

Inhibition of histamine-induced airway constriction by ascorbic acid

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We studied the effect of ascorbic acid on histamine-induced airway constriction in 17 healthy subjects; we also investigated its effect on guinea pig tracheal strips in vitro. Ventilatory function was measured by recording partial expiratory flow-volume (PEFV) curves on which maximum flow rates at 50 per cent VC and at 25 per cent VC were calculated. Following oral administration of 500 mg. ascorbic acid, the mean reductions of \dot{V}_{max} at 50 per cent VC and \dot{V}_{max} at 25 per cent VC after histamine inhalation were significantly smaller in comparison with placebo administration ($P < 0.01$). In the guinea pig trachea preparation, ascorbic acid reduced contractions induced by histamine and relaxed this tissue in the absence of other agents. Propranolol did not block the effect of ascorbic acid in man (80 mg. orally), but in vitro relaxations of tracheal strips by ascorbic acid were reduced by 2.5 μ g propranolol. Ascorbic acid probably has a direct effect on airway smooth muscle; in the guinea pig trachea its effect may be mediated by β -adrenergic receptors.

In 1804, Reisseissen¹ described airway smooth muscle in the human lung and attributed "convulsive asthma" to its contraction. The same author² later observed symptoms of "convulsive asthma" in patients with severe scurvy. More recently, Dawson and West³ reported that ascorbic acid protects guinea pigs against anaphylactic shock. These observations suggest that ascorbic acid might have a therapeutic effect in asthma, but clinical reports on this subject are contradictory.^{4, 5}

We have found that ascorbic acid can inhibit the airway constrictor effect of histamine in healthy human subjects and also in the isolated trachea of the guinea pig. Thus, the therapeutic use of ascorbic acid in asthma may warrant further investigation.

SUBJECTS AND METHODS

Experiments in man

Seventeen healthy subjects (14 men, 3 women, 21 to 38 years of age; 12 nonsmokers and 5 regular smokers) participated in the study. Each subject inhaled identical histamine aerosols on 2 days, a "placebo day" and an "ascorbic acid day." The aerosols were produced by a Dautrebande⁶ D-30 nebulizer with an air pressure of 15 p.s.i. Histamine concentrations in

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the aerosolized liquid varied from 15 to 40 mg. per milliliter for different subjects (histamine dihydrochloride dissolved in 0.9 per cent saline; pH = 7.4; doses expressed as mg. base per milliliter). These were chosen on the basis of symptoms and reduction of ventilatory function during a preceding series of experiments with different histamine concentrations. Identical conditions of inhalation were used in all experiments. The duration of inhalation was 30 seconds, and on each day inhalations were performed at 9 A.M., 12 noon, and 3 P.M. (hereafter referred to as 0, 3, and 6 hours, respectively). Ventilatory function was measured immediately after inhalation (0'), and again after 2', 4', and 6', by recording PEFV curves.⁸ The subject inspired to about 70 per cent vital capacity and then performed a forced expiratory maneuver to residual volume (RV), immediately followed by a maximal inspiration to total lung capacity (TLC). Expiratory flow-volume curves were recorded on a Brush 500 High Performance XY Recorder (Gould, Inc.), with lung volume changes on the abscissa and expiratory flow rate on the ordinate. Maximum flow rates at 50 per cent and 25 per cent of the control vital capacity (i.e., VC at 0 hour) were measured from the PEFV curves. On each curve a constant volume (50 per cent and 75 per cent of the control VC) was subtracted from total lung capacity (TLC) and expiratory flow was read at these volumes (Fig. 1). Three control blows were made before histamine inhalation, and the mean of the 2 curves with the highest flows was used.

The comparison of maximum flow assumes that TLC is not changed by the inhalation of histamine. We confirmed that this is so by determining TLC separately in an air-conditioned volume-displacement body plethysmograph before and after histamine inhalation (30 mg. and 20 mg. per milliliter). No changes were recorded in TLC, while residual volume (RV) was increased (2 subjects).

After the first histamine inhalation (0 hour) on the ascorbic acid day, 500 mg. of ascorbic acid dissolved in water was administered orally. Histamine inhalation and ventilatory function measurements were repeated 3 and 6 hours later. After 4 to 5 days, the same procedure was repeated with a placebo identical in taste to ascorbic acid solution (water with acetic acid added). At the time of the ventilatory function measurements, heart rate was also measured.

The *t* test for paired samples was used for statistical analysis.

Experiments in guinea pigs

Sixteen female guinea pigs (Hartley strain; 200 to 250 grams) were used for the spirally cut trachea preparations.⁸ The strips of tissue were suspended in 10 ml. Tyrode's solution at 37° C. and constantly aerated with 95 per cent O₂ and 5 per cent CO₂. Contractions and relaxations were recorded isometrically on a pen recorder. Drugs were dissolved in Tyrode's solution, and ascorbic acid was neutralized to pH 6.5 to 7.5 with sodium bicarbonate. The buffering capacity of Tyrode's solution is sufficient to prevent pH changes on addition of the ascorbic acid solution. Final bath pH was 7.4 in the experiments reported here. In separate experiments, similar results were obtained with pH values ranging from 7.2 to 7.6, kept constant throughout each experiment. Doses of drugs are expressed in μ g base per milliliter final bath volume.

RESULTS

Experiments in man

Histamine aerosol inhalation causes a decrease of expiratory flow rates on the PEFV curve (Fig. 1, *A*). After pretreatment with ascorbic acid, flow rates decreased less after inhalation of the same histamine aerosol dose (Fig. 1, *B*).

The measurement of maximum expiratory flow rates at constant lung volumes ($\dot{V}_{\max 50}$ and $\dot{V}_{\max 25}$) allows a quantitative assessment of these drug-induced changes (Table 1). Under control conditions (0 hour) the reduction of $\dot{V}_{\max 50}$ and $\dot{V}_{\max 25}$, after histamine inhalation, was similar on both days (with placebo or ascorbic acid). Following the administration of ascorbic acid, histamine

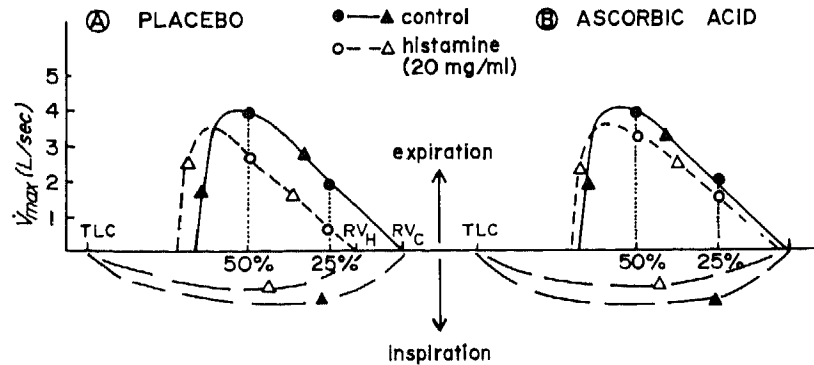


FIG. 1. PEFV curves from healthy subject on placebo day (A) and on ascorbic acid day (B). Lung volume (abscissa) in percentage of vital capacity (TLC, 100 per cent; RV, 0 per cent). RV_c = residual volume on control curves; RV_H = residual volume after histamine. Ordinate: Expiratory flow rate (L./sec). Measurement of \dot{V}_{max} at 50 per cent VC and \dot{V}_{max} at 25 per cent VC indicated by ● for control and ○ after histamine inhalation.

TABLE I. Maximum expiratory flow rates on PEFV curves

	Time (hr.)	\dot{V}_{max} at 50% VC (L./sec.)					\dot{V}_{max} at 25% VC (L./sec.)				
		Control ± S.E.*	After histamine (min.)				Control ± S.E.*	After histamine (min.)			
			0'	2'	4'	6'		0'	2'	4'	6'
Placebo day	0	4.20 ± 0.34	2.61	2.64	2.86	3.06	1.78 ± 0.23	1.05	0.97	1.15	1.26
	3	3.94 ± 0.29	2.56	2.84	2.71	2.97	1.67 ± 0.20	1.01	1.07	1.12	1.04
	6	3.85 ± 0.27	2.54	2.55	2.73	3.02	1.57 ± 0.18	0.88	0.93	1.11	1.08
Ascorbic acid day	0	4.49† ± 0.28	2.71†	2.96†	3.19†	3.19†	1.87† ± 0.18	0.99†	1.12†	1.22†	1.12†
	3	4.06† ± 0.20	2.99†	3.49†	3.58†	3.77†	1.61† ± 0.15	1.13†	1.36†	1.51†	1.58†
	6	3.77† ± 0.19	3.12†	3.28†	3.47†	3.53†	1.51† ± 0.13	1.19†	1.26†	1.32†	1.45†

*Mean ± standard error.

†Not significantly different from the corresponding value on the placebo day ($P > 0.05$).

‡Significantly different ($P < 0.01$) from corresponding value on placebo day.

reduced flow rates less than after administration of placebo, and the differences were statistically significant in all but one instance (the 0' value at 3 hours).

Figs. 2 and 3 represent the data in all subjects, expressed as a percentage of the control values at 0, 3, and 6 hours, on both days. After placebo, the effect of histamine on \dot{V}_{max} 50 and \dot{V}_{max} 25 remains unchanged at 3 and 6 hours. After ascorbic acid the effect of histamine is reduced, and the reduction is maintained 6 hours after ascorbic acid ingestion. Although ascorbic acid reduced the effect of histamine, the decreases of flow rates were in most instances still significant compared to the control value before histamine inhalation.

On the placebo day, as well as on the ascorbic acid day, control values for \dot{V}_{max} 50 and \dot{V}_{max} 25 were lower at 3 and 6 hours than at 0 hours (Table I).

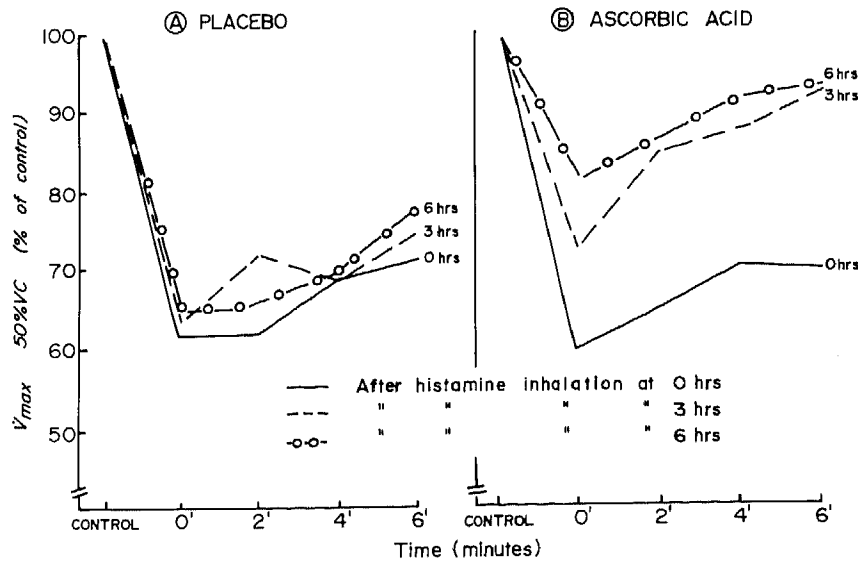


FIG. 2. Mean changes (all subjects) in \dot{V}_{max} at 50 per cent VC on PEFV curves after histamine inhalation as a percentage of control values, on placebo day (A) and on ascorbic acid day (B).

TABLE II. Control flow rates in 10 subjects

Time	\dot{V}_{max} at 50% VC (L./sec.)			\dot{V}_{max} at 25% VC (L./sec.)		
	Placebo day ($\bar{X} \pm S.E.$)	Ascorbic acid day ($\bar{X} \pm S.E.$)	Day without drugs ($\bar{X} \pm S.E.$)	Placebo day ($\bar{X} \pm S.E.$)	Ascorbic acid day ($\bar{X} \pm S.E.$)	Day without drugs ($\bar{X} \pm S.E.$)
9 A.M.	3.64 ± 0.37	3.98 ± 0.35	3.39 ± 0.23	1.44 ± 0.20	1.54 ± 0.19	1.21 ± 0.21
12 noon	3.64 ± 0.42	3.74 ± 0.21	3.23 ± 0.26	1.45 ± 0.23	1.40 ± 0.17	1.10 ± 0.21
3 P.M.	3.48 ± 0.35	3.56 ± 0.23	3.49 ± 0.26	1.34 ± 0.27	1.43 ± 0.14	1.26 ± 0.22

$\bar{X} \pm S.E.$ = Mean \pm standard error.

Since this might be a result of a circadian rhythm in ventilatory function, we restudied 10 of our subjects, without administration of drugs, at the same 3 hour intervals. Table II compares the average control flow rates on the 3 days of study in these 10 subjects. Only minor and insignificant changes of flows occurred on the day without any drugs.

Heart rate increased consistently after histamine inhalation, and this was not changed by administration of ascorbic acid. The increase was most marked (117 to 140 per cent of control value) immediately after histamine inhalation. Six minutes after inhalation the heart rate had returned to control values.

We tested the effect of propranolol on the protective action of ascorbic acid in 4 subjects. After 3 control blows, histamine aerosol was inhaled (15 to 20 mg. per milliliter), and PEFV curves were recorded immediately after inhalation and 2, 4, and 6 minutes later. Propranolol was then given, 80 mg. orally.

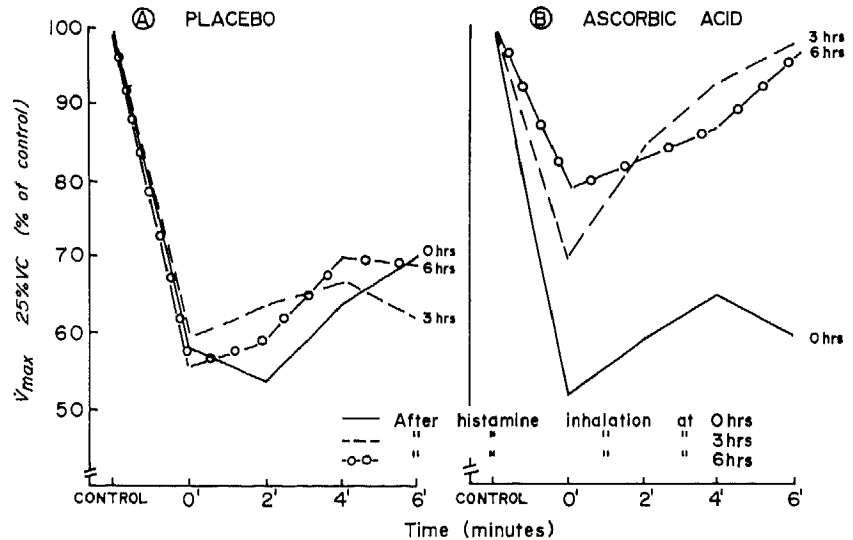


FIG. 3. Mean changes (all subjects) in \dot{V}_{max} at 25 per cent VC on PEFV curves after histamine inhalation as a percentage of control values, on placebo day (A) and on ascorbic acid day (B).

After 75 minutes, the histamine inhalation was repeated and flow-volume curves recorded according to the same schedule. Ascorbic acid was then administered in a dose of 500 mg., and histamine inhalation was repeated 2 and 3 hours after ascorbic acid.

After propranolol, the effect of histamine was enhanced (Table III). This potentiation of the histamine effect was abolished by administration of ascorbic acid, which resulted in a histamine response similar to the one before administration of propranolol. Since oral propranolol in this dose acts during several hours,^{9, 10} and the heart rate remained below control values throughout the experiments, we conclude that ascorbic acid can exert its protective action in the presence of β -receptor blockade by propranolol.

Guinea pig experiments

Ascorbic acid produced dose-related relaxations of the uncontracted preparation (Fig. 4, A), with a threshold concentration of 35 to 55 μg per milliliter. Tachyphylaxis was observed when concentrations higher than 100 μg per milliliter were used. With 1 to 25 μg per milliliter doses of ascorbic acid, no effect on the baseline of the uncontracted trachea was observed. These low doses also did not affect histamine contractions produced immediately after treatment. Ascorbic acid did produce dose-related relaxations in preparations previously contracted by submaximal concentrations of histamine (1 μg per milliliter, Fig. 4, A). The threshold concentration for this effect varied from 20 to 30 μg per milliliter.

When ascorbic acid was added to the organ bath, the muscle first relaxed. After the ascorbic acid was washed out, the muscle returned to the original

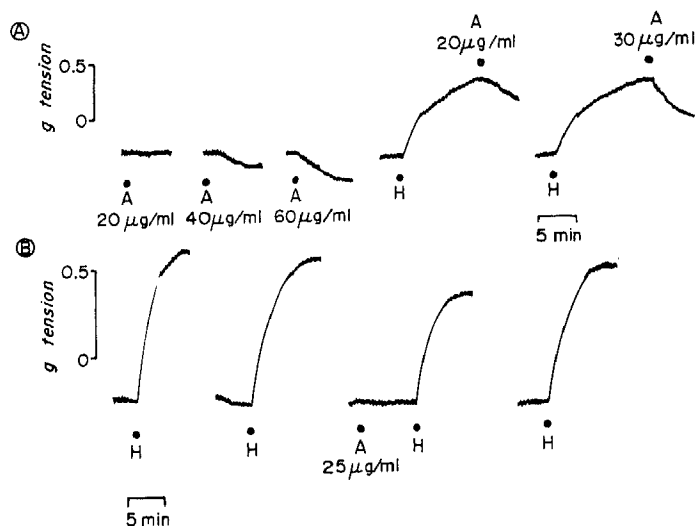


FIG. 4. Top, Effect of ascorbic acid (A) on uncontracted and contracted trachea of the guinea-pig, in vitro. Contractions are produced by 1 μg per milliliter histamine (H). Bottom, Effect of subthreshold ascorbic acid (A) on histamine (1 μg per milliliter, H) contractions.

TABLE III. Effect of propranolol and ascorbic acid on airway smooth muscle contraction after histamine inhalation

	\dot{V}_{max} at 50% VC (L./sec.)					\dot{V}_{max} at 25% VC (L./sec.)				
	Control ± S.E.*	After histamine (min.)				Control ± S.E.*	After histamine (min.)			
		0'	2'	4'	6'		0'	2'	4'	6'
Histamine	4.21 ± 0.60	3.45 ± 0.62	3.53 ± 0.50	3.62 ± 0.46	3.86 ± 0.48	1.85 ± 0.47	1.48 ± 0.41	1.61 ± 0.47	1.35 ± 0.41	1.68 ± 0.46
% of control		81.9	83.8	86.0	91.7	80.0	87.0	73.0	90.8	
Heart rate	82	92	88	86	83					
Propranolol	4.16 ± 0.68	2.73 ± 0.86	3.30 ± 0.75	3.05 ± 0.53	3.58 ± 0.67	1.78 ± 0.39	1.15 ± 0.39	1.68 ± 0.52	1.33 ± 0.52	1.63 ± 0.51
% of control		65.6	79.3	73.3	86.0	64.6	94.4	74.7	91.6	
Heart rate	70	73	71	66	67					
Ascorbic acid†	4.15 ± 0.68	3.63 ± 0.53	3.77 ± 0.53	3.72 ± 0.69	3.93 ± 0.69	1.85 ± 0.46	1.50 ± 0.35	1.68 ± 0.54	1.75 ± 0.36	1.65 ± 0.52
% of control		87.5	90.8	89.6	94.7	81.1	90.8	94.6	89.2	
Heart rate	68	74	69	70	68					
Ascorbic acid‡	4.08 ± 0.62	3.82 ± 0.60	4.12 ± 0.74	3.85 ± 0.54	4.08 ± 0.65	1.77 ± 0.50	1.70 ± 0.45	1.90 ± 0.48	1.86 ± 0.50	1.92 ± 0.37
% of control		93.6	101.0	94.4	100.0	96.0	107.3	105.1	108.5	
Heart rate	78	75	71	70	72					

*Mean ± standard error.

†Mean changes 2 hours after ascorbic acid.

‡Mean changes 3 hours after ascorbic acid.

baseline in 20 to 25 minutes. When ascorbic acid was added to the previously contracted preparation, the muscle returned to the original baseline somewhat more quickly (5 to 10 minutes).

Prior administration of ascorbic acid in concentrations of 30 to 50 μg per

milliliter reduced contractions mediated by histamine by 20 to 45 per cent (Fig. 4, B).

Propranolol (2.5 μ g per milliliter incubated for 10 minutes) completely prevented relaxation mediated by isoproterenol on strips previously contracted by histamine. The same dose of propranolol reduced the relaxation produced by ascorbic acid both in the histamine-contracted and in the uncontracted trachea (6 preparations). Propranolol was effective for at least 4 hours.

DISCUSSION

The measurement of maximum expiratory flow rates on PEFV curves allows a sensitive assessment of relatively slight degrees of airway constriction induced by pharmacologic agents.⁷ Changes of these flow rates reflect caliber changes in small airways, for instance, in bronchial asthma and in byssinosis. In general, decreased flow rates reflect airway constriction, while increased flows indicate dilatation of small airways.¹¹ The method lends itself to controlled trials with drugs in which each subject serves as his own control, as in the present study.

With this technique to assess airway caliber changes after histamine inhalation, we have shown that a single oral dose of 500 mg. ascorbic acid inhibits the constrictor effect of histamine on airways of human subjects *in vivo*. This effect lasts at least 6 hours.

Reductions of expiratory flow rates accompany the acute symptoms of byssinosis in textile workers. Recently, Valic and Zuskin (unpublished material) demonstrated that ascorbic acid (500 mg. orally) prevented this effect of exposure to textile dusts to a large extent. The acute constrictor effect of textile dust in man is caused, at least in part, by the presence of a water-soluble histamine-releasing agent in these dusts.^{12, 13} The action of ascorbic acid in preventing the acute effect of dust exposure in textile workers is thus consistent with the results of the present study. Both actions can be explained by an antagonistic action of ascorbic acid against the effect of histamine on human airway smooth muscle.

Experiments with anesthetized guinea pigs³ suggested that ascorbic acid has a direct relaxant effect on airway smooth muscle, and our results confirm this (Fig. 4, A). In the guinea pig, ascorbic acid antagonized not only histamine, but also 5-hydroxytryptamine and bradykinin.³ Thus, ascorbic acid does not appear to have a specific antihistamine action on airway smooth muscle.

The evidence on a possible role of β -adrenoreceptors in the action of ascorbic acid is to some extent contradictory. Dawson and West³ found that pronethalol did not alter the inhibitory effect of ascorbic acid on airway constrictor responses *in vivo*. In the present study, propranolol reduced the relaxant effect of ascorbic acid on the guinea pig trachea *in vitro*. On the other hand, the experiments with human subjects (Table III) suggest that ascorbic acid can protect against histamine-induced airway constriction even in the presence of β -receptor blockade induced by propranolol. There may be species differences in the mechanism of action, or it may be that the dose of ascorbic acid used in man (500 mg.) is sufficient to overcome the competitive block of β -receptors by propranolol.

A large dose of ascorbic acid is excreted for the most part in about 4 to 5 hours.^{14, 15} To maintain the desired effect on airway smooth muscle, it may be useful to administer smaller doses (i.e., 250 mg.) at 3 hour intervals. Such a dosage schedule might be of use in preventing the acute symptoms of byssinosis in textile workers, and may also be of interest for the treatment of bronchial asthma. Since ascorbic acid in these doses has no important side effects,¹⁶ controlled clinical trials to test the efficacy of this drug in bronchial asthma and in byssinosis may be in order.

The decrease of control flow rates at 3 and 6 hours (Table I) is intriguing and cannot be explained adequately by circadian variation (Table II). It seems more likely that the decrease is caused by a residual effect of the previous histamine inhalation experiments. Histamine is a short-acting drug, and its direct action on airways lasts at most 30 to 45 minutes,¹⁷ but restitution of airways to control conditions may take longer.

It should be emphasized that the present experiments concern doses of histamine aerosols that can safely be given to healthy subjects since they elicit at most slight dyspnea and wheezing. The use of a sensitive method of assessing the functional effects of histamine enabled us to obtain significant results with the doses employed. We have not investigated the action of ascorbic acid against more severe degrees of histamine-induced airway constriction. Thus, at present the possible therapeutic implications of our study are limited to mild degrees of bronchial asthma.

Recently, Pauling¹⁸ suggested that man requires 2.0 to 2.6 Gm. ascorbic acid daily for optimal health. It is conceivable that minimal airway smooth muscle tone is one aspect of optimal health, but our study was not designed to test this hypothesis. Pauling has also summarized controlled trials that suggest a protective action of ascorbic acid against clinical effects of common cold viruses.¹⁹ The inhibition of airway constriction by ascorbic acid, which we demonstrate in the present paper, might have a beneficial effect in the common cold, but unfortunately little is known about the functional aspects of acute airway responses in these viral diseases. The study of their therapy might be facilitated by inclusion of objective lung function tests, such as flow-volume curves, in the protocol of clinical trials.

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