Marihuana: New Support for Immune and Reproductive Hazards

The movement to lessen penalties for the possession of small quantities of marihuana is gaining steam throughout the country. Already, six states (Alaska, Oregon, Ohio, Colorado, California, and Maine) have reduced the penalty to a misdemeanor or civil offense that does not bring the possessor a criminal record, and several other states are considering such laws. Concurrent with this effort, many scientists have redoubled their efforts to identify potential hazards associated with marihuana use, often in an effort to forestall further liberalization of the laws. While these scientists have produced new evidence of such hazards, it is by no means obvious that these findings should be a roadblock in the path of liberalization.

Many reputable individuals and organizations argue, with some justification, that the social effects resulting from the felony convictions of hundreds of thousands of young people each year for possession of marihuana are far more serious than the health effects that have so far been identified. They also argue that a disproportionately large amount of the efforts of police and the courts is directed toward marihuana possessors, leaving them less time to deal with more serious crimes. Such proponents thus contend that the legal debate about liberalization of marihuana laws should be separated from the medical debate about the potential hazards of marihuana use. But even most of the firmest advocates of change in the marihuana laws agree that every possible effort should be made to discourage teenagers and young women who are or may be pregnant from using the drug.

Although some of the recent findings support the generally well-accepted conclusion that use of marihuana and its derivatives—known collectively as cannabis—is strongly deleterious to the lungs, most of the findings bear on the possibility that cannabis can cause impairment of both the immune system and the reproductive system. Serious concern about these possibilities was aroused a little more than a year ago [*Science*, 23 Aug. 1974, p. 683] when Gabriel G. Nahas and his associates at the Columbia University College of Physicians and Surgeons reported that lymphocytes from regular users of cannabis showed an impaired capacity to proliferate in the presence of agents that stimulate mitosis (mitogens). About the same time, Robert C. Kolodny of the Reproductive Biology Research Foundation in St. Louis reported that he had observed sharply lower levels of testosterone and reduced sperm counts in young men who used cannabis frequently.

These reports evoked a great deal of publicity in the media, but produced mainly skepticism among other investigators. A few hastily published experiments that contradicted these results were reported shortly thereafter, and the results of Nahas and Kolodny were generally discounted. Further work has been performed in the last year, however, and several investigators have corroborated the results of Nahas and Kolodny.* It should be emphasized, however, that cannabis is much like other drugs, such as tobacco and liquor, in that the greatest potential hazard exists for those who abuse it. All of the observed adverse effects of cannabis have been found in individuals who have used large quantities of it for prolonged periods. There is still no convincing evidence that casual, infrequent use of marihuana produces any ill effects.

There have always been many apparent contradictions and inconsistencies in cannabis research, for a variety of reasons (see box). One of the best examples of how such inconsistencies arise is to be found in research on the effects of cannabis on the reproductive system. Shortly after Kolodny reported observing lowered testosterone levels in 20 young men who used cannabis on their own, Jack H. Mendelson and his associates at the Harvard Medical School's Alcohol and Drug Abuse Research Center in Belmont, Massachusetts, reported on a study in which 30 cannabis users lived in a hospital ward for a month. After a 5-day period without drugs, the subjects were permitted to use as much cannabis as they wanted for 21 days. During the period of cannabis use, Mendelson observed no change in testosterone levels.

Kolodny subsequently collaborated with investigators conducting a study of 20 cannabis users who lived in a University of California at Los Angeles hospital ward for nearly 3 months. The men used no drugs for the first 11 days, then were given an average of five "joints" a day for 9 weeks. Kolodny also observed no change in testosterone levels during the first 4 weeks of cannabis use. After 4 weeks, though, he observed substantial decreases in the concentration of luteinizing hormone in the subjects' blood; luteinizing hormone is thought to stimulate the testes to produce testosterone. During the fifth week of smoking, the subjects' testosterone levels began dropping and continued to drop throughout the rest of the study.

After the subjects had smoked for 8 weeks, Kolodny also observed significant decreases in their blood levels of follicle-stimulating hormone; this hormone stimulates sperm production. (Both luteinizing hormone and follicle-stimulating hormone were first described and named by their activity on ovulation. It has subsequently been shown that they also affect the male reproductive system.) At the end of 9 weeks of smoking, the subjects' average testosterone levels had fallen by 33 percent. The averages were still within the normal range for men of that age, but the concentrations for several of the men fell to the point where impotence or infertility could result.

A similar contradiction arose in studies of the acute effects of cannabis on testosterone levels. Kolodny reported a transient depression of testosterone levels after a male subject had smoked one cannabis cigarette, but Carl F. Schaefer of the University of Oklahoma reported that he found no effect. Schaefer observed testosterone concentrations for only 90 minutes after smoking, however, whereas Kolodny observed depressed testosterone levels only after 120 to 180 minutes.

Kolodny's results have received some support from other investigators. Wylie C. Hembree III of the Columbia University College of Physicians and Surgeons, for example, studied five cannabis users in an experiment much like Mendelson's. He also observed no ill effects during the 4 weeks of cannabis use, but he found that the subjects had an average 58 percent reduction in sperm count in a medical examination after the end of the smoking period. He attributes this delayed response to the fact that sperm production normally requires at least 65 days. And Costas N. Stefanis of the University of Athens in Greece has observed a variety of abnormalities in the sperm of men who have smoked cannabis for many years. These abnormalities include changes in lipid concentrations, protrusion of chromatids from the nucleus, and marked changes in the balance of acidic and basic amino acids in the histone proteins that encapsulate the sperm DNA. The significance of these changes is unclear, however, as Stefanis

*Much of the work reported here was presented at a recent symposium in Helsinki. The proceedings will be published early next year by Springer-Verlag.

has found no ill effects definitely associated with them.

A biochemical basis for these clinical observations has been offered by Alexander Jakubovic of the University of British Columbia. Jakubovic studied the effects of several different constituents of cannabis (cannabinoids) on cultured rat testis tissues. He found that the cannabinoids inhibit the incorporation of amino acids into proteins, inhibit the incorporation of nucleosides into both RNA and DNA, and inhibit the metabolism of glucose—the primary source of energy for the production of sperm. He says that the inhibition of DNA and RNA synthesis results from a block in the conversion of purines and pyrimidines to nucleotides, whereas the inhibition of protein synthesis results from both the inhibition of RNA synthesis and the decreased concentrations of adenosine triphosphate within the cell. Any of these effects could reduce proliferation of sperm cells.

Similar biochemical effects from exposure to cannabinoids have been observed in other cell types. Nahas, for example, has observed reduced proliferation and an inhibition of macromolecule synthesis in cultured human lymphocytes. R. Dean Blevins of East Tennessee State University has observed similar effects in several lines of cultured cells, including human fibroblasts and mouse and human neuroblastoma cells. Jacques Huot and Simone Radouco-Thomas of the University of Laval in Quebec have observed a reduction in proliferation in cultured monkey kidney cells and neuroblastoma cells. And Arthur M. Zimmerman of the University of Toronto and Stanley Bram of the Pasteur Institute in Paris have independently observed inhibition of proliferation in unicellular species.

The investigators provide different explanations for their results. Nahas and Zimmerman suggest that the cannabinoids inhibit the transport of amino acids and of purines and pyrimidines across the cell membrane. Blevins suggests that these substrates are transported into the cell at normal rates, but that they do not stay in the cell. Huot finds that very low concentrations of cannabinoids stimulate the activity of adenylate cyclase, an enzyme that mediates the synthesis of adenosine 3′,5′-monophosphate (cyclic AMP). Other investigators have observed that increased concentrations of cyclic AMP are associated with reduced proliferation.

The inhibition of proliferation of lymphocytes is one way in which the human immune system could be impaired, but recent work suggests that other facets may also be affected. Nahas and Eliot Osserman of the Columbia University College of Physicians and Surgeons, for example, have observed decreased concentrations of the circulating antibody immunoglobulin G in cannabis users who smoked in a hospital ward. Harris Rosenkrantz of the Mason Research Institute observed a similar suppression of circulating antibodies in rats exposed to cannabis smoke. He also harvested spleen cells from the rats and found that they had a decreased capacity to produce antibodies when exposed to antigens.

Suppression of circulating antibodies

Inconsistencies and Contradictions: Why?

Research on cannabis has generally been characterized by a great number of inconsistencies and contradictions, with workers from different laboratories often reporting widely differing results. There are many reasons for these problems, and most of them are discussed in some detail in a new book, *Marihuana and Health Hazards*.* This book is the proceedings of a conference on methodological issues in cannabis research that was sponsored by the Drug Abuse Council.

Among the problems in cannabis research discussed in the book are:

▶ Definitions. Some investigators have defined heavy use of cannabis as five to ten joints per week; others may define it as five to ten joints per day. It is not surprising that they then get different results.

▶ Retrospective studies. It is difficult to draw conclusions about potential hazards when the investigator must rely on the subject's own account of his drug use. Even if the subject is truthful, he probably cannot provide accurate information about his nutrition, health care, radiation exposure, and the true identity of the presumed "cannabis" that he has purchased on the street.

▶ Baselines. Cannabinoids are very lipophilic, so that they accumulate in fatty tissues from which they may be slowly released when the subject is not using cannabis. It is thus difficult to get an assessment of the subject's baseline metabolic characteristics, even if he stops smoking for a few days at the start of the tests.

▶ Solubility. Most cannabinoids are very insoluble in aqueous solution, so it is quite difficult to apply them to cultured cells. Some investigators claim to have used concentrations of cannabinoids well above their solubility, so that it is quite difficult to interpret their results.

▶ Tetrahydrocannabinol. Many investigators use only tetrahydrocannabinol when looking for potential hazards because it is the primary psychoactive component of cannabis. But it is at least equally likely that ill effects—if there are any—might be caused by other cannabinoids consumed at the same time.

▶ Dosage. There are no accurate techniques to measure the amount of cannabinoids absorbed from a cannabis cigarette by an individual. The amount absorbed may thus vary from individual to individual. There may also be a wide variability in metabolism of the cannabinoids and in genetic susceptibility to their effects, as is the case with tobacco. Good tests to measure cannabinoid concentrations in the blood are one of the most pressing needs of cannabis research.

▶ Animals. It is often difficult to extrapolate results obtained with animals to effects on humans, whether for cannabis or for other drugs. Completely different results have often been found in closely related species at the same time that identical results were found in widely varying species. Comparison of dosages in animals and humans is also difficult because of differences in metabolic rates. Animal work usually does, however, point to areas of concern that should be studied more closely in humans.

▶ Duration. Most of the research conducted so far has consisted of short-term projects aimed at an immediate answer. There is a great need for studies in which cannabis users are followed for long periods. Only in that way will the long-term effects of cannabis become apparent.

The book also explains the rationale behind some of the current studies of cannabis hazards, explains the possible results of those hazards, and explores the techniques and difficulties of individual experiments. But since the conference was held last January, the book does not contain some of the more recent results discussed in the accompanying text.—T.H.M.

*J. R. Tinklenberg, Ed., *Marihuana and Health Hazards* (Academic Press, New York, 1975).

was also observed in mice given high oral doses of tetrahydrocannabinol (THC)—the principal psychoactive ingredient of cannabis—by Albert Munson of the Medical College of Virginia. The high doses also suppressed the capacity of certain lymphocytes known as T cells to destroy skin grafts, to destroy transplanted tumor cells, and to proliferate in the presence of mitogens. The doses Munson gave to the rats were much higher than would be used by humans.

Alan Mellors of the University of Guelph in Ontario has found that smaller doses of THC given to rats and guinea pigs inhibit the production of migration inhibition factor, a substance that attracts scavenger lymphocytes (macrophages) to the site of interaction between a T cell and an antigen. He has also shown that THC inhibits the response of guinea pig lymphocytes to mitogens and alters the membranes of the lymphocytes by inhibiting the conconavalin A–stimulated incorporation of choline into phospholipids.

Macrophages in the lungs of rats are also affected by THC, according to Aviva Chari-Bitron of the University of Tel-Aviv. Chari-Bitron observed that the compound irreversibly inhibits the motility of the cells and causes them to release degradative enzymes, such as β-glucuronidase and β-galactosidase, that are normally contained in lysosomes within the cell. Mellors similarly observed that lysosomes from rat lymphocytes are split open in vitro by THC, and that their fragility is increased in vitro. He suggests that the effects on lysosomes are very similar to those produced by high doses of vitamin A.

Very little contradictory evidence has surfaced. Bernard W. Janicki and his associates at the Veterans Administration Hospital in Washington, D.C., observed no impairment in the function of lymphocytes from long-term cannabis users. But their subjects had used cannabis an average of only 3.4 times per week, so it is perhaps not surprising that they found no effects. Phyllis J. Lessin and Melvin J. Silverstein of the University of California School of Medicine at Los Angeles found no impairment of immunity among cannabis users in a standard skin patch test. But their criteria for selection of "chronic" smokers was use at least three times a week for at least 6 months. Again, this is not what most investigators would classify as either long-term or heavy use.

The major problem with all of these findings based on work with animals or cultured cells is extrapolating them to humans. There is a little evidence, such as that presented last year by Kolodny, that some long-term cannabis users suffer from sexual impotence and infertility, but there is no evidence of any widespread occurrence of this phenomenon in either the United States or foreign cultures where cannabis use is even more common. Similarly, there is no evidence for an increased incidence of infectious diseases or cancer among these populations, as would be expected if there were an impairment of immunity.

There is, however, also no evidence that such effects are not occurring. The only information which suggests that the effects do not occur comes from the clinical impressions of physicians who treat cannabis users and from studies on small numbers of subjects in other cultures. Both approaches are grossly ineffective in cases, such as this, where the observed effect might be manifested as only an increase in incidence of a few percentage points, even though the absolute numbers of people affected could be quite large. It is thus clear that a great deal more needs to be learned about the biochemical effects. Furthermore, most investigators agree that large-scale epidemiological studies such as those used to determine the relationship of cancer and smoking need to be conducted in this country. Only then will it be possible to get a true assessment of the risks associated with cannabis use.

—THOMAS H. MAUGH II

An Escalation of Potency

If there are any hazards associated with cannabis, it has become clear, they arise from prolonged use of large quantities of the more potent forms of the drug. Many investigators have in the past not been particularly concerned about cannabis use in this country because there were only limited quantities of the drug available and what was available contained only very small amounts of tetrahydrocannabinol (THC), the primary psychoactive ingredient. In the last few years, though, there have been marked changes in the quantity and quality of cannabis available in the United States. The nature of this change was indicated earlier this year at hearings of the Senate Subcommittee on Internal Security.* This subcommittee has often been accused of having a very strong anticannabis bias, but the facts pointed out at the hearing appear to have a strong foundation.

Before 1970, most marihuana consumed in the United States was a very weak domestic variety with an average THC content of about 0.2 percent. As consumption increased after 1970, Mexican marihuana edged out the domestic variety; this imported weed had an average THC concentration of about 1.5 to 1.8 percent. Beginning about 2 years ago, the predominant variety in the eastern United States became Jamaican and Colombian marihuana with an average THC concentration estimated by the Drug Enforcement Administration to be 3 to 4 percent. Even this may not be the end. If Mexican marihuana were harvested with a bit more sophistication, says Coy Waller of the University of Mississippi, its THC content could be increased to 5 percent or more.

The amount of marihuana that comes into the country has also increased dramatically. In 1970, federal authorities seized about 90,000 kilograms of marihuana. In 1974, they seized a little more than 900,000 kilograms. Officials estimate that only about 10 to 15 percent of incoming marihuana was seized in 1970 and about 15 to 20 percent last year. Estimates from various sources indicate that the total amount of marihuana imported last year was enough to prepare 6.5 to 8 billion cigarettes.

Even more alarming to many people is the increase in importation of hashish oil, a more concentrated—and thus more readily smuggled—form of cannabis. Hashish oil may contain as much as 90 percent THC, although the average is probably closer to 40 or 50 percent. In 1970, federal seizures of hashish oil totaled about 2800 kilograms. In 1974, total seizures were about 23,000 kilograms. The percentage of hashish intercepted is probably lower than that of marihuana.

Data from other surveys indicate that the total number of cannabis users, in contrast, has increased by only about 35 percent since 1971. The number of individuals who use it at least once a day, however, has grown from about 0.5 million in 1971 to more than 3 million today. These results strongly suggest that the average user is not only using a more potent form of cannabis, but also is using more of it.—T.H.M.

**Marihuana-Hashish Epidemic and Its Impact on United States Security: The Continuing Escalation* (Government Printing Office, Washington, D.C. 20402, 1975). Stock number 052-070-03019-4, $1.35.