EXPERIMENTAL
approaches

Marijuana, Tetrahydrocannabinol, and Pulmonary Antibacterial Defenses*

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Although marijuana is now consumed extensively, little is known of its biologic effects on the lung. To study this problem, the intrapulmonary inactivation of an aerosolized challenge of *Staphylococcus aureus* was quantified in rats exposed to graded amounts of fresh marijuana smoke. Controls inactivated 85.1 percent ± 0.3 percent of the bacteria six hours after inoculation. Following an *in vivo* accumulative exposure to smoke from progressively increasing numbers of marijuana cigarettes for periods of ten minutes each hour for five consecutive hours, intrapulmonary bacterial inactivation was impaired in a dose-dependent manner. Evaluation of the effects of parenterally administered delta-9-tetrahydrocannabinol (THC) or of exposure to fresh smoke from THC-extracted marijuana placebo cigarettes indicated that the cytotoxin in marijuana was not related to the primary psychomimetic component. Thus, marijuana smoke is toxic to the lung and impairs the pulmonary antibacterial defense system in a dose-dependent manner.

Marijuana consumption is widespread in the United States today, with an estimated 50 million or more sporadic users and a lesser number of regular consumers. The potential health hazards to the marijuana smoker are not well known.[1] As long as possession of marijuana remains illegal, this problem cannot be studied adequately by epidemiologic techniques in human populations. Furthermore, an understanding of any potential disease risk associated with marijuana use is complicated by frequent contamination of the illicit product, especially most recently with the herbicide paraquat,[2] making isolated case reports of the adverse effects of marijuana difficult to interpret. Marijuana, like tobacco, is consumed primarily by smoke inhalation. In that lung injury and the subsequent development of emphysema, chronic bronchitis, and lung cancer have been attributed by epidemiologic associations to tobacco smoking,[3] it would be of primary importance to know the biologic effects of marijuana smoke inhalation, as well as some of its component ingredients, on the lung. The primary psychoactive agent in marijuana is delta-9-tetrahydrocannabinol (THC). This cannabinoid has several potentially useful therapeutic applications in medicine, including its use as a potent bronchodilator. The effect of THC on the lung, independent of the effects of whole marijuana smoke, is not fully understood. The purpose of this communication is to report that under experimental conditions, the acute inhalation of marijuana smoke impairs the antibacterial defenses of the lung in a dose-dependent manner, and this effect does not appear to be associated with the primary psychoactive component.

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Materials and Methods

White male specific pathogen-free rats (CD strain, Charles River Breeding Laboratories, Wilmington, Massachusetts), weight-matched at 125 to 150 gm body weight and maintained on commercial rodent chow (Wayne Lab-Blox, Allied Mills, Inc, Chicago) and water *ad libitum*, were used in all experiments. All animals were acclimatized to laboratory conditions for at least one week prior to use. The experimental design for the research reported herein is shown in Figure 1. In groups of 36, the rats received an aerosolized intrapulmonary bacterial inoculation of *Staphylococcus aureus* labeled with radioactive phosphorus P 32 (FDA 209 P, bacteriophage type 42D) in a closed vented chamber.[4,5] In each of several sets of experiments, 12 of the initial 36

inoculated animals were chosen randomly and killed immediately after bacterial challenge for quantification of zero-hour bacterial and isotope deposition, using previously reported techniques.[4-6] All animals were killed with intraperitoneal administration of pentobarbital sodium (50 mg/kg of body weight, Abbott Laboratories, North Chicago) and exsanguinated by aortic transection. Within five minutes after completing the staphylococcal inoculation, and additional 12 randomly selected animals out of the initial group of 36 rats were exposed to the fresh whole smoke of marijuana cigarettes (marijuana research cigarettes, National Institute on Drug Abuse) using a 30 port smoking machine.[7,8] These marijuana research cigarettes contained an average of 2.2 mg/100 ml THC per unit, as determined by gas chromatographic analyses. The remaining 12 animals of the initial 36 inoculated animals served as unexposed controls.

The smoking machine was set to deliver aerosol-stabilized smoke in a 10:1 volume-to-volume air dilution to individually restrained animals. The performance of the machine was calibrated to meet the criteria established by the Hunter Committee for experimental animal smoke inhalation studies,[9] with smoke generated from each cigarette via a puff-volume of 35 ml over 2-second duration, with 58 sec between puffs. The puff-profile was square-shaped in character. A more detailed description of the use and characteristics of this smoke exposure system has been published previously.[8] By loading in each different experiment, the 30 port smoking machine with 3, 6, 10, 15, or 30 cigarettes for each exposure, each group of 12 experimental animals was exposed to fresh smoke over an eight minute period at five consecutive hourly intervals after bacterial inoculation for an accumulative exposure to the smoke generated from 15, 30, 50, 75, or 150 marijuana cigarettes, respectively. All cigarettes were preconditioned at 24°C and 60 percent relative humidity for 48 hours and were burned to a constant butt length. The cigarettes were weighed prior to use, but not selectively rejected. The final butt length after smoking was measured, the number of puffs per cigarette counted, and the burning characteristics calculated. To evaluate the effect on intrapulmonary antibacterial defenses of restraint immobilization *per se* in the smoking apparatus, an additional group of animals was sham-smoked under conditions identical to those of the smoke-exposed animals, except that the marijuana cigarettes were excluded.

At six hours post-bacterial inoculation, the 12 smoke-exposed and the 12 control (nonsmoked) animals from each experiment were sacrificed (Fig 1). At the time of sacrifice, 2 to 3 ml of blood were removed from the abdominal aorta of all animals for determination of carboxyhemoglobin with a CO-oximeter (Instrumentation Laboratories, Lexington, Mass) adapted and calibrated for rat hemoglobin. The lungs of all animals were removed aseptically, homogenized in nutrient broth, and processed for quantification of remaining viable intrapulmonary bacteria on replicate pour plate dilutions.[4-6] An aliquot of each homogenate was cultured on blood agar plates to determine the purity of the test microbe. The radioactivity of a hydroxide-digested aliquot of the homogenate was quantified by deep well scintillation counting (tri-carb liquid scintillation spectometer, Packard Instrument Company, Inc, Downers Grove, Ill). Bacterial and isotope counts were corrected for dilution and expressed as the number of viable staphylococci or the disintegrations per minute of radioactivity in the total lung tissue of each animal, respectively.

In an additional set of studies designed to evaluate the effect of the active psychomimetic component of fresh marijuana smoke, THC, 12 experimental animals were exposed, in a manner identical to that described above (Fig 1), to a dosage of the smoke from a total of an accumulative 75 THC-extracted marijuana placebo cigarettes (National Institute on

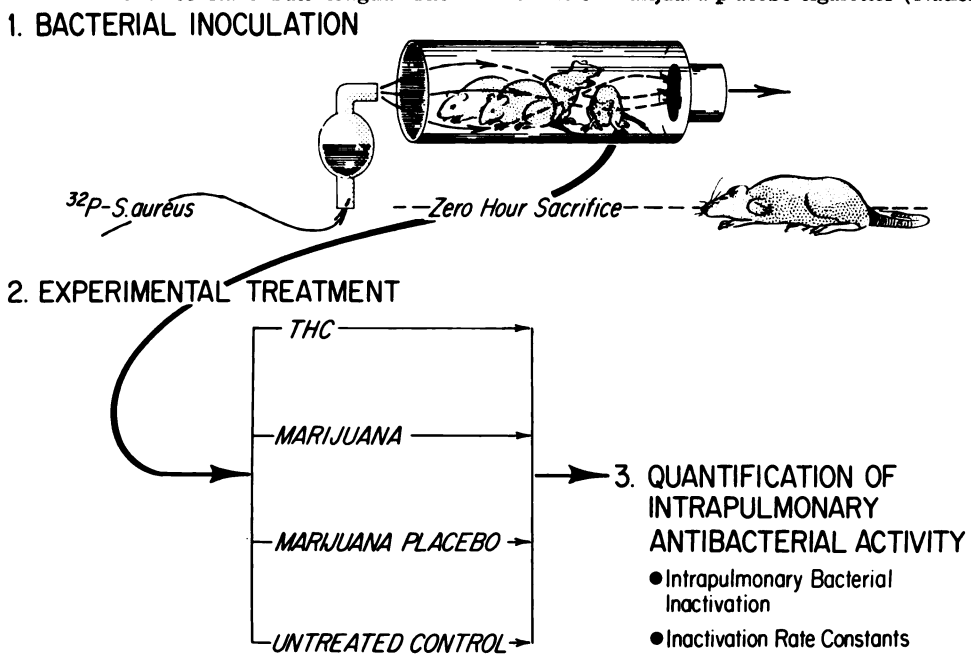

FIGURE 1. Experimental design to study individual effects of marijuana smoke in varying doses, marijuana placebo (THC-extracted) smoke, or systemic tetrahydrocannabinol (THC) administration on antibacterial defenses of lung. Following the aerosol inoculation of 36 animals with *S aureus* labeled with radioactive phosphorus P32 in each experiment, 12 animals were sacrificed for zero-hour deposition measurements, 12 animals were set aside as untreated shelf controls, and 12 animals were treated with the experimental regimen or appropriate sham procedure. The bactericidal capacity of lung was quantified by calculation of bacterial inactivation and rate factors.

Drug Abuse) over five exposure sequences and processed for quantification of viable intrapulmonary bacteria and radioisotope activity. In additional experiments of identical design (Fig 1), the effect of systemic intraperitoneal administration of saline-diluted tetrahydrocannabinol (purified THC in dehydrated ethanol, National Institute on Drug Abuse) on intrapulmonary bacterial inactivation was determined by administering dosages of 4 mg/kg or 10 mg/kg of body weight of the drug to 12 experimental animals immediately after staphylococcal aerosol inoculation. In these studies, the sham-treated animals received an intraperitoneal injection of dehydrated ethanol in isotonic saline in a volume equivalent to that delivered as a carrier for systemic THC administration. In all experiments, intrapulmonary bacterial inactivation was determined over the six-hour period between completion of the 30-minute aerosol staphylococcal inoculation and the time of killing. All experiments for each experimental variable were repeated three times, providing 36 animals for analysis in each treatment group.

In selected experiments, marijuana cigarettes were laced with decachlorobiphenyl (DCBP), a biologically inert, water-insoluble chlorinated hydrocarbon used as a nonreactive tracer of the particulate phase of smoke.[8,10] Total marijuana particulate matter, as well as carbon monoxide concentrations, delivered to the animal exposure system were quantified in all exposures. Our methods of quantification, and the dosimetric extrapolation on an individual animal basis in this study, for chromatographic determination of this tracer in the animal exposure apparatus and in the lungs of the smoke-treated animals, as well as the monitoring of carbon monoxide and carboxyhemoglobin levels, were identical to those procedures used in related studies.[8,10]

Pulmonary antistaphylococcal activity, defined as the change in the proportion of remaining viable to total bacteria initially deposited in the lungs of individual animals, was calculated by a modification of the group mean method of Laurenzi and co-workers.[4] In this method, intrapulmonary bactericidal activity was determined by counting the number of bacterial colony-forming units in the lungs of animals at zero hours and six hours after the aerosolized staphylococcal inoculation, with the percentage of viable organisms at the six-hour period expressed in reference to the mean number of colony forming units in zero hour animals. In a similar manner, the physical clearance or transport out of the lung of the inhaled microbial challenge was determined by quantifying the decline in radioactive phosphorus activity at six hours relative to the mean zero hour isotope activity for any given aerosol exposure. Each experiment was repeated a minimum of three times for each of the five levels of exposure to fresh marijuana smoke, exposure to smoke from the marijuana placebo units, or parental administration of purified THC.

The average value expressed for each level of exposure was obtained by pooling all experiments involving the same treatment and dose, and calculating a "weighted average" by the following formula [11]:

$$\text{Pooled mean} = \Sigma\, \bar{M}_c \bar{N}_c/\sigma^2{}_c/\Sigma N_c/\sigma^2{}_c$$

where $\bar{M}_c$ is average for the c^{th} experiment; N_c, number of animals in treatment group in c^{th} experiment; and $\sigma^2{}_c$; variance in treatment group c^{th} experiment. Thus, each experimental mean was "weighted" by the reciprocal of its variance in the averaging, yielding an unbiased minimum variance estimate for the treatment group.[8,11] All data thus expressed are presented as the weighted mean, plus or minus one standard error of the mean, with comparisons made according to Student's *t*-test.[11]

An additional measure of intrapulmonary bacterial inactivation, the *impairment factor*, was calculated for each treatment and dose, based on the assumption of a negative exponential curve characterizing bacterial inactivation over time, according to the following relationship:

$$B_t = B_0 e^{-kt}$$

where B_t is the number of viable staphylococci at time t, B_0 is the number of staphylococci culturable at time zero, t is time (six hours in these experiments), and k is the inactivation rate constant. This measure is interpreted as the theoretical amount of time required by animals given a particular treatment and dose to inactivate the same percentage of bacteria inactivated by matched untreated animals. For example, an impairment factor of 30 percent would mean that the treated animals require 30 percent longer to inactivate the same percentage of bacteria as the untreated animals. Further details on the calculation of the impairment factor are in press.[8]

Results

Compared to our experience with research and commercial tobacco cigarettes, the NIDA reference marijuana cigarettes were considerably more variable in packing consistency and friability. Their burning in the smoking machine left a gummy residue in the exposure system, which required frequent cleaning. The data derived from the measurements on pyrolyzation of the marijuana reference and marijuana placebo cigarettes are presented in Table 1. The mean concentration of marijuana particulate matter in the exposure system with the passage of the bolus generated by each smoking puff was 4.8 μg

Table 1—*Marijuana and Marijuana-Placebo Cigarette Pyrolyzation*

Parameter	Marijuana	Marijuana-Placebo
Cigarette length (mm)	85	85
Cigarette weight (gm)	0.79	0.77
Puffs/cigarette	8.0	8.0
Butt length (mm)	12.0	12.9
Burning rate (mm/min)*	7.3	7.2
Total particulate matter (mg)**	12.8	12.2
TPM/puff (mg)	1.6	1.5
Carbon monoxide (ppm)†	2600	2300
TPM exposure (μg/ml)††	4.8	4.6

*Combined burning rate for mainstream and sidestream smoke generation per minute.

**Total particulate matter (TPM) in mainstream smoke per cigarette.

†Average CO (ppm) concentration per puff delivered to the animal exposure rack in the intermittent exposure periods with each bolus of smoke.

††Concentration of total particulate matter (TPM) in the animal exposure rack during the passage of the bolus of smoke in the intermittent exposure periods.

Table 2—*Extrapolation of Experimental Lung Deposition Equivalents to Theoretical Human Consumption*

Scaling Variable	Scaling Factor	Marijuana Equivalent per Accumulative Exposure (μg)* 200	400	670	1000	2000
Weight calculations						
Body weight (kg)	70/0.125=560**	79	158	264	396	792
Lung weight (gm)	758/1.44=526**	74	149	248	372	744
Volume calculations						
Lung capacity (ml)	4830/5.9=819**	115	232	386	579	1158
Lung capacity (ml)	4830/8.5=568†	67	133	222	333	666

*Total particulate matter deposition equivalent in man (mg TPM per lung per day).
**Weight/weight and volume/volume scaling factors calculated from reference values.[12]
†Laboratory measured value (Huber, unpublished data).

/ml, and the corresponding carbon monoxide concentration was 2,600 ppm. There was an average loss of approximately 3 percent (range: 2 percent to 5 percent) of the total particulate matter generated to the walls and conducting tubing of the animal exposure system. Slightly less than 5 percent of the total smoke delivered to the exposure rack was retained by the exposed animals, with no significant differential by position in the exposure rack. The accumulative marijuana particulate smoke exposure in the animal rack for pyrolyzation of a total of 15, 30, 50, 75, or 150 cigarettes over the five-exposure sequence was 200 μg, 400 μg, 670 μg, 1,000 μg, and 2,000 μg, respectively. The corresponding average carboxyhemoglobin (COHb) concentrations measured in aortic blood of animals following these accumulative particulate exposures were 1.1 percent ± 0.3 percent COHb, 2.3 percent ± 1.0 percent COHb, 4.2 percent ± 0.7 percent COHb, 5.6 percent ± 1.9 percent COHb, and, in those animals that survived in the highest exposure, 9.4 percent ± 2.7 percent, respectively. Animals exposed to the smoke from THC-extracted marijuana placebo cigarettes had a COHb of 4.9 percent ± 1.7 percent. Control and sham-treated animals had COHb levels of less than 0.1 percent. Based on measurements of DCBP in the lung homogenates, smoke-exposed animals retained 19.8 μg ± 4.3 μg of marijuana per gram of lung per puff of cigarette. No animals died with accumulative particulate exposures of 1,000 μg or less. At the highest level of exposure (2,000 μg), the acute animal mortality was 43 percent. The comparable extrapolated marijuana lung deposition equivalents for humans, for each experimental animal exposure level, are presented in Table 2. Values reported by others for human and rat body weights, lung weights, and total lung capacity were used in these calculations,[8,12] as well as measurements from our laboratory on animal lung volumes obtained by water displacement.

The effect of acute exposure to fresh whole marijuana smoke on the antibacterial defenses of the lung is presented in Table 3. The dose-dependent nature of this response, and the corresponding calculated accumulative smoke exposures (total accumulative micrograms), are shown in Figure 2. Control

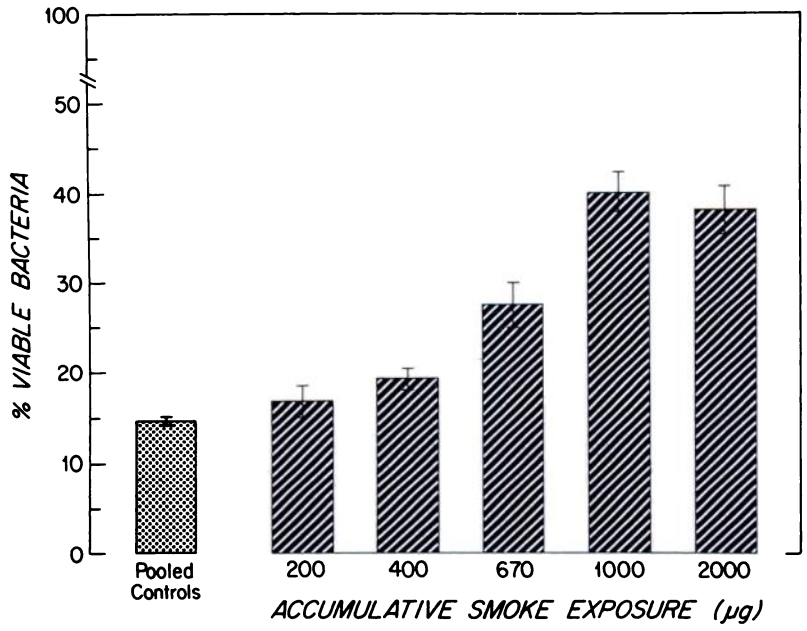

FIGURE 2. Effect of exposure to marijuana smoke on pulmonary defenses, with percentage of viable staphylococci remaining in lungs after exposure to progressively increasing amounts of smoke. Accumulative exposures are expressed as total marijuana particulate matter in exposure system.

Table 3—*Effects of Marijuana and Marijuana-Placebo Smoke on Pulmonary Antibacterial Defenses**

	Control	Marijuana Cigarettes Pyrolyzed					Placebo
	0 Units (n=126)	15 Units (n=36)	30 Units (n=36)	50 Units (n=36)	75 Units (n=36)	150 Units (n=36)	75 Units (n=36)
Bacterial inactivation (percent)	85.1±0.3	83.5±1.0	80.9±0.6	72.6±0.7	60.0±1.3	63.8±2.6	73.5±0.7
Radioisotope clearance (percent)	11.5±1.6	13.6±2.1	8.8±1.5	8.8±1.6	9.2±2.8	8.4±1.1	9.5±2.3
Impairment factor (percent)	. . .	24.0	29.7	49.7	68.4	64.1	67.6

*Number (n) in parentheses equals total number of pooled animals in each group. All data are presented as the weighted mean (see text) ±1 SD.

animals, pooled from data generated from all experiments, inactivated all but 14.9 percent ± 0.3 percent of the initial intrapulmonary staphylococcal challenge over the six-hour period of study. Sham exposure, in the absence of burning cigarettes, had no effect on intrapulmonary bacterial inactivation, with 16.5 percent ± 1.2 percent of the staphylococci remaining in the lungs of sham-treated animals versus 15.4 percent ± 0.9 percent in matched controls ($P > 0.05$). Although there was in some instances a suggestion of a consistent trend towards a reduction in physical clearance of the radioisotope following exposure to marijuana smoke (Table 3), none of the experimental values differed significantly from their individual matched control group or from the average values for pooled controls.

The comparative effect on pulmonary antibacterial defenses of fresh smoke from THC-containing marijuana cigarettes and THC-extracted marijuana placebo cigarettes, delivered to the experimental animals at comparable exposure levels, is presented in Figure 3. The percentage of staphylococci remaining in the lungs of animals from the two exposure conditions did not significantly differ. The effect of systemic administration of THC on the bactericidal activity of the lung is summarized, with intrapulmonary bacterial inactivation values, radioisotope clearance, and relative impairment factors presented in Table 4. When corrected for the effects of the ethanol carrier, as determined by evaluation of antibacterial defenses in ethanol sham-treated animals, systemic administration of the THC psychomimetic did not impair the antibacterial defenses of the lung.

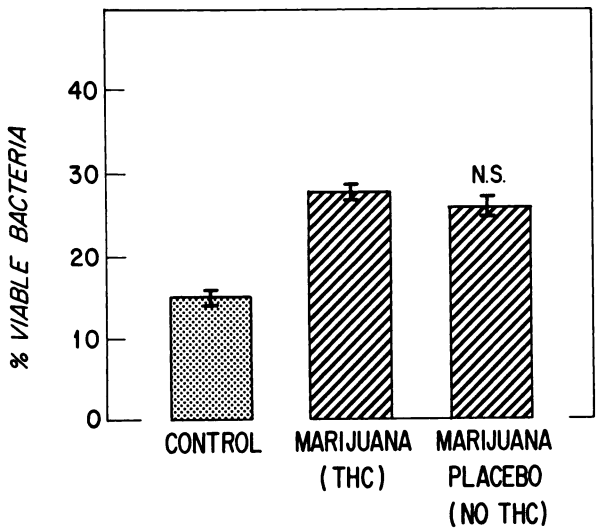

FIGURE 3. Effect of exposure to marijuana and marijuana placebo cigarette smoke on host defenses, with percentage of viable bacteria remaining in lungs of control and treated animals. Marijuana cigarettes contained 2.2 percent THC. The THC was extracted from marijuana placebo cigarettes. Biologic effects of THC-containing and THC-extracted marijuana smoke were not significantly (NS) different.

DISCUSSION

These studies characterize an experimental animal inhalation model that is a sensitive bioassay for evaluating the biologic effects of marijuana smoke on the intrapulmonary antibacterial defense system. The development and application of this model have been described in greater detail elsewhere.[8,13] The multiportal smoke generating apparatus used in these studies performed in an extremely reliable manner, dependably delivering fresh whole marijuana smoke as a stable aerosol in its gas phase in highly controllable and quantifiable exposure conditions. Compared to tobacco reference cigarettes,[8,10] however, the marijuana research cigarettes burned more rapidly and delivered significantly less total particulate matter and carbon monoxide. The THC-containing marijuana cigarettes differed only slightly in their pyrolyzation characteristics from the THC-extracted marijuana placebo units.

The amount of whole marijuana smoke particulate matter retained by the experimentally-exposed animals was a physiologically realistic dose, as based on the amount of chlorinated hydrocarbon tracer (DCBP) recoverable from each lung, the measured carboxyhemoglobin values, and the calculated lung deposition equivalents.[8,12] It is very difficult, however, even under ideal conditions, to extrapolate

Table 4—*Systemic Tetrahydrocannabinol and Pulmonary Antibacterial Defenses**

	Control (n=72)	THC 4 mg/kg (n=36)	THC Sham (n=36)	THC 10 mg/kg (n=36)	THC Sham (n=36)
Bacterial inactivation (percent)	85.1±0.3	78.3±2.2	78.3±3.9	51.4±3.2	59.4±3.9
Radioisotope clearance (percent)	11.5±1.6	11.3±2.8	9.5±3.0	10.0±3.9	13.7±4.1
Impairment factor (percent)	. . .	34.0	34.0	75.8	69.1

*Number (n) in parentheses equals the total number of pooled animals in each group. All data are presented as the weighted mean (see text) ±1 SD.

accurately exposure and dosimetry conditions from one species to another, including possible projections from experimental animals to man.[8,14] This difficulty was even more complicated in these studies, as the delivery and retention of marijuana smoke by human consumers of the drug is not known. It is important, nevertheless, as was done in these studies, to carefully control and quantify exposure to and retention of the smoking product to better compare cross-species inhalations and to assure research reproducibility and maximum potential interpretation of biologic significance. Scaling factors, as developed by Stahl,[12] can be utilized to project relevance to human consumption. Our data suggest, based on these calculated extrapolations, that the exposure conditions employed in these studies range from a low of five or fewer marijuana cigarettes per day (200 μg accumulative exposure) to 50 or more at the sublethal (43 percent mortality) highest dosage (2,000 μg accumulative exposure), assuming a 12 mg marijuana particulate delivery per cigarette.

The intrapulmonary bactericidal capacity of the host is dependent on a complex defense network with several component parts.[13] One primary line of defense is the physical transport or removal from the lung by the mucociliary stream of any organisms landing on the airways. Although the bacteria delivered in our aerosol inoculating system are carried for the most part within droplet nuclei and as such are deposited on the alveolar surfaces and nonciliated air spaces, previous studies have demonstrated that there is some agglomeration of the aerosol and a resultant deposition of approximately 10 percent to 15 percent of the initial inoculum on ciliated surfaces.[4-6] Experimental exposure to whole marijuana smoke, or to THC-extracted marijuana placebo smoke, or the systemic administration of THC did not impair this component of the antibacterial defenses of the lung, as radioisotope clearance did not differ significantly from controls in any smoke-exposed animals. The radioactive label incorporated by the bacteria is tightly bound to the DNA of the staphylococci, and its activity in lung homogenates represents an index of physical transport by mucociliary removal.[13]

Most inhaled bacteria delivered under the physiologic conditions used in these experiments are carried within droplet nuclei that land on the alveolar spaces, not the airways,[4,13] where they are inactivated by ingestion and intracellular digestion by pulmonary alveolar macrophages. Our results demonstrate that the alveolar component of pulmonary antibacterial defenses is impaired acutely, within a range of minimum and maximum threshold values, in a dose-dependent manner following exposure to increasing amounts of whole marijuana smoke. Extraction of the primary psychoactive component from the marijuana smoke, tetrahydrocannabinol, did not reduce this relative toxicity, indicating, as Vachon[15] has emphasized, that the "smoke" in marijuana smoking is by itself very important in considering the health effects on the consumer. Systemic administration by parenteral injection of THC did not appear to impair pulmonary antibacterial defenses beyond that effect expected by administration of the ethanol carrier alone.[16]

The mechanism of the dose-dependent impairment in pulmonary bactericidal activity by marijuana smoke is not known. Previous studies indicate that a water-soluble gas-phase component of marijuana smoke is cytotoxic to isolated alveolar macrophage recovered from the lungs of animals not previously exposed to marijuana.[17,18] Green and co-workers,[19-22] in a series of papers, have suggested that acrolein, acetaldehyde, and possibly other oxidants in the gas phase of tobacco smoke alter the activity of certain sulfhydryl-containing enzymes in macrophages and depress the energy-producing pathways required for phagocytosis. Whether or not similar oxidants in the gas phase of marijuana smoke act in a comparable manner is not yet known. In addition, these hypotheses need further clarification in that the metabolic events associated with alveolar macrophage phagocytosis and intracellular bacterial killing and digestion are not as yet clearly understood.

These studies have several clinical implications. Marijuana is now so widely consumed by smoking in

our society, especially by the nation's younger generations, that its potential adverse health effects deserve the most careful consideration. Several studies suggest that, dose for dose, marijuana smoke is highly more toxic to the lung than tobacco smoke.[8,15,23] Several reports, some of which have not been confirmed, associating marijuana and THC with adverse pulmonary or systemic responses, are conflicting and controversial.[24-32] Bronchitis, an enhanced susceptibility to pulmonary infection, alterations in protein synthesis by lung explants, and depressed immunity induced by marijuana have been suggested. Our results from the experimental animals reported herein support the concern for potential associations of lung disease with marijuana smoking. Unfortunately, as long as possession of marijuana remains illegal, reliable confirmation through epidemiologic investigations in human populations will be difficult to obtain.

Although the smoke in marijuana smoking may carry a significant associated health hazard,[15] preliminary reports imply that the psychoactive cannabinoids, in contrast, have several potentially useful therapeutic applications. Tetrahydrocannabinol is a potent bronchodilator with possible therapeutic implications in the management of asthma, a promising agent in the treatment of glaucoma, a possibly effective antiemetic to cancer chemotherapy, a possible pain reliever, a potential sedative and anesthetic, and has several other clinically important uses.[33-35] Our results, however, indicate that any therapeutic benefit of marijuana will require delivery of the pharmacologically active ingredient by a means other than marijuana smoke inhalation.

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