cigarette smoking and cancer. India's experience, indeed, underlines the fact that *some* are not immune from unfortunate consequences, but the majority of users do not satisfy any desire *we* might have to pin down a definitive and *generally* applicable set of unavoidable adverse consequences.

We are also aware that not all of the young are using alcohol and/or marijuana, and/or downers or uppers, and/or heroin with discretion. Where do they get their marijuana? Do their suppliers supply other drugs? Sooner or later we must face the social decision of whether or not to regulate the marketing and recreational use of drugs. If there is an appetite for a given chemical — marijuana — is that appetite conveying with it more or less social damage? That is to say, when multiple drugs are being sold, are there really selective, controlled, self-contained patterns of marijuana use? Are there ways of obtaining marijuana that reinforce these self- and group-regulated patterns of use? Or is our social policy *encouraging* multiple drug use and some of the more adverse and undesirable consequences of the current drug scene?

All of these problems involve different orders of inquiry; they start with the molecular and end with the social. If we are to find answers to any of these questions, support for research cannot be pulled in and out at will. In brief, if we want to know something, we have to have the research tools, the expertise, and the *sustained* inquiry to build a body of knowledge that will serve our different needs. Somehow the notion has gotten abroad that because a drug is commonly self-prescribed and used, its pharmacology and biopsychological effects can be readily grasped and understood. The very forefront of scientific probing, of laboratory findings of the moment become slogans, entrails for augury in the ongoing debate about social values — in this instance, to smoke or not to smoke marijuana. Guilt by "word association" — chromosomes equal genetics, equal fetal damage, etc. — rather than judicious assessment of biological sense and fact is the media fashion.

As a result of our discussions I feel I have a better grasp on what some of the new findings *might* mean. I am aware that it will require several years

of work, replication, and methodological development to begin to make any kind of definitive assessment. I encourage such pursuit. But at this point I see no major new medically or psychologically relevant reason to be disturbed about marijuana, after taking into consideration the fact that there is always reason to be disturbed about the use of any drug.

Finally, we will not get the research done if someone doesn't start reasserting the importance of research without an immediate payoff. This search for immediate gratification is exactly what strikes those concerned with the development of young people; they need to be encouraged and tutored to delay gratification for greater psychological strength and growth and more satisfactory social relationships. No scientist should wish to add to complexities, but no society should retreat from the inevitable complexity — which involves delay in gratification — with which biology and nature present us. Moral decisions have not yet been replaced by the purported miracles of science. When we deal with those concerned with public policy and public information, our theme must be the need to search and sort, the need for sustained work, in order to provide the kind of information that is wanted. We cannot lend our findings to the moods of the moment; rather we must place them in the appropriate context for our nonscientific colleagues to judge — explaining, often, their explicitly preliminary nature. If this kind of sermonizing does no good, that is not really relevant. For these facts about the way we come to know a fact are the truth, and that is what binds us together!

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