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Marijuana and Tobacco Smoke Gas-Phase Cytotoxins[1]

GARY L. HUBER[2]

Division of General Internal Medicine, University of Texas Health Science Center, Tyler, TX 75710

MELVIN W. FIRST AND OTTO GRUBNER

Department of Environmental Health Sciences, Harvard School of Public Health, Boston, MA 02215

HUBER, G. L., M. W. FIRST AND O. GRUBNER. *Marijuana and tobacco smoke gas-phase cytotoxins*. PHARMACOL BIOCHEM BEHAV **40**(3) 629–636, 1991.—To evaluate the in vivo versus in vitro paradoxical effects of marijuana and tobacco smoke on pulmonary defenses, the responses to smoke constituents were assessed with an alveolar macrophage tissue culture bioassay. A dose-response impairment of macrophage bactericidal activity was associated with water-soluble, gas-phase constituents. A model airway surface was constructed to examine the behavior of specific gas-phase constituents removed as they passed over wetted surfaces simulating the characteristics of the human respiratory system. Chemical analyses in the bioassay flask and in the model airway were compared. Gas-phase cytotoxins were measured after passage over wetted surface areas analogous to the trachea between the larynx and second-order bronchus. A wetted surface comparable to only 5% of the human airway, or less than 0.05% of the gas-exchanging surface of the entire lung, was capable of complete detoxification of the highly water-soluble gas-phase cytotoxins. In conclusion, gas-phase cytotoxins demonstrable by in vitro bioassays may have no cytotoxic potential when inhaled by humans.

Marijuana Tobacco Cytotoxins Bioassay Pulmonary alveolar macrophages Lung injury Chemical analyses

VARIANTS of *Cannabis sativa* have been consumed as marijuana, hashish, and hashish oil for centuries for their psychoactive constituents. In most Western cultures, especially in Europe and North America, marijuana, like tobacco, is consumed primarily by inhalation of smoke. Literally thousands of publications now have addressed the biological effects of these smoking products on human consumers and, under more highly controlled conditions, on experimental animals. Research publications on marijuana have been heavily weighted towards the effects of the cannabinoids, often exclusive of the many other constituents in marijuana smoke or in other preparations. Most of the scientific contributions on tobacco have focused on the potential role of cigarette smoke in the development of various lung diseases, including lung cancer and the chronic obstructive pulmonary diseases. Much less information is available on the effects of marijuana smoke on the lung or in the development of lung disease.

A burning tobacco cigarette has been described as a "miniature chemical factory" (21). There is no reason to believe that burning marijuana is much different. At least 6,000 chemical constituents have been identified and well characterized in fresh, whole tobacco smoke, and the number of trace components is several thousand-fold greater. Marijuana smoke has been far less investigated, and it is unlikely that, in the future, very much additional new information will be forthcoming. Marijuana smoke, at least as it is consumed in everyday life, is far more difficult to characterize because of the diverse nature of the product used. In addition, the manner in which shredded marijuana and tobacco are wrapped, and the very nature of the wrapping paper or other material used to contain the fragmented leaves, alter the burning characteristics during smoking, and in so doing alter the chemical nature of the smoke product.

Following pyrolization, marijuana and tobacco share a large number of common smoke constituents (3, 12, 18, 20, 33, 34, 36, 46, 48–51, 54, 59, 61, 68, 71); some of these are summarized in Table 1. Except for their psychoactively related components, fresh whole smoke from burning marijuana and burning tobacco is qualitatively quite similar. In most studies of these pyrolization products, it has been a common practice to divide the generated smoke into a particulate phase and a gas or vapor phase. The smoke particulates of research marijuana cigarettes and research tobacco cigarettes generate aerosols that are quite comparable, but not quite identical, in size (19,25); burning marijuana leaves a more "gummy residue," in comparison to the "tar" of tobacco cigarettes (19). The aerosol particulates are initially about a quarter of a micron, or slightly less, in diameter

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[2]Requests for reprints should be addressed to Gary L. Huber, M.D., UT Health Science Center, Box 2003, Tyler, TX 75710.

TABLE 1

COMPARISON OF MARIJUANA AND TOBACCO SMOKE CONSTITUENTS*

Constituent†	Marijuana	Tobacco
Whole Smoke		
Burning rate (mm/min/g)	11.6	5.7
pH (3rd to 10th puffs)	6.56–6.58	6.14–6.02
Moisture (%)	10.3	11.1
Particulate Phase		
Total particulate (mg/puff)	1.6	2.4
Phenol (μg)	76.8	39.0
o-Cresol (μg)	17.9	24.0
m- and p-Cresol (μg)	54.4	65.0
2,4- and 2,5-Dimethylphenol (μg)	6.8	14.4
Naphthalene (μg)	3000	1200
Benz(a)anthracene (μg)	75	43
Benz(a)pyrene (μg)	31	22
Nicotine (μg)	—	2850
Delta-9-tetrahydrocannabinol (μg)	820	—
Cannabinol (μg)	400	—
Cannabidiol (μg)	190	—
Gas Phase		
Carbon monoxide (ppm/cig)	2600	4100
Ammonia (μg)	228	198
Hydrogen cyanide (μg)	532	498
Isoprene (μg)	83	310
Acetaldehyde (μg)	1200	980
Acetone (μg)	443	578
Acrolein (μg)	92	85
Acetonitrile (μg)	132	123
Benzene (μg)	76	67
Toluene (μg)	112	108
Dimethylnitrosamine (ng)	75	84
Methylnitrosamine (μg)	27	30

*Literature composite (3, 12, 18, 20, 33, 34, 36, 46, 48–51, 54, 59, 61, 68, 71).

†Weights expressed as grams (g), milligrams (mg), micrograms (μg), or nanograms (ng) per cigarette or per other denominators, as indicated.

and are suspended in their surrounding gas phase as approximately 2 to 5 billion liquid microdroplets per ml. About 10% of the total fresh, whole smoke weight is in the monodispersed particulate phase, and 90% or greater is in the vapor or gas phase. The psychoactive constituents—the cannabinoids in marijuana smoke and nicotine and its related products in tobacco smoke—are initially in the particulate phase, but for the most part probably vaporize into the gas phase prior to absorption within the lung. This appears at least to be true for nicotine, but less information is available on the transfer kinetics of the psychoactive constituents of marijuana smoke. Regardless, significant early research on the biological effects of marijuana smoke and tobacco smoke on the lung focused on gas-phase constituents that were potentially cytotoxic to pulmonary tissues (6, 16, 26, 27, 37–42, 45, 57). The purpose of this paper is to address the nature of some of the potential cytotoxins in the gas phase that are common to marijuana smoke and tobacco smoke.

METHOD

The effects of fresh, whole smoke and the gas phase of fresh smoke generated from research marijuana units and research tobacco cigarettes on an in vitro bactericidal bioassay system employing pulmonary alveolar macrophages (PAM) were studied; this bioassay has been described extensively (16, 27, 28). In brief, PAM were recovered from specific pathogen-free male, 150–200-gm rats (Charles River Breeding Laboratories, Wilmington, MA) by seven sequential bronchoalveolar lavages (BAL) with 7-ml aliquots of isotonic saline, heparinized (10 U/ml) at 23°C (35,57). Approximately 1×10^6 viable PAM/ml were distributed to tissue culture flasks agitated on a shaker bath at 37°C containing a balanced salt solution, 20% normal rat serum, and 5% strain-specific antiserum (57). A strain of *Staphylococcus epidermidis* (BCH No 71-55065) was used in all studies (35,57). Intracellular bactericidal activity of PAM, quantified after 3 hours of incubation, was expressed relative to the bacterial colony-forming unit capacity of the zero hour control, corrected for bacterial replication within the system over the same period of total incubation, by standard procedures (45).

Fresh whole mainstream smoke was generated from research marijuana cigarette units (2.1% delta-9-tetrahydrocannabinol or THC), or from reference tobacco cigarettes. The THC-containing research marijuana units were supplied by the National Institute on Drug Abuse; the reference tobacco cigarettes (2RI nonfiltered) were supplied by the Tobacco and Health Research Institute at the University of Kentucky. After cold storage at 1°C, all marijuana units or reference cigarettes were preconditioned at 60% relative humidity and 23°C for 24 h prior to study. The research marijuana units or the reference tobacco cigarettes were fitted closely with an inert connector to a 50-ml gas-tight glass syringe. After lighting, smoke was generated in 35 ± 0.5 ml aliquots drawn at 60-s intervals as a 2 ± 0.2-s "puff" into the syringe. The first three such "puffs" were discarded. Fresh whole smoke from the fourth "puff" generated was injected in either 2-, 4-, or 8-ml aliquots into the bioassay tissue culture flasks. In additional experiments, whole smoke from each product was separated into particulate- and gas-phase constituents following passage of the smoke through an absolute Cambridge-like filter pad (27). In further experiments, the gas phase of each smoking product was further partitioned by its passage through a distilled water trap fitted with a fitted gas dispersion filter distal to the absolute filter; the resultant effluent thus contained only water-filtered gas-phase constituents. Either the complete gas phase or the water-filtered gas phase was introduced in 8-ml aliquots in the bioassay system, in the same manner as described for whole smoke above.

The concentrations of two specific gas-phase cytotoxins, acrolein and acetaldehyde, specifically reported to be important to impairment of alveolar macrophage bactericidal activity in this bioassay system (15, 17, 53), were quantified in the freshly generated tobacco cigarette smoke under standard analytical automated smoking conditions; variations in marijuana were so extensive from unit to unit that partitional analyses could be undertaken reliably only with reference tobacco cigarettes. Each cigarette was smoked with 35 ± 0.5-ml puffs of 2 ± 0.2 s duration to a 23 ± 0.5-mm butt length with a five-port analytical smoking machine. The gas phase of the smoke was separated from the particulate phase by passage of the whole smoke through a Cambridge-like absolute filter pad. Two cigarettes were smoked per each determination, and six determinations were made in this set of experiments.

Additional studies were performed to compare the gas-phase cytotoxin concentrations generated from cigarettes smoked with a standard analytical automated smoking machine to the concentrations of the same constituents generated by smoking the cigarettes manually with a glass syringe, as is conventionally employed in delivering smoke or smoke components to the in vitro bioassay system. Manual smoke generation was undertaken with a 50-ml gas-tight glass syringe, fitted with a three-way inert valve. Smoke was generated through Cambridge-like absolute filter

TABLE 2

INTRACELLULAR KILLING OF BACTERIA BY PULMONARY MACROPHAGES (27)*

Smoke Product	Marijuana†	Tobacco†
No Smoke (0 ml)	78.8 ± 1.4	78.6 ± 4.1
Fresh Gas Phase Only (8 ml)	24.8 ± 4.1	21.9 ± 3.4
Water-Filtered Gas Phase (8 ml)	72.6 ± 1.4	76.9 ± 4.1
Fresh Whole Smoke		
2 ml	67.1 ± 7.1	50.4 ± 9.4
4 ml	23.7 ± 7.0	37.4 ± 13.0
6 ml	20.5 ± 7.0	22.6 ± 17.7
8 ml	11.4 ± 7.6	17.7 ± 0.7

*Macrophage bactericidal function is expressed as intracellular inactivation rates after 3 h incubation, relative to zero hour control values (27). Volume of whole smoke or smoke product was introduced into the tissue culture flasks at time zero.

†Data are expressed as the arithmetic mean of the percentage of bacteria killed, plus or minus one standard error of the mean.

= Volume of whole smoke or smoke product introduced into the tissue culture flasks at time zero.

TABLE 3

CARBONYL COMPOUNDS GENERATED FOR THE BIOASSAY SYSTEM

		Acrolein*			Acetaldehyde*		
Method of Smoke Generation	No. of Puffs	Per Cig.	Per Puff	Per Vol.	Per Cig.	Per Puff	Per Vol.
Mechanically Smoked	10.9	87	8.0	0.23	913	84	2.4
Manually Smoked†	20	162	8.1	0.23	1727	86	2.5

*All results are expressed in micrograms of the carbonyl compound, per total reference cigarette (per cig.), per puff of the gas phase of the reference cigarette (per puff), or per milliliter of gas phase (per vol.).

†Puff and volumetric were data generated from the fourth manually generated puff.

pads to separate the gas phase from the fresh whole smoke. Twenty cigarettes were smoked for each determination, and four determinations were made in this set of experiments. Consistent with the smoke-generating protocol for the in vitro bioassay system, only gas-phase components from the fourth puff were analyzed.

In additional experiments designed to measure the uptake of acrolein and acetaldehyde from the gas phase of cigarette smoke by the tissue culture medium of the in vitro bioassay system, the plastic tissue culture flasks from this system were fitted with drilled-out plastic caps with teflon-lined septums. This permitted the direct injection of 8 ml of fresh gas-phase smoke from the manually smoked fourth puff of the research cigarettes directly into the tissue culture flask. Each tissue culture flask contained 2 ml of the balanced salt solution. In separate experiments, attempts were made to carry out these analyses with serum present in the balanced salt solution. These analyses did not meet with success, in that serum proteins were not compatible with the reagents used in the determination of these carbonyl compounds. Thus, to facilitate chemical analyses, specific antiserum and fresh antibody were not added to the liquid milieu in any studies involving chemical analyses. In two separate experiments, the gas-phase smoke was permitted to remain in contact with the balanced salt solution within the tissue culture flask for one minute or for five minutes before the solution was removed and analyzed for the carbonyl compounds. For the one-minute study, three determinations were made; for the five-minute study, five determinations were made.

Finally, in additional experiments with the tissue culture flasks, attempts were made to quantify the amount of acrolein and acetaldehyde remaining in the gas phase above the balanced salt solution within the flask after five minutes of contact of the gas phase with the balanced salt solution. To do this, the tissue culture flasks were fitted with an inert stopcock at the end opposite the cap. The gas phase of freshly generated smoke was injected via the cap into the flask, as described above. After five minutes, a needle connected to a nitrogen source was inserted through the septum, and the gas phase above the liquid tissue culture milieu was swept into a trap for analysis.

To further study the behavior and interaction of water-soluble constituents of the gas phase common to these smoking products with wetted surfaces comparable to those lining the airways to the lung, we developed a model airways surface. The detailed description of this model airway surface, as well as the specific methodologies used in the gas-phase chemical analyses, will be reported separately. A very similar airway model had been used in the past for the study of various environmental air pollutants. In brief, the model airway surface consisted of four fixed lengths of wetted wall tubing that simulated some of the characteristics of the human upper respiratory system. The four serially connected components of the model airway were glass tubes 30 cm in length, with an internal diameter of 8 mm, lined with all-glass microfiber filter material (MSA 1106B, Mine Safety Appliances Co., Pittsburgh, PA) moistened with the same balanced salt solution employed in the in vitro PAM bactericidal bioassay system and in the tissue culture flask chemistry studies, noted above. The gas phase of cigarette smoke was partitioned by passage of whole smoke generated from standard 35 ± 0.5-ml, 2 ± 0.2-s "puffs" through an absolute Cambridge-like filter pad, as described above. The gas phase of tobacco smoke or of marijuana smoke could be passed through each or all of the four tubes as they were bare and dry, or it could be passed through a varying number of tubes that were lined with moistened filter paper. In order to evaluate the differential changes in bioactivity of the water-soluble, gas-phase cytotoxins, none, one, two, or four of the tubes were wetted; tubes not lined and wetted were left in place, so that the configuration of the glass apparatus and the characteristics of the transient passage of the gas phase introduced into the model airway surface remained unaltered throughout all experiments. Gas-phase effluent samples were sampled with tight-fitting glass syringes from the sampling ports at the end of each tube. These gas-phase samples were used for the collection of aliquots of effluent, 8 ml of which were introduced into the in vitro PAM-bactericidal bioassay, as described above.

When lined and wetted, each of the four tubes in the model airway surface had an effective internal surface area of about 75 cm^2. Thus the four tubes together comprised an area comparable to the surface area of the human trachea from the larynx to the second order bronchus (72). The total volume of the airway surface simulator was 120 ml, thus requiring at least four 35-ml puffs to fully fill the apparatus. A 2.5×30-cm strip of all-glass microfiber sheet was wetted with the balanced salt solution, at pH 7.4, used in the in vitro bioassay system. To be consistent with the procedures employed in the analyses from the tissue culture flasks, serum proteins were not added to the solution pipetted onto the microfiber sheets. To measure differential chemical changes in the quantity of water-soluble constituents of gas-phase smoke issuing from the apparatus, none, one, two, or four tubes were lined and wetted. Tubes not lined or wetted were left

TABLE 4

UPTAKE OF CARBONYL COMPOUNDS BY THE BIOASSAY SYSTEM

	Acrolein*			Acetaldehyde*		
Contact Duration	Per Flask	Per ml	Percent Recovery†	Per Flask	Per ml	Percent Recovery†
1 min	0.44	0.05	22	5.7	0.7	28
5 min	0.48	0.06	26	6.3	0.8	32

*Results are expressed as micrograms (μgm) of the carbonyl compound within the flask (per flask) or within the balanced salt solution (per ml).

†Percent recovery was calculated on the basis of carbonyl compounds recoverable from this tissue culture balanced solution relative to amounts potentially available (from Table 3).

in place, as was done in related experiments in which the effluent was introduced into the bioassay system. Gas samples of varying amounts (usually 0.5 ml) were extracted with a gas-tight glass syringe from the end of the fourth tube for chemical analyses by gas-liquid chromatography, with a flame ionization detector operating at 250°C.

The arithmetic mean and, where appropriate, standard error of the mean were calculated for all data; comparisons were made using the Student's *t*-test for unpaired data.

RESULTS

As summarized in Table 2, whole marijuana smoke and whole tobacco smoke depressed the ability of pulmonary alveolar macrophages to kill bacteria in the bioassay system employed in these studies; essentially identical results were obtained with other smoking products reported independently from our laboratories (26–28, 45). This depression of in vitro bactericidal activity was dose dependent, and the responsible cytotoxic fraction was present as water-soluble constituents of the gas phase. Although there were slight trends in differences of the arithmetic means of the depression of bacterial killing rates induced by the different smoking products, these trends were not consistent, and none were statistically significant.

The quantification of two key macrophage-known cytotoxins, acrolein and acetaldehyde, generated from the research cigarettes either under standard analytical smoking conditions or with manual smoking conditions, is summarized in Table 3. These results represent data generated from analyses of the gas phase only. The number of total puffs per cigarette, and thus the total amount of carbonyl delivery per cigarette, differed as a function of the method of smoking. The amount of acrolein and the amount of acetaldehyde, however, were essentially identical when expressed on a puff per puff or volume per volume basis. Thus, by extrapolation, 1.84 μg (0.23 μg/ml × 8.0 ml) of acrolein and 20 μg (2.5 μg/ml × 8.0 ml) of acetaldehyde were introduced from the fourth smoking puff as gas-phase constituents into the bioassay flasks when the filtered smoke was added to the tissue culture system.

TABLE 5

REMOVAL OF GAS-PHASE CONSTITUENTS BY THE MODEL AIRWAY SURFACE

Carbonyl Compound	One Tube (75 cm^2)	Two Tubes (150 cm^2)	Four Tubes (300 cm^2)
Acrolein	22–47	74–80	93–100
Acetaldehyde	25–42	46–59	64–72

*All data expressed as a percent (%) removal of the carbonyl compounds, calculated for the amount of acrolein or acetaldehyde in the effluent of each wetted tube relative to zero wetted tubes.

TABLE 6

INTRACELLULAR KILLING OF BACTERIA BY PULMONARY MACROPHAGES*

Wetted Surface Area	Marijuana†	Tobacco†
0 cm^2	18.3 ± 1.5	17.4 ± 1.6
75 cm^2	35.0 ± 2.3	38.2 ± 1.8
150 cm^2	71.5 ± 1.9	77.8 ± 2.4
300 cm^2	85.1 ± 2.1	88.3 ± 2.9

*Macrophage bactericidal function expressed as intracellular inactivation rates after 3 h of in vitro incubation, relative to zero hour control values (27).

†Data expressed as the arithmetic mean of the percent of bacteria killed, plus or minus on standard error of the mean, after the introduction of gas-phase effluent from the model airway surface into the tissue culture flasks following exposure to varying wetted surface areas.

The quantitative uptake of acrolein and acetaldehyde by the balanced solution within the tissue culture flasks, following exposure periods of 1 min or 5 min, is presented in Table 4. Small amounts of balanced salt solution were lost either to the tissue culture flask surfaces or in transfer, and thus the quantities within the tissue culture flasks listed in Table 4 may be lower than the amounts actually present within the tissue culture milieu. In reference to the quantities initially introduced with the gas phase into the tissue culture flask (Table 3), 26% of the acrolein available and 32% of the acetaldehyde available were absorbed into the balanced salt solution within 5 min of exposure; the additional data presented in Table 4 indicates that this absorptive transfer occurred, for the most part, within 1 min of exposure. In those studies designed to quantify the amounts of acrolein and acetaldehyde remaining within the tissue culture flask in the gas phase above the balanced salt solution, no acrolein or acetaldehyde could be detected. That is, within 5 min of exposure within the flask, 74% of the acrolein and 68% of the acetaldehyde introduced into the flasks could not be detected, presumably due to a combination of absorption into the tissue culture milieu, adsorption to the tissue culture flask walls, and loss through chemical reactions with other gas-phase constituents present.

The relative removal of acrolein and acetaldehyde following passage over increasing increments of wetted surface area within the model airway surface is presented in Table 5. Based on repetitive experiments, the range of removal of the carbonyl gas-phase constituents, acrolein and acetaldehyde, is expressed relative to the quantities of these constituents in the effluent portal when no wetted surfaces were present within the model airway surface. Thus, following passage of the gas phase over a wetted surface area equivalent to just 5% or less of the human airway or less than 0.05% of the entire gas-exchanging surface of the entire lung (72), 93% to 100% of the acrolein and 64% to 72% of the acetaldehyde were removed. The detailed chemical analyses of 17 constituents of the gas phase during passage within this model airway surface will be reported independently.

Table 6 summarizes the capacity of pulmonary alveolar macrophages to kill bacteria following the introduction of 8 ml of

residual effluent gas phase into the in vitro bioassay tissue culture flasks after passage through zero (0 cm^2), one (75 cm^2), two (150 cm^2), or four (300 cm^2) wetted tubes of the model airway surface. Exposure of the gas phase to 150 cm^2 to 300 cm^2 of wetted surface resulted in a near-total reduction in gas-phase cytotoxicity for both smoking products, as assessed in this in vitro bioassay system.

DISCUSSION

The results of these and other studies indicate that marijuana and tobacco smoke depress the intracellular killing of bacteria by pulmonary alveolar macrophages when these smoke products are delivered to an in vitro bioassay system (15–17, 26–28). The cytotoxin appears to be water-soluble constituent(s) of the gas phase. Marijuana and tobacco whole smoke and gas phase are quite comparable in their cytotoxicity, when evaluated in this manner. Previous research has indicated that the cytotoxin(s) in whole smoke or in the gas phase need be in contact with the in vitro bioassay system for only a few seconds to exert their maximum cytotoxicity (15–17, 26–28). Stale marijuana or tobacco smoke, held for several seconds prior to its introduction into the in vitro bioassay, rapidly loses all of its cytotoxic properties (27). Our previous results also indicate that the cytotoxic effect of THC-extracted marijuana placebo cigarettes did not significantly differ from THC-containing marijuana whole smoke or from tobacco whole smoke (27). Furthermore, the administration, in relatively high dosage, of purified tetrahydrocannabinol directly to the in vitro bioassay system did not adversely affect the capacity of pulmonary alveolar macrophages to kill bacteria under these conditions (27).

Pathomechanistic studies indicate that glycolysis and, specifically, glyceraldehyde-3-phosphate dehydrogenase activity, in pulmonary alveolar macrophages are directly impaired by gas-phase constituents of tobacco smoke (15,53); comparable mechanistic studies with marijuana smoke apparently have never been conducted. A dose-dependent depression of phagocytosis of staphylococci by pulmonary alveolar macrophages exposed to tobacco smoke under these in vitro conditions has paralleled the depression of intracellular activity of phagocytosis-related glyceraldehyde-3-phosphate dehydrogenase (53); phagocytosis was equivalent to intracellular killing of the bacterial species used as the test microbe in our bioassay system (35,57). Generally, the pulmonary alveolar macrophage derives its cellular energy for phagocytosis primarily from glycolysis, whereas intracellular killing of this strain of bacteria appears to be linked to a concurrent burst of hexose-monophosphate shunt activity (7–10).

In this bioassay system, several studies suggest that a highly water-soluble constituent of the gas phase of marijuana smoke, tobacco smoke, or other smoking products adversely affects sulfhydryl groups on glyceraldehyde-3-phosphate dehydrogenase and alters its associated role in phagocytosis (15, 17, 53). This in vitro effect is not confined in its toxicity to pulmonary alveolar macrophages, as the gas phases of marijuana and of tobacco smoke also have similar in vitro toxicity on human and animal lung tissue cultures and lung explants (37–42). Acrolein, acetaldehyde, formaldehyde, and other gas-phase potential cytotoxins have been implicated as responsible for these alterations, and their adverse effect on the pulmonary alveolar macrophage bioassay system or on other lung tissue culture preparations can be reduced or fully eliminated by the addition of sulfhydryl-protecting agents to the in vitro preparations (15, 17, 38, 40).

Essentially all of the data reported in the literature for the cytotoxicity of the gas phase of marijuana smoke, tobacco smoke, or other smoking products are derived almost exclusively from in vitro cell culture systems or from in vitro bioassays of whole or gas-phase marijuana smoke and tobacco smoke. Exposures of the intact host to these smoking products paradoxically reveal little or no acute toxicity, except at unrealistically high doses (22–26). Furthermore, pulmonary alveolar macrophages from experimental animals or from humans chronically exposed to marijuana or tobacco whole smoke are not metabolically depressed and, depending on the test conditions, often appear to have little or no significant depression in their antibacterial capacity (7–10, 22–26, 44). Hypothetically, then, it may well be that these differences are due to the removal of highly water-soluble, gas-phase cytotoxins high within the airways of the human or animal respiratory system, long before they ever potentially could reach the pulmonary macrophages in the alveolar spaces deep within the distal lung.

With present technologies available, it will be most difficult to test in humans, or even in experimental animals, the hypothesis that the proximal airways remove most or all gas-phase cytotoxins within the upper airways. For that reason, we constructed a model airways surface to examine the amount and nature of specific gas-phase constituents removed as they were passed through wetted surfaces simulating the characteristics of the human respiratory system, including retention times and flow rates. Chemical analyses of additional gas-phase constituents, quantified by gas-solid chromatography, will be further reported independently. In this model airways surface, acrolein and acetaldehyde were greatly diminished in concentration after exposure to a wetted surface area analogous to or less than that of the human trachea between the larynx and the second-order bronchi (72). When the potential cytotoxicity of the effluent gas phase from this model airways system was tested in our bioassay system, no significant impairment in pulmonary alveolar macrophage bactericidal capacity was demonstrable. Thus a physiologically wetted surface, comparable to only 5 percent or less of the human airway (72), or less than 0.05% of the gas-exchanging surface of the entire lung (72), was capable of complete detoxification of highly water-soluble gas-phase cytotoxins, including acrolein and acetaldehyde. Under other experimental conditions, important gas-phase constituents appear to be removed following exposure to the wetted surfaces of the mouth, posterior pharynx, and trachea (5,32).

The data reported herein from analyses of acrolein and acetaldehyde within the tissue culture flasks indicate that the concentration of these carbonyl compounds delivered by manual generation of the gas phase was comparable to those values, on a puff-by-puff basis, reported for the gas phase generated under analytical smoking conditions. Related data from mass balance analyses indicate that the tissue culture solution absorbed these carbonyl compounds very rapidly from the gas phase within the flask, for the most part within one minute and certainly by five minutes of exposure. If these cytotoxins, in fact, do exert their adverse effect in depressing alveolar macrophage bactericidal activity by impairing phagocytosis of the microorganisms, this effect would be exerted very rapidly, well before significant numbers of microorganisms could be ingested.

The human smoker generates a smoke puff volume of less than 35 ml to 50 ml or more from tobacco cigarettes (21); the volume generated by marijuana smokers is less well characterized. This puff volume is diluted 10- to 20-fold by the smoke inhalation volume in tobacco smokers (21); the dilutional volume in marijuana smoking is not well appreciated, but is probably equal to or greater than that reported for tobacco cigarette consumption. Thus exposure by humans to gas-phase cytotoxins in marijuana or tobacco smoking would be diluted by a factor of at least one log or more in the conventional use of these smoking products, relative to the volumetric concentrations delivered to the in vitro bioassay system described in this report. In addi-

tion, the diluted water-soluble, gas-phase constituents would be delivered in the human lung to a wetted surface area at least two logs greater in size than the surface area that effectively removed these cytotoxins in the model airways surface employed in our studies. Thus the adverse effects of these cytotoxins on pulmonary alveolar macrophages in the in vitro bioassay tissue culture flask represent an artifactually and unrealistically high exposure level, relative to the potential exposure of alveolar macrophages to those cytotoxins deep within the lungs of human marijuana or tobacco smokers. These results may explain, to a significant degree, the discrepancies that exist in the literature between in vitro and in vivo cytotoxicity of marijuana and tobacco smoke. These results also raise the consideration that the potentially injurious effects of water-soluble, gas-phase constituents on alveolar macrophages on lung explants may not be relevant to the health and integrity or the lung of the intact smoker in everyday life.

There are several reports that inhalation of marijuana whole smoke and tobacco whole smoke by humans or experimental animals induces alterations in the lung in a differential manner (2, 22, 29, 30, 44, 47, 55, 56, 58, 62–67, 69, 70, 73) including an apparent differential response in the airways and in recoverable pulmonary alveolar macrophages (1, 14, 24–26, 55). The specific constituent(s) responsible for these differential alterations are not known, although the gas-phase cytotoxins have received much of the earlier investigative attention; the data presented herein, however, would appear to render them less suitable culprits. Recent attention has focused on oxidant-related smoke injury, including the potential adverse effect of free radicals directly on the airways, directly on the lung parenchyma, or indirectly on the lung via neutrophil-medicated alterations in antiprotease activity (4, 13, 31, 60). It must be emphasized, however, that no precise mechanisms of lung injury have been established as yet, and that the exact role of specific marijuana or tobacco smoke constituents in the pathogenesis of lung disease remains hypothetical (13, 31, 52). In that, except for the psychoactive constituents, the pyrolyzation products of marijuana and tobacco are similar in many ways, differential pulmonary responses may be due, in part, to the different manner in which these two substances are consumed.

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