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Antitussive Action of Aerosolized Terpenes in Guinea Pigs and Humans

By S. J. DESALVA, R. A. EVANS, and R. B. BUTLER

The present study has established with both animal and human assays that the use of aerosolized terpenes does produce significant antitussive effects, and these effects are related to the concentration of the materials in the exposed atmosphere. Moreover, it would appear that the effectiveness of this type of therapy is related to an action on the smooth muscles of the bronchi and bronchioles.

INHALATION THERAPY, *e.g.*, vaporizers, mist, and aerosols, for the treatment of respiratory disturbances has been accepted as a reasonable procedure (1). This is particularly evident in the legendary use of volatile oils for the relief of coughs. The basis for this type of treatment has been mainly empirical. It appeared pertinent at this time to undertake an investigation of the antitussive effectiveness of certain of these volatile substances, given as aerosols,[1] with controlled conditions such as the ammonia-induced cough assay in guinea pigs (2) and the citric acid-induced cough assay in humans (3). This report is a summary of our findings which offers to the reader animal and human data obtained through the efforts of the same investigators.

EXPERIMENTAL

Antitussive Assays in Guinea Pigs

Method.—The method employed is a modification of that described by Winters and Flataker (2). Test animals (350 to 450 Gm. body weight) were individually placed for 1 minute in a 1-L. exposure chamber—a plastic cylinder—into which ammonia vapors were introduced. Vapors were delivered at 10 p.s.i. from an Ohio nebulizer, which contained 200 ml. of 0.5% ammonia water (Merck 28% stock) in the reservoir. The concentration of ammonia in the exposure chamber was relatively constant under the experimental conditions used.

Ammonia-induced coughs were recorded on a paper oscillograph (Grass model 5) *via* a pressure transducer (PT 5-A) which was connected to the exposure chamber and the oscillograph. The transducer was capable of recording respiratory rate as well as body movements and by proper manipulation of the sensitivity gain of the preamplifier, the gross body movement measurements could be made less apparent. Prior to the antitussive evaluation, the animals were screened to determine their susceptibility to ammonia vapors. Animals that coughed at a frequency of between 10–40 coughs per minute were selected for the drug study which was generally performed at least 2 hours afterwards. In any particular group, the test animals were selected in a manner such that their cough frequency response did not vary more than 10 per minute. This arbitrary selection helped to diminish wide variability within any test group. For the most part, the variations in mean cough frequency response among the test groups were within the range of 10 coughs per minute.

To facilitate the statistical processing of the data, the changes in cough frequency responses between pretreatment and treatment measurements were converted to percentiles of pretreatment values. Generally, five animals per test level sufficed but in some instances, it was necessary to increase this number. Throughout the animal study a control nontreated group of animals was always evaluated simultaneously with treated animals. This monitoring was performed in order to eliminate any variable within the laboratory which might influence the assay and could not otherwise be detected.

Received September 25, 1963, from the Colgate-Palmolive Research Center, Research and Development, New Brunswick, N. J.
Accepted for publication November 7, 1963
[1] Marketed as Congestaid by the Colgate Pharmaceutical Laboratories.

TABLE I.—CONSTITUENTS OF AEROSOLIZED TERPENES (CONGESTAID)–FREON FORMULATION

Menthol	Dipropylene glycol
Thymol	Triethylene glycol
Camphor	Alcohol SDA-40
Eucalyptol	Propellant 11
Medicated odor	Propellant 12

Control animal data have therefore been accumulated for over 1000 animals. From this collection, it was established on the basis of a distribution pattern that the mean is skewed to the left and that there are two peaks for the frequency responses. In this study, the peak with the range of 10–40 coughs per minute was selected. The determination of statistical significance of the antitussive data is based upon the assumption that the cough frequency response of each group of animals may be treated as a binomial effect and as such analyzed by the method of Mainland and Murray (4).

Effects of Freon Propelled Formulation of Mixtures of Terpenes, Glycols, and Alcohol.—The antitussive effects of a freon-propelled formulation of terpenes, glycols, and alcohol, as shown in Table I, were evaluated in guinea pigs. The formulations were prepared to be delivered by a metered valve system. A 10-L. rectangular plastic exposure chamber was first sprayed either for 1 second or 10 seconds with the test formulation and then the animal was placed within for 3 minutes and then removed to be placed in the challenging chamber. The results are illustrated by the example in Fig. 1. The aerosolized terpenes formulation significantly reduced the induced ammonia coughs by 33 and 41% at the 60 to 120 minute intervals, respectively. Not only was there a reduction in cough paroxysmals but the general character of the respiratory pattern of the animals was improved (Fig. 1). Respiration was more even, deeper, and unrestrained.

Concentration in Exposure Chambers.—The effects of chamber concentration of aerosolized terpenes was determined by placing guinea pigs for 3 minutes in chambers of different volumes (see Table II) in which a 1-second spray (2 Gm. of total spray) was dispersed and evaluating the antitussive effects over a period of 4 hours. In the case of the maximum chamber volume, a 10-second spray (27 Gm. of total spray) was used.

The results as shown in Table II illustrate that the antitussive activity occurs immediately and generally persists for about 1 to 2 hours. The concentration effect of the different chamber volumes was noted in the duration of antitussive activity. In the chamber volume of 1 liter, the treated animals retained their protection for as much as 4 hours whereas those treated in the large volume chambers had a correspondingly shorter duration of protection.

Bronchospasms

Fifty guinea pigs were employed in this study and the bronchospasms were induced by either exposing the animals to aerosolized 0.5% histamine dihydrochloride or 1.0% acetylcholine hydrochloride in a 10-L. chamber. The respiratory responses of the animals were recorded as described for the antitussive study and in addition an observer with a stop watch also noted the onset time for bronchospasm to occur. The bronchospasm was witnessed by the observer as a spasm of the diaphragm which correlated with the oscillographic recording (Fig. 2).

In these studies it was technically impossible to use the same animal as its own control or for more than one test level evaluation. Therefore, separate groups of control nontreated animals were employed for each study. It was determined, that aerosolized histamine dihydrochloride produced bronchospasms in 45.2 seconds and 60% of the animals died and for the survivors their respirations were severely embarrassed. Animals exposed to a 10-second spray of aerosolized terpenes for 3 minutes in a 10-L. chamber were able to tolerate the histamine challenge; and the bronchospasm did not occur until 57 seconds of exposure (Fig. 2) and only 20% of the animals died with the survivors manifesting only mild to moderate respiratory distress.

Acetylcholine-induced bronchospasm occurred in control animals after 48 seconds and the animals were severely affected. Exposure to a 10-second spray of aerosolized terpenes did not alter or influence the acetylcholine-induced bronchospasm.

Antitussive Studies in Humans

Method.—The method used is an adaptation of that described by Bickerman (3). Essentially, it involves for each subject the determination of the supraminimal exposure time to citric acid aerosol

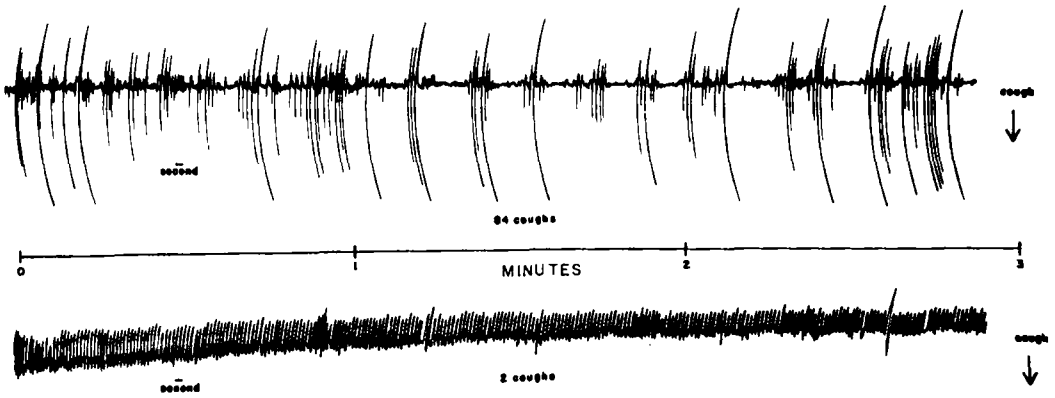

Fig. 1.—Oscillographic recording of respiratory rate and depth of guinea pigs exposed to ammonia vapors before treatment (top) and effects of aerosolized terpenes treatment on respiration of ammonia vapor-treated animals (bottom). The respiration is irregular and the depth shallow during ammonia vapor exposure and the animal exhibited cough paroxysmals. Following aerosolized terpenes treatment, this animal only exhibited one cough and the respiration was regular and deep as compared with 84 coughs prior to treatment.

TABLE II.—INFLUENCE OF EXPOSURE CHAMBER VOLUME ON THE ANTITUSSIVE EFFECTS OF AEROSOLIZED TERPENES (CONGESTAID) IN GUINEA PIGS TREATED WITH NEBULIZED AMMONIA VAPORS

Exposure Chamber Volume (liter)	N[a]	Per Cent Inhibition of Cough — Minutes after Treatment				
		0	30	60	120	240
1[b]	40	...	51	88	53	48
10[b]	50	47	76	73	22[d]	17[d]
235[b]	70	95	62	55	39	42
25,000[c]	20	44	28[d]	...	...	...

[a] N = Number of animals evaluated. [b] 1-second spray of aerosolized terpenes. [c] 10-second spray of aerosolized terpenes. [d] Nonsignificant effect; all other effects were significant at the 95% confidence level.

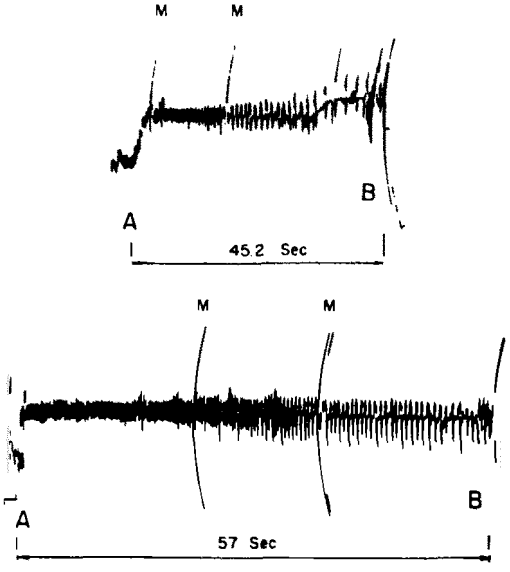

Fig. 2.—Oscillographic recording of histamine-induced bronchospasms in guinea pigs and the delaying effects of aerosolized terpenes toward the bronchospasms. The control record (top) shows at A the initiation of histamine exposure and at B the occurrence of bronchospasm after 45.2 seconds. Following aerosolized terpenes treatment (bottom), the respiration was more regular and the occurrence of bronchospasm after 57 seconds was delayed. Bronchospasm was always characterized by a reduction in the inspiratory volume followed by an explosive expiration. This feature contrasts bronchospasm activity (B) from gross body movements (M).

(25% aqueous solution) for the production of a cough response. The exposure time is regulated by a solenoid valve (Hoke valve) which can be manually activated through a timer (Eagle signal, 1–5 seconds) so that the volume of irritant delivered is fixed and reaches the subject at the onset of inspiration. The amount of aerosolized citric acid delivered for each exposure time employed has been determined and this relationship is curvilinear as illustrated in Fig. 3.

In this study, a panel of 12 male college students (21–25 years) was selected. They represented a group of trained subjects who had been used for at least 15 other determinations and were capable of reliably distinguishing between the antitussive effects of placebo and codeine phosphate (15 and 30 mg. *per os*). The subjects were seated in a comfortable position and a face mask (Willson mono mask) was securely fastened over the nose and mouth. During the pretreatment test periods, each subject was exposed to various amounts of citric acid until the supraminimal threshold amount was determined. Once the supraminimal threshold, as expressed as exposure time, was established, the subject was then given five challenges at intervals of 30 seconds per period of testing. The cough responses were recorded oscillographically by means of a pressure transducer (PT5) and a Grass model 5 recorder.

After each period of citric acid challenging, the subject was asked to rinse his mouth with the tap water. A 10-second spray of aerosolized terpenes was applied to a 4 × 4-in. gauze pad and the subject inhaled deeply the vapors for 5 minutes. The antitussive effects of the aerosolized terpenes were then determined 30 and 60 minutes afterwards. The assay was repeated twice within 24 hours and the results are shown in Tables IIIa and IIIb.

It was observed that the best effects occurred 60 minutes after treatment although at 30 minutes about half of the subjects exhibited antitussive benefits. The most obvious changes were in the cough frequency and cough numbers. Cough frequency refers to the subject's response to a challenge of citric acid. For example, each subject was given five exposures of aerosolized citric acid per test intervals. If, after treatment with aerosolized terpenes, the subject responded with a coughing episode to only three challenges, his cough frequency response was reduced by 40 per cent. Cough numbers refers to the total coughs recorded for all five citric acid challenges. Thus if a subject responded twice to each challenge of citric acid he would exhibit 10 coughs per test; if after aerosolized terpenes the

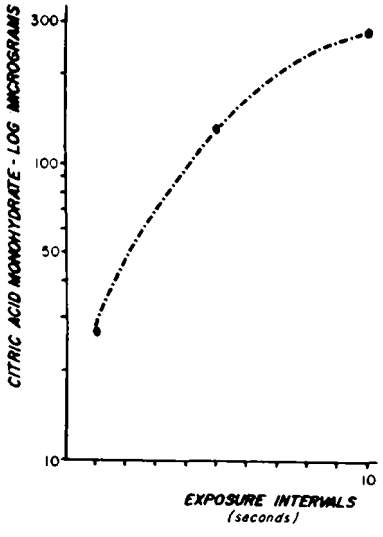

Fig. 3.—Relationship of delivered aerosolized citric acid and exposure intervals used in inducing coughs in humans.

TABLE IIIa.—FIRST TEST—MEAN PER CENT CHANGE FROM CONTROL[a]

Subject	No.	30 Minutes				60 Minutes			
		Frequency	Coughs	Velocity	Onset	Frequency	Coughs	Velocity	Onset
N. P.	1	0	10	0	0	0	−10	−8	8
J. H.	2	0	0	0	0	−20	0	0	0
W. A.	3	0	−30	−34	11	0	−20	−34	33
E. W.	4	−20	−58	0	+33	−20	−50	0	27
R. C.	5	0	17	0	−13	0	−17	0	−13
R. M.	6	0	0	0	0	0	0	0	0
S. S.	7	−20	−50	−23	−7	−40	−50	6	23
M. W.	8	−40	−75	13	23	−80	−90	13	62
J. K.	9	0	25	0	0	−20	−38	0	0
A. H.	10	−80	−33	−33	−27	−60	−67	−36	−13
B. K.	11	−20	−6	−6	9	−40	−13	5	14
P. E.	12	−80	−29	−29	−18	−80	−82	−29	−18

[a] A positive result indicates per cent increase over the control reading; a negative result indicates per cent decrease from the control reading. The above data were determined by taking the average of five challenges, one every 30 seconds, at each time interval.

TABLE IIIb.—SECOND TEST—PER CENT CHANGE FROM THE CONTROL

Subject	No.	30 Minutes				60 Minutes			
		Frequency	Coughs	Velocity	Onset	Frequency	Coughs	Velocity	Onset
N. P.	1	0	0	0	11	0	9	0	0
J. H.	2	0	17	0	0	20	−17	80	7
W. A.	3	0	−33	−20	0	0	−33	−20	0
E. W.	4	−20	−56	9	−25	−60	−67	0	−25
R. C.	5	20	0	67	0	0	−13	67	0
R. M.	6	0	0	0	0	0	25	0	7
S. S.	7	20	−34	−39	0	−40	−70	−22	0
M. W.	8	0	−39	0	0	0	−28	0	0
J. K.	9	0	25	0	0	−20	−37	0	0
A. H.	10	−20	−25	24	−13	−50	−50	−16	−6
B. K.	11	−20	−11	56	46	0	0	82	15
P. E.	12	−47	−56	−22	−41	−60	−63	−22	−41

total cough number was only five his cough was reduced by 50%. As shown in the Tables IIIa and IIIb, at the 60-minute period, the cough frequency was reduced in 8 out of 12 and 5 out of 12 subjects, not affected in 4 out of 12 and 6 out of 12 subjects, and in one instance increased. Similarly, it was found that the cough number was reduced in 10 out of 12 and in 9 out of 12 subjects. Therefore, aerosolized terpenes were capable of suppressing both the subject's cough response in terms of cough frequency and cough number.

The effects of aerosolized terpenes on cough velocity and onset time were not clearly established. In almost 50% of the subjects, the cough velocity was decreased whereas in several instances it was appreciably elevated in spite of obvious reductions in cough frequency and in cough numbers. The onset time to cough was not appreciably altered by aerosolized terpenes, although there was some delay.

DISCUSSION

The results of this investigation have demonstrated that the introduction of aerosolized terpenes, alcohol, and glycols in an atmosphere into which animals are exposed will afford a significant antitussive action capable of suppressing ammonia-induced coughs. Moreover, the dispersement of a combination of these materials in a freon-propelled system is equally antitussive and antihistaminic in animals exhibiting bronchospasm following exposure to histamine dihydrochloride aerosols. Similarly, it was found that inhalation of aerosolized terpenes vapors by human volunteers also reduced the frequency and severity of citric acid-induced coughs. These findings agree with those observed by Sadove (5) and Calesnick (6). By way of comparison, we have observed that the effects of aerosolized terpenes were greater than those obtained with 15 and 30 mg. *per os* of codeine phosphate.

It has been taught that the volatile oils were capable of stimulating the production of mucin secretion by the goblet cells in the epithelium of the bronchi and that this action produced a protective layer over the sensitive bronchial surfaces. However, in addition to this it may be deduced from our animal data that this is not the only action of the volatile oils. Since it was demonstrated (Fig. 1), that following an exposure to aerosolized terpenes, the respiratory rate and depth and ease of breathing were facilitated, it must be concluded that some other pharmacological effects are also elicited. Among the possibilities is that of producing a relaxation of the smooth muscle of the bronchi and bronchioles. Justification for this may be inferred by the demonstration of an antihistaminic action following the use of aerosolized terpenes. The data do not permit us to make any strong claim for assuming that there is generalized smooth muscle relaxation since aerosolized terpenes did not antagonize the effects of aerosolized acetylcholine hydrochloride. Nonetheless, it can be stated that following the aerosolized terpenes treatment, the flow of air in the respiratory passages is enhanced as is evident in the animal data and in the human data obtained by us and Sadove (5).

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