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Rapid method for measurement of bronchial responsiveness

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ABSTRACT A rapid, simple method for measuring bronchial responsiveness to inhaled histamine is described. The method was used to obtain dose response curves in 50 atopic subjects with varying respiratory and nasal symptoms. The cumulative dose of histamine which caused a 20% fall in the one second forced expiratory volume ($PD_{20\text{-}FEV_1}$) varied between 0·046 and greater than 3·9 μmol and correlated with the severity of symptoms. The reproducibility of the $PD_{20\text{-}FEV_1}$, determined from duplicate measurements in 15 subjects with varying degrees of bronchial responsiveness was found to be satisfactory. When the $PD_{20\text{-}FEV_1}$ from this rapid method was compared with that obtained from the dosimeter method no significant difference was found. The dose delivered by this method was shown to be cumulative.

Increased bronchial responsiveness to histamine and methacholine is one of the hallmarks of asthma. Measurement of the degree of hyperresponsiveness is an important tool in the clinical management of patients with asthma, in epidemiological studies, and in research relating to the causes of asthma. The degree of hyperresponsiveness has been shown to correlate well with the severity of asthma.[1 2]

Although several techniques for the measurement of bronchial hyperresponsiveness have been described, two are used widely. The first, described by Cockcroft *et al*,[1] uses the Wright nebuliser with tidal breathing of different concentrations of the agonist. The second method, described by Chai *et al*,[3] uses a De Vilbiss No 42 nebuliser attached to a dose metering device to deliver discrete amounts of agonist. Both methods measure bronchial responsiveness by administering increasing doses of the provoking agent, recording the change in forced expiratory volume in one second (FEV_1), and plotting a dose response curve. From this curve the dose or concentration of provoking agent which causes a 20% fall in FEV_1—that is, $PD_{20\text{-}FEV_1}$ or $PC_{20\text{-}FEV_1}$—is obtained. Both methods are relatively time consuming (about 30 minutes being needed for a dose response study) and require an external air source to drive the nebuliser, so the equipment is cumbersome.

The importance of measurement of bronchial responsiveness in epidemiological studies and diagnostic clinics is now generally recognised, and there is a need for a simple, rapid, and portable method for evaluating airway reactivity. This paper outlines such a method and describes the reproducibility of results and also their comparability with the response obtained by the dosimeter method. We describe response to histamine using the method in five groups of subjects with various symptoms.

Methods

SUBJECTS

Subjects were patients from the allergy clinic of the Royal Prince Alfred Hospital and volunteers from the staff of that hospital or the University of Sydney. They all gave informed consent and the study was approved by the ethics committee of Royal Prince Alfred Hospital. All had two or more positive responses to skinprick tests with a battery of 13 commonly inhaled allergens. Subjects were divided into five groups according to their symptoms and clinical history (table 3). Group I subjects had asthma which required daily medication; group II subjects had less severe asthma requiring intermittent medication; group III subjects had allergic rhinitis and a history of wheezing but had no current symptoms and were taking no medication; group IV

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subjects had allergic rhinitis but had never had wheezing, episodes of dyspnoea, or chest tightness; group V subjects had no history of respiratory or nasal symptoms. All subjects refrained from taking bronchodilator treatment for at least six hours and antihistamines for 48 hours before any challenge.

STUDY DESIGN

All subjects underwent a histamine inhalation test by the hand operated technique. On a second occasion, 15 subjects had this test repeated, 11 subjects had a histamine inhalation test using the dosimeter, and seven subjects were given the total cumulative dose of histamine administered in the first study as a single dose. The single dose study was to determine whether the doses administered by the rapid technique were cumulative. The single dose was administered via the hand operated nebuliser and the FEV_1 was measured initially after inhalation of normal saline and then at 30 second intervals for three minutes after inhalation of histamine. The second study was carried out within 10 days of the first study and at about the same time of day.

LUNG FUNCTION MEASUREMENT

FEV_1 was measured with a Vitalograph dry spirometer, each measurement being repeated until two values were reproducible to within 100 ml. The higher of these was recorded.

PREPARATION OF HISTAMINE SOLUTIONS

Five grams of histamine diphosphate salt were weighed and dissolved in 100 ml normal saline to make a 50 mg/ml solution. Solutions of 25, 6·25, and 3·13 mg/ml concentration were then produced by serial dilution.

HAND OPERATED TECHNIQUE

Five De Vilbiss No 40 glass nebulisers (De Vilbiss Co, Pennsylvania) were used to administer saline or histamine by hand. To determine the output of each nebuliser, 1 ml saline was placed in the nebuliser, the stoppers were placed in the holes, and the unit was weighed. The stoppers were removed and the bulb of the nebuliser was squeezed firmly 10 times. With the stopper replaced to reduce evaporation the unit was reweighed. This procedure was repeated 10 times for each nebuliser to determine the mean volume delivered per squeeze.

After baseline FEV_1 had been established the subject inhaled three breaths of normal saline from the first nebuliser. The mouthpiece of the nebuliser was placed between the teeth of the subject, who exhaled to slightly below functional residual capacity (FRC) and then inhaled slowly over one to two seconds towards total lung capacity (TLC), where the breath was held for three seconds. At the beginning of inspiration the operator gave the bulb of the nebuliser one firm squeeze. The FEV_1 was measured after 60 seconds and the higher of two values that were reproducible to within 100 ml was recorded.

The subject then took one inhalation of 3·1 mg/ml histamine, as listed in the dose schedule in table 2. This was considered to be equivalent to 0·03 μmol histamine since the mean output of the nebuliser was 0·003 ml per squeeze. The FEV_1 was recorded 60 seconds after each dose and followed immediately by the next dose. When a dose required more than one inhalation, these were given in consecutive breaths. The challenge was stopped when the FEV_1 fell by more than 20% from the postsaline value or when the maximum dose of 3·9 μmol was reached (7·8 μmol in one subject showing a 19% fall in FEV_1 with 3·9 μmol). For subjects with no history of increased airway responsiveness who had shown no response to the previous dose the test was sometimes shortened by combining dose 3 with dose 4 and dose 5 with dose 6. The test usually took seven to eight minutes. Bronchodilator aerosol was given to aid recovery.

DOSIMETER TECHNIQUE

This procedure was carried out with a De Vilbiss No 646 nebuliser attached to a dosimeter using compressed air. With the dosimeter set to give a nebulisation time of 0·6 seconds, the nebuliser was weighed before and after five nebulisations. The procedure was repeated 10 times. After baseline FEV_1 had been measured the subject inhaled five breaths of normal saline from the nebuliser attached via a nebulisation dosimeter (Rosenthal-French, USA) to compressed air at 20 lb/in^2 (138 kPa). Inhalations were taken slowly from slightly below FRC towards TLC with a nebulisation time of 0·6 seconds. As with the hand operated technique the inhalation time was one to two seconds followed by a breath hold of three seconds. FEV_1 was measured 60 seconds later. The challenge began with five breaths of 0·3 mg/ml histamine (0·006 μmol histamine) and continued with doubling concentrations to 10 mg/ml. The challenge was stopped when the FEV_1 had fallen by more than 20% or when the maximum dose had been reached. The study took 25–30 minutes to complete. Bronchodilator aerosol was used to aid recovery.

EXPRESSION AND ANALYSIS OF DATA

On the basis of the estimated volume delivered by the nebuliser and the known concentration of the solution, the dose of histamine delivered to the mouth of the subject was calculated in terms of μmoles of the salt. Response was measured as per-

Table 1 *Output of nebulisers (values (ml) represent one discharge)*

	De Vilbiss No 40					*De Vilbiss No 646*
	1	*2*	*3*	*4*	*5*	*1*
Mean	0·0026	0·0038	0·0033	0·0022	0·0029	0·0101
SD	0·0002	0·0003	0·0001	0·00012	0·0003	0·0010

centage change in FEV_1 from the postsaline value. This was plotted on a linear scale against log dose histamine to enable the dose of histamine causing a 20% fall in FEV_1 PD_{20-FEV_1} to be determined.

Variability in the output of the nebulisers was determined by analysis of variance. The PD_{20-FEV_1} values were logarithmically transformed and all subsequent analysis was performed on the log values. The significance of differences between groups was determined by Student's *t* on geometric means, and differences between the results obtained by the two methods by paired *t* tests. The line of regression relating the results obtained by the hand operated method on two separate days was determined by the method of least squares and compared to the line of identity using analysis of covariance. The 5% probability level was taken to indicate a significant difference.

Results

Table 1 shows the mean output from each of the five No 40 nebulisers and the 646 nebuliser. The mean output for the No 40 nebulisers was 0·003 (SD 0·0007) ml. The outputs of two nebulisers (Nos 2 and 4) were significantly different from the mean. The No 646 nebuliser had a larger output (0·01 ml). Table 2 shows the doses of histamine administered by the No 40 hand operated nebuliser for each concentration of histamine.

Baseline data for the five groups of subjects are shown in table 3; dose response curves for each subject are shown in figure 1. All those with current asthma and subjects with rhinitis who had wheezed in the past showed a 20% or greater fall in FEV_1 and their PD_{20} values were less than 3·9 μmol, except for the one subject in group III given 7·8 μmol histamine, whose PD_{20-FEV_1} was 4·9 μmol. The geometric mean values for PD_{20-FEV_1} for groups I, II, and III (table 4) are significantly different from each other, although considerable overlap exists between subjects in group II (mild asthma) and group III (past wheezing). Atopic normal subjects and subjects with rhinitis who had never wheezed had less than a 20% change in FEV_1 after the highest dose (3·9 μmol) of inhaled histamine.

Figure 2 shows the PD_{20-FEV_1} values for the two study days for 15 subjects with varying degrees of bronchial responsiveness. Good agreement between the values on the two days is shown. The line of regression was not significantly different from the line of identity.

In seven subjects the change in FEV_1 after the final dose of histamine in the first study was compared with the change in FEV_1 after the total cumulative dose of histamine given as a single dose (fig 3). There was no significant difference between

Table 2 *Dose schedule for the rapid histamine inhalation test*

Dose No		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Histamine concentration (mg/ml)		3·13	3·13	6·25	6·25	25	25	25	50
No of inhalations		1	1	1	2	1	2	4	4
Cumulative dose delivered:	mg	0·009	0·019	0·037	0·075	0·15	0·30	0·6	1·2
	μmol	0·029	0·061	0·122	0·244	0·488	0·977	1·954	3·91

Table 3 *Details of subjects studied*

Group		*No*	*M:F*	*Age range (y)*	*Mean (SD) baseline FEV_1 (% pred)*
I	Asthmatic subjects requiring daily bronchodilator treatment	10	5:5	25 (13–34)	69 (15)
II	Asthmatic subjects requiring bronchodilator treatment not more than once a week	10	3:7	25 (18–35)	97 (17)
III	Subjects with allergic rhinitis who have wheezed in the last 10 years but have never required bronchodilator treatment	10	3:7	27 (20–36)	106 (11)
IV	Subjects with allergic rhinitis who have never wheezed	10	8:2	36 (21–55)	108 (7)
V	Atopic normal subjects with no history of rhinitis, wheeze, or airways disease	10	3:7	25 (18–37)	102 (13)
	All subjects atopic (two positive skinprick test responses)				

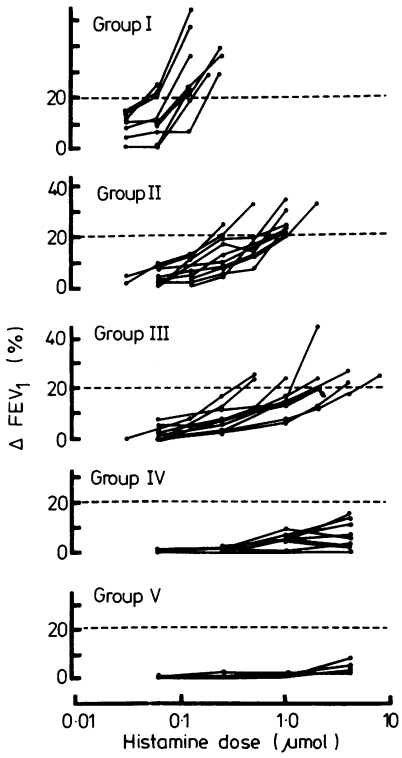

Fig 1 *Dose response curves for histamine in 50 subjects divided into groups according to symptoms (see table 3).*

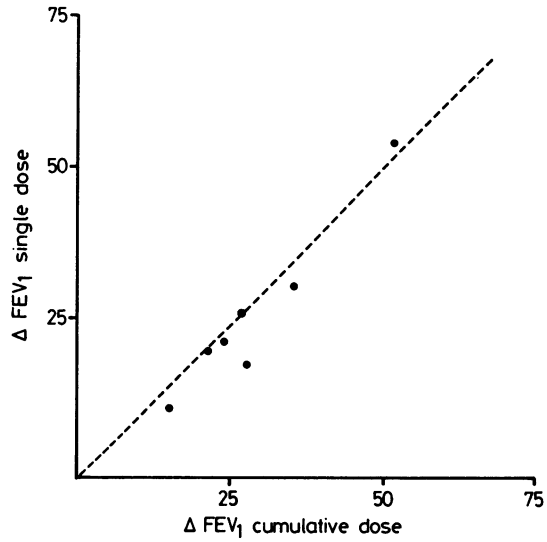

Fig 3 *Percentage change in FEV_1 in seven subjects in response to histamine given cumulatively compared with the response to the same amount given as a single dose.*

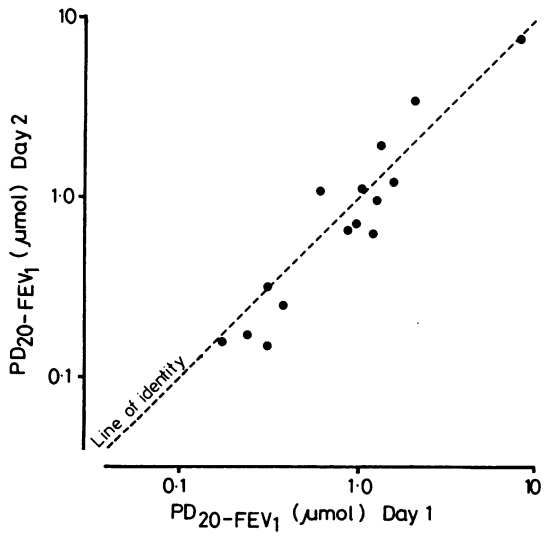

Fig 2 *Histamine PD_{20-FEV_1} values for 15 subjects using the hand operated De Vilbiss No 40 nebuliser on two separate days.*

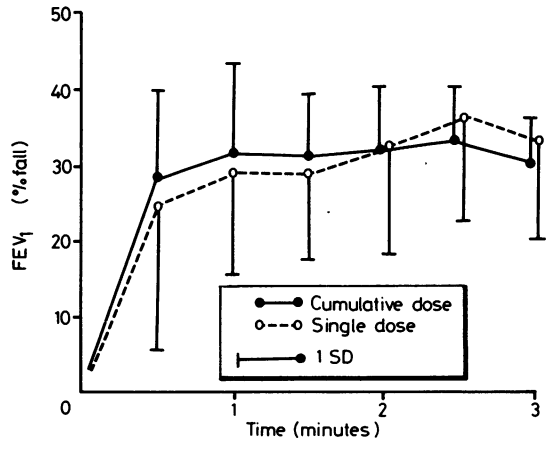

Fig 4 *Mean and standard deviation of percentage change in FEV_1 during three minutes of recovery after a cumulative dose and a single dose of histamine.*

Table 4 *Geometric mean and range of $PD_{-20}FEV_1$ values for groups I–III*

Group	Geometric mean $PD_{-20}FEV_1$ (μmol)	Range	
I	0·085	0·046–0·205	Group I *v* group II p<0·001
II	0·62	0·56 –1·0	Group II *v* group III p<0·01
III	1·53	0·30 –5·00	

the values (paired *t* test). Figure 4 shows that the mean values for percentage change in FEV_1 during recovery for these seven subjects was similar whether the histamine was given as a single dose or in a cumulative fashion.

The PD_{20-FEV_1} values obtained by the hand operated technique were not significantly different from those obtained with the dosimeter (fig 5).

There were no appreciable side effects from any dose of histamine.

Discussion

This study has shown that this simple, rapid method for measuring bronchial responsiveness produces reproducible results similar to those obtained with the standard dosimeter method. Bronchial responsiveness to histamine, as measured by this method, correlates well with the clinical severity of symptoms, as shown previously for other methods.[1 2]

To compare this method with the dosimeter method it was necessary to estimate the volume of solution delivered from the two types of nebuliser and calculate the actual dose of histamine delivered.

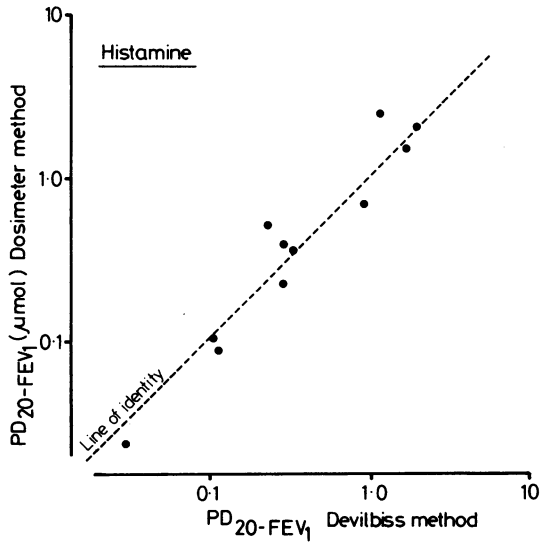

Fig 5 *Histamine PD_{20-FEV_1} values obtained with the hand-operated De Vilbiss No 40 nebuliser compared with those obtained by the dosimeter technique in 11 subjects.*

We chose to compare this method with that described by Chai *et al*,[3] since both methods use discrete doses of histamine. It has, however, been shown that results obtained with the dosimeter are comparable to those obtained with a method which uses continuous generation of an aerosol inhaled by tidal breathing.[4 5] Since the technique of inhalation—tidal breathing or discrete inspirations—appears not to affect the response it should be possible to compare actual dose response curves from different studies provided that the volume delivered is known and the concentration of solution is accurate. Despite some variability in the output of the No 40 nebulisers the results were reproducible and similar to those obtained with the dosimeter method.

In this study the dose of histamine was calculated in terms of μmoles of histamine diphosphate salt. This was done so that in future it will be possible to compare the potency of histamine with that of other agonists with different molecular weights, such as methacholine. We have shown that the effects of the doses of histamine delivered by this method are cumulative. In practice, this has made it possible to decrease the amount of time taken to administer the challenge by combining some of the doses. On some occasions doses 3 and 4 and doses 5 and 6 (both pairs requiring one inhalation followed by two inhalations) were combined in a single dose of three inhalations of the appropriate concentration. The total dose delivered remained unchanged. We emphasise that this was done only in subjects with no history suggesting heightened bronchial responsiveness and only when they had shown no response to the previous dose. Since it is important to plot a dose response curve so that the level of responsiveness can be accurately determined, doses were combined only when it was clear that this would not jeopardise our ability to get at least three points on the curve.

Examination of the time course of the response to histamine showed that the response was maximal at 60 seconds and maintained for at least three minutes (fig 4). The FEV_1 should be measured after 60 seconds and provided that the next dose is given no more than three minutes after the previous one the effect is shown to be cumulative.

There was a clear relationship between PD_{20-FEV_1}

and respiratory symptoms in these 50 subjects. All subjects with current respiratory symptoms had $PD_{20\text{-}FEV_1}$ values of less than 1·0 μmol. Subjects who had never had respiratory symptoms did not have a 20% fall in FEV_1 after the highest dose (3·9 μmol) of histamine. This was, however, a small population, selected on the basis of clinical symptoms. Future studies of larger groups may help to determine whether these ranges for $PD_{20\text{-}FEV_1}$ are of diagnostic value. Studies on large numbers of subjects would be facilitated by this simple, rapid method of assessing histamine responsiveness.

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