

# Measurement of airway responsiveness to methacholine: relative importance of the precision of drug delivery and the method of assessing response

J R Beach, C L Young, A J Avery, S C Stenton, J H Dennis, E H Walters, D J Hendrick

## Abstract

**Background** The value of measuring airway responsiveness in asthma research is currently limited by the number of different methods used by different investigators, by the lack of a standardised method of expressing precision, and by an inability to equate the results of one method with those of another.

**Methods** Two pairs of measurements of airway responsiveness to methacholine were performed in 20 asthmatic subjects, one pair using a dosimeter method (AR-D) and one pair using the conventional Wright nebuliser tidal breathing method (AR-W). The two methods normally use different techniques for quantifying changing levels in forced expiratory volume in one second (FEV<sub>1</sub>) after each dose of methacholine (the mean of the highest three of six measurements for AR-D, the lower of two measurements for AR-W), and different techniques for expressing measurements of airway responsiveness (the provoking dose (PD<sub>20</sub>) and the provoking concentration (PC<sub>20</sub>) respectively responsible for a 20% decrement in FEV<sub>1</sub>).

**Results** The coefficient of repeatability (and hence precision) for the measurement of airway responsiveness was significantly better for AR-D (3.0) than for AR-W (10.9), but the technique for quantifying FEV<sub>1</sub> contributed more to this than the technique for delivering methacholine. A PC<sub>20</sub> of 1 mg/ml with AR-W was equivalent to a PD<sub>20</sub> of 103 µg with AR-D.

**Conclusions** It is practical as well as desirable to compare the precision of different techniques for the measurement of airway responsiveness and to derive conversion factors so that results may be equated.

(Thorax 1993;48:239-243)

Airway responsiveness provides a useful concept in understanding asthma and its measurement is valuable in asthma research. Of the various bronchoconstrictor stimuli used, nebulised methacholine is probably the most popular. At present several different methods of methacholine delivery are employed together with various different methods of

expressing or measuring airway responsiveness. This limits the value of such measurements because the results from one laboratory cannot readily be compared with those from another. There is consequently a need to establish some means of defining precision in measurement—for example, coefficient of repeatability—and of equating results between methods.

Of the two measurement methods which are currently most popular, that using the Wright nebuliser has become the conventional one throughout much of Canada, Australasia and Europe.<sup>1</sup> Aerosol is generated continuously over successive periods of two minutes from doubling concentrations of methacholine and is inhaled by the test subject during tidal breathing. The dose delivered consequently depends on tidal volume and ventilatory frequency as well as on aerosol output and so is not readily quantified, although it may be closely repeatable for the individual subject. As a result airway responsiveness is expressed by the provoking concentration of methacholine (rather than the delivered dose) which is estimated to provoke a 20% decrement in the forced expiratory volume in one second (FEV<sub>1</sub>)—that is, PC<sub>20</sub>. The alternative method, which is currently popular in the USA and some European countries, uses a "dosimeter" and attempts to deliver a precise dose of methacholine during part of a single inspiratory manoeuvre.<sup>2</sup> This allows airway responsiveness to be expressed by the actual dose provoking a 20% decrement in FEV<sub>1</sub> (PD<sub>20</sub>). There is little evidence, however, that PD<sub>20</sub> provides a more accurate measure of airway responsiveness than does PC<sub>20</sub>.<sup>3</sup>

Bronchoconstriction provoked by methacholine and other non-specific stimuli has little stability and successive measurements of ventilatory function over a short time may vary considerably.<sup>4,5</sup> The precise technique used for identifying the bronchoconstrictor response may therefore exert a considerable influence on the value obtained for the measurement of airway responsiveness and on its precision.

These factors have influenced the development of our own dosimeter method.<sup>6-9</sup> In this investigation we have used it as an example of the dosimeter method in general and have compared it with the conventional Wright nebuliser tidal breathing method in order (a) to evaluate the relative importance of the precision of methacholine delivery and the

Chest Unit, Newcastle General Hospital, University of Newcastle upon Tyne, Newcastle upon Tyne NE4 6BE

J R Beach  
C L Young  
A J Avery  
S C Stenton  
J H Dennis  
E H Walters  
D J Hendrick

Reprint requests to:  
Dr D J Hendrick

Received 22 November 1991

Returned to authors  
11 March 1992

Revised version received  
26 May 1992

Accepted 18 September 1992

method of assessing response, and (b) to show how the results from different methods of measurement can be compared and contrasted.

## Methods

### STUDY DESIGN

Twenty asthmatic subjects (sequential volunteers from a "research panel") completed four methacholine tests on separate days at a standardized time ( $\pm$  one hour) within 10 days, the majority being performed on consecutive days. Two tests used the dosimeter method (AR-D) and two the Wright nebuliser tidal breathing method (AR-W). The four tests for each subject were performed in a random but balanced order (Latin square design) by the same pair of investigators, each performing one test by each method. Each investigator was blind to the results obtained by the other. Baseline FEV<sub>1</sub> values for each test were at least 60% of the predicted values. Inhaled  $\beta$  agonists were withheld for a minimum of 12 hours and oral  $\beta$  agonists and theophyllines for a minimum of 24 hours before each test. The protocol was approved by the Joint Ethics Committee of Newcastle Health Authority and the University of Newcastle upon Tyne.

### METHACHOLINE ADMINISTRATION

A locally designed microprocessor controlled dosimeter was used to generate aerosols sequentially from 10 Turbo jet nebulisers (Medic Aid, Pagham, UK), each having been calibrated to release 10  $\mu$ l ( $\pm$  10%) of aerosol during an individually timed activation of approximately two seconds at a driving pressure of 20 psi (138 kPa).<sup>6</sup> Aerosol release into a mouthpiece was activated by a pressure transducer during inspiration from functional residual capacity to inspiratory capacity and five inhalations (total of 50  $\mu$ l) were taken for each incremental dose. A Wright nebuliser (MG Electrics, Colchester, UK) was calibrated to give a total output by weight—that is, aerosol of methacholine solution plus water vapour—of 130 mg/min.<sup>11</sup> Generated aerosol was delivered into a loosely fitting face mask and was inhaled during two minutes of tidal breathing. A nose clip was used in all cases. Doubling concentrations of methacholine from 0.0625 to 32 mg/ml were used for the dosimeter method, the cumulative dose available for administration ranging from 3.125 to 6400  $\mu$ g. With the Wright nebuliser the same concentrations of methacholine were used with the addition of one further dilution of 0.03125 mg/ml. For both methods sequential doses were administered at five minute intervals until a decrement in FEV<sub>1</sub> exceeding 20% of baseline was achieved.

### EXPRESSION OF AIRWAY RESPONSIVENESS

Ventilatory function was assessed by measurement of FEV<sub>1</sub> with a Jaeger Screenmate pneumotachometer (Erich Jaeger UK Ltd,

Market Harborough, UK) with an Apple Macplus PC running software by Collingwood Measurement Ltd (Packington, Leicestershire, UK). Two alternative methods were used to estimate FEV<sub>1</sub> at each time point, the mean of the highest three of six measurements ( $M_{3/6}FEV_1$ ) and the lower of two measurements made one minute apart ( $L_2FEV_1$ ). The first is the method we use to estimate AR-D, baseline FEV<sub>1</sub> being measured as the overall mean from measurements at -10, -5, and 0 minutes—that is, the mean of nine measurements. The second is the method recommended to estimate AR-W, baseline FEV<sub>1</sub> being defined as the lowest of the first three measurements at zero minute which are reproducible within 5%.<sup>1</sup> In order to allow both methods of FEV<sub>1</sub> determination to be used with both methods of aerosol delivery, a total of seven FEV<sub>1</sub> measurements were taken during each five minute dose cycle at the following times after the start of methacholine delivery: 150 seconds ( $\times$  1); 210 seconds ( $\times$  1); 210–240 seconds ( $\times$  5)—that is, the first two for AR-W and the last six for AR-D. No control inhalation of diluent was used with either method of drug delivery as we have shown that the control inhalation does not affect PD<sub>20</sub> measurement by the dosimeter method (and so we do not use it) and we wished to use the same protocol throughout.<sup>6</sup> In not using a control inhalation of the diluent and in using seven rather than two measurements of FEV<sub>1</sub> we acknowledge that our protocol for AR-W showed minor differences from that recommended.<sup>1</sup> Incremental challenges with methacholine were continued until there was a decrement in both  $M_{3/6}FEV_1$  and  $L_2FEV_1$  exceeding 20%. Airway responsiveness was then expressed by the dose (PD<sub>20</sub>) or concentration (PC<sub>20</sub>) calculated to provoke a decrement in FEV<sub>1</sub> of exactly 20% using the method of linear interpolation from a dose response plot.<sup>12</sup>

### STATISTICAL METHODS

Log transformed data were used for all analyses of PD<sub>20</sub> and PC<sub>20</sub>. The coefficient of repeatability for each method of measurement of PD<sub>20</sub> and PC<sub>20</sub> together with a 95% confidence interval was calculated from the within subject standard deviation and this was used to assess the precision of measurement of airway responsiveness.<sup>13</sup> The coefficient of repeatability (CR) was calculated as  $\text{antilog}(2s)$ , where  $s = \sqrt{(\sum d_i^2/n)}$ ,  $d_i$  is the difference in paired values of log PD<sub>20</sub> (or log PC<sub>20</sub>) for the  $i^{\text{th}}$  subject, and  $n$  is the number of subjects. An approximate 95% confidence interval for the CR is then given by  $\text{antilog}(2s \pm 2s \sqrt{(2/n)})$ . The CR itself yields an approximate 95% confidence interval for the result of the second of a further pair of PD<sub>20</sub> or PC<sub>20</sub> measurements given the result of the first (lower limit = first measurement/CR, upper limit = first measurement  $\times$  CR). CR has a minimum value of 1; the closer it is to 1 the more repeatable (and precise) is the method of measurement.

Table 1 Geometric mean values and 95% confidence intervals for PD<sub>20</sub>, PC<sub>20</sub>, and mean baseline FEV<sub>1</sub> for the paired methacholine tests in 20 subjects

	Dosimeter M3/6 FEV <sub>1</sub> (AR-D)		Tidal breathing L/2 FEV <sub>1</sub> (AR-W)	
	PD <sub>20</sub> (µg)	Baseline FEV <sub>1</sub> (l)	PC <sub>20</sub> (mg/ml)	Baseline FEV <sub>1</sub> (l)
Initial test	32.4 (15.4-66.4)	2.60 (2.18-3.01)	0.253 (0.107-0.598)	2.45 (2.04-2.87)
Repeat test	36.9 (19.9-68.4)	2.55 (2.09-3.02)	0.444 (0.205-0.963)	2.44 (2.02-2.85)
Mean	34.6 (18.0-66.4)	2.57 (2.17-2.98)	0.335 (0.190-0.592)	2.44 (2.04-2.85)

Table 2 Coefficient of repeatability (CR) for measurements of airway responsiveness and 95% confidence intervals (CI).

	PD <sub>20</sub> M3/6 FEV <sub>1</sub> (AR-D)	PC <sub>20</sub> M3/6 FEV <sub>1</sub>	PD <sub>20</sub> * L/2 FEV <sub>1</sub>	PC <sub>20</sub> L/2 FEV <sub>1</sub> (AR-W)
n	20	20	15	20
CR	3.04	3.93	4.64	10.89
95% CI	(2.14-4.31)	(2.55-6.06)	(2.65-8.13)	(5.12-23.6)

\*For five subjects L/2 FEV<sub>1</sub> gave a greater than 20% decrement after the first dose of methacholine, thereby preventing calculation of PD<sub>20</sub>.

**Results**

Twenty asthmatic subjects (16 women) of median age 34 (range 18-65) years were recruited. The mean baseline FEV<sub>1</sub> for the group before all four methacholine tests was 85% of that predicted. A decrement of at least 20% in FEV<sub>1</sub> was achieved with all tests in all subjects. Five subjects showed a greater than 20% decrement in FEV<sub>1</sub> after the first dose of methacholine with one or other of the dosimeter tests when the L/2FEV<sub>1</sub> (but not the M3/6FEV<sub>1</sub>) was used to quantify FEV<sub>1</sub>. Linear interpolation could not, therefore, be used to calculate a PD<sub>20</sub> from L/2FEV<sub>1</sub> for these particular tests. There was no difference in results between the two investigators.

Geometric means for PD<sub>20</sub> and PC<sub>20</sub> are shown in table 1 together with the mean values for baseline FEV<sub>1</sub>. The overall mean PD<sub>20</sub> with AR-D was 34.6 µg and the overall mean PC<sub>20</sub> with AR-W was 0.335 mg/ml. A PC<sub>20</sub> of 1 mg/ml (based on L/2FEV<sub>1</sub>) was consequently equivalent to a PD<sub>20</sub> of 103 µg (based on M3/6FEV<sub>1</sub>). After standardisation of the method of drug delivery, use of the M3/6FEV<sub>1</sub> compared with the L/2FEV<sub>1</sub> increased the airway responsiveness measurement by about 2.3 fold (2.1 for

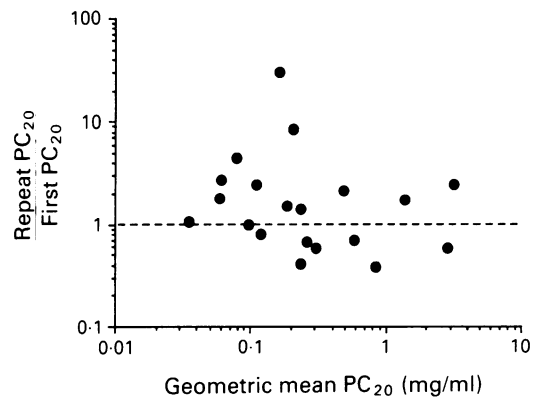


Figure 2 Duplicated measurements of airway responsiveness (PC<sub>20</sub>) in all subjects with the Wright nebuliser tidal breathing method.

PD<sub>20</sub> and 2.5 for PC<sub>20</sub>).

The CR values in table 2 show the combined effects of the method of drug delivery and the method of quantifying FEV<sub>1</sub> on the precision of airway responsiveness measurement. The CR was significantly smaller with the dosimeter and with M3/6FEV<sub>1</sub> (that is, AR-D) than with the Wright nebuliser and L/2FEV<sub>1</sub> (that is, AR-W). Of the two factors, the use of M3/6FEV<sub>1</sub> contributed more to this superior precision than the use of the dosimeter.

Fig 1 shows the ratio of the repeat to the initial measurement of AR-D for each individual (a measure of repeatability) plotted against the geometric mean of the two measurements (the best estimate of the true value). The closer the scatter to the line y = 1, the more closely repeatable is the method of measurement.<sup>12</sup> Fig 2 illustrates the data for AR-W, the scatter being significantly wider. Neither plot showed evidence of heteroscedasticity.

Fig 3 shows the mean FEV<sub>1</sub> values of each of the seven sequential expiratory manoeuvres (all tests, all subjects) after the last dose of methacholine—that is, the point at which decrements in FEV<sub>1</sub> just exceeded 20%. It shows progressive and highly significant (p < 0.001, F test) increases in FEV<sub>1</sub> following this critical time point, most of which occurred between the second and third manoeuvres. They are probably due to “deep breath bronchodilatation.” M3/6FEV<sub>1</sub> (which

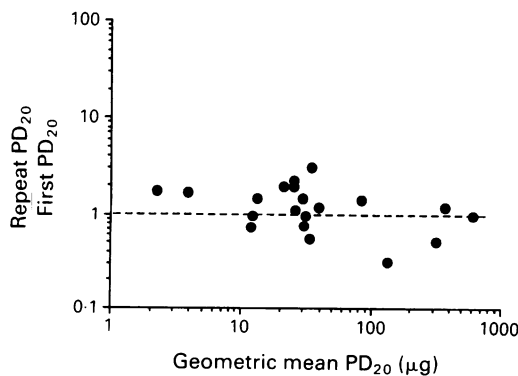


Figure 1 Duplicated measurements of airway responsiveness (PD<sub>20</sub>) in all subjects with the dosimeter method.

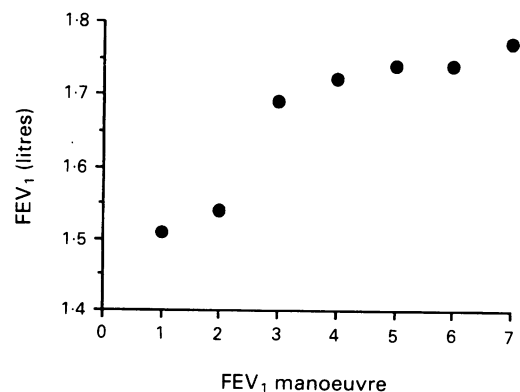


Figure 3 Mean FEV<sub>1</sub> of all subjects for each of the seven expiratory manoeuvres after the last dose of methacholine of all four tests (SE differences of means = 0.015).

depends usually on measurements 5–7 and is more affected by deep breathing) consequently differs appreciably from  $L/2FEV_1$  (which depends usually on the first measurement and is less affected by deep breathing).

### Discussion

Precision in quantifying airway responsiveness may be assessed by the closeness of duplicated measurements in individual test subjects and can be quantified usefully by the CR when duplicated measurements have been carried out in a sufficiently large number of subjects. Values of CR from about 2 to 10 or more have been reported (or can be deduced), CR often differing considerably between different investigators using apparently identical methods, including AR-W.<sup>3,7,8,14–16</sup> Comparative values for CR in the present study indicate that the dosimeter method (using the Newcastle dosimeter) coupled with the  $M3/6 FEV_1$  method of measuring change in ventilatory function provides significantly greater precision than the conventional Wright nebuliser method using  $L/2FEV_1$ . The method of estimating  $FEV_1$ , however, contributed more to this improvement in precision than the method of methacholine delivery. The contribution of the dosimeter itself was relatively modest and this needs to be weighed against its greater cost (£1000–2000 compared with £50–60).

In earlier work using AR-D we obtained values of less than 2 for CR for both methacholine and histamine, but in these investigations the subjects were selected to have closely reproducible baseline measurements of  $FEV_1$ .<sup>7,8</sup> Some had undergone previous measurements using AR-D and all were studied by the same investigator. These factors may have produced artificially optimistic results for the precision of  $PD_{20}$  measurements and we have since shown that, if peak expiratory flow is used to assess changing ventilatory function and if the study population is elderly, the CR is less satisfactory.<sup>17</sup>

In the present study subjects were selected without regard to the repeatability of baseline  $FEV_1$  measurements and most had never previously undergone a methacholine test. Different investigators were responsible for the paired measurements, the result of the initial test being unknown to the second investigator. Furthermore, 53 of the 80 methacholine tests were performed just 24 hours after the previous test and so may have been affected by the recently suggested phenomenon of refractoriness to methacholine.<sup>18,19</sup> If this was relevant, the balanced ordering of the methacholine tests should have inflated all CR values equally. We could not identify any systematic bias which might have affected AR-D differently from AR-W, and values for geometric mean  $PD_{20}$  or  $PC_{20}$  did not differ significantly between the paired tests. We consequently doubt if refractoriness (or our minor deviations from the standard AR-W protocol) could have exerted much influence over our comparison of AR-D with

AR-W. A CR of about 3 in this particular subject group may consequently be considered satisfactory for the AR-D method. These factors may, however, help to explain why the CR for the AR-W method was relatively high in our hands compared with its originators, although other investigators have reported a similar experience to our own with AR-W.<sup>14</sup>

Not only does the CR provide a confidence limit for quantifying airway responsiveness but it defines a range beyond which changed levels can be recognised—that is, in the present study population a subsequent  $PD_{20}$  (by AR-D) of less than a third of the initial measurement suggests a significant increase in airway responsiveness while one that is three times or more greater suggests a significant decrease. Such recognition may be invaluable in detecting adverse environmental factors of relevance to asthma or the beneficial effects of therapeutic agents. This would be much less easy with the AR-W method and a CR of about 10.

Our results suggest that a  $PC_{20}$  of 1 mg/ml with the conventional Wright nebuliser method (with  $L/2FEV_1$ ) is equivalent to a  $PD_{20}$  of the order 100  $\mu\text{g}$  with our dosimeter (with  $M3/6FEV_1$ ). This is fully consistent with clinical experience, substantial airway responsiveness being expressed by  $PC_{20} \leq 0.125$  mg/ml and  $PD_{20} \leq 12.5$   $\mu\text{g}$ , moderate responsiveness by  $PC_{20} 0.125$ – $1.0$  mg/ml and  $PD_{20} 12.5$ – $100$   $\mu\text{g}$ , and mild responsiveness by  $PC_{20} 1.0$ – $8.0$  mg/ml and  $PD_{20} 100$ – $800$   $\mu\text{g}$ .<sup>1</sup> We would also recognise a further category of minimal but nevertheless measurable responsiveness with a  $PD_{20}$  of 800–6400  $\mu\text{g}$  with the AR-D method. We conclude that it is practical as well as desirable to derive such equivalents for other techniques of measurement.

We are grateful to Mr G Flanagan and Mr E Thompson, Department of Medical Physics, University of Newcastle upon Tyne, and to Mr P Byrne, Regional Department of Medical Physics, Newcastle General Hospital, Newcastle upon Tyne, for their help with the design and construction of the dosimeter used in this study.

The work was supported by grants from the Medical Research Council (No SPG 8714757), National Asthma Campaign (No 99), and the Newcastle District Research Committee.

- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235–43.
- Chai H, Farr RS, Froehlich LA, Mathison DA, Mclean JA, Rosenthal RR, *et al.* Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323–7.
- Ryan G, Dolovich MB, Roberts RS, Frith PA, Juniper EF, Hargreave FE, *et al.* Standardisation of inhalation provocation tests—two techniques of aerosol generation and inhalation compared. *Am Rev Respir Dis* 1981;123:195–9.
- Orehek J, Nicolini MM, Delpierre S, Beaupre A. Influence of the previous deep inspiration on the spirometric measurement of provoked bronchoconstriction in asthma. *Am Rev Respir Dis* 1981;123:269–72.
- Wheatley JR, Pare PD, Engel LA. Reversibility of induced bronchoconstriction by deep inspiration in asthmatic and normal subjects. *Eur Respir J* 1989;2:331–9.
- Hendrick DJ, Fabbri LM, Hughes JM, Banks DE, Barkman HW, Connolly MJ, *et al.* Modification of the methacholine inhalation test and its epidemiologic use in polyurethane workers. *Am Rev Respir Dis* 1986;133:600–4.
- Connolly MJ, Avery AJ, Walters EH, Hendrick DJ. The use of sequential doses of inhaled histamine in the measurement of bronchial responsiveness: cumulative effect and distortion produced by shortening the proto-

- col. *J Allergy Clin Immunol* 1988;**82**:863-8.
- 8 Connolly MJ, Avery AJ, Walters EH, Hendrick DJ. The relationship between bronchial responsiveness to methacholine and bronchial responsiveness to histamine in asthmatic subjects. *Pulmonary Pharmacol* 1988;**1**:53-8.
  - 9 Connolly MJ, Stenton SC, Avery AJ, Walters EH, Hendrick DJ. Refractory period following bronchoconstriction provoked by histamine in asthmatic subjects. *Thorax* 1989;**44**:146-50.
  - 10 Dennis JH, Stenton SC, Beach JR, Avery AJ, Walters EH, Hendrick DJ. Jet and ultrasonic nebuliser output: use of a new method for direct measurement of aerosol output. *Thorax* 1990;**45**:728-32.
  - 11 Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;**33**:705-10.
  - 12 Eiser NM, Kerrebijn KF, Quanjer PH, eds. Guidelines for standardisation of bronchial challenges with (nonspecific) bronchoconstricting agents. SEPCR Working Group on Bronchial Reactivity. *Bull Eur Physiopathol Respir* 1983;**19**:495-514.
  - 13 Bland MJ, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**i**:307-10.
  - 14 Britton J, Mortagy A, Tattersfield A. Histamine challenge testing: comparison of three methods. *Thorax* 1986;**41**:128-32.
  - 15 Nieminen NM, Lahdensuo A, Kellomaeki L, Karvonen J, Muittari A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. *Thorax* 1988;**43**:896-900.
  - 16 Trigg CJ, Jhalli N, Herdman MJ, Cundell DR, Thomas JM, Davies RJ. The daily variability of bronchial responsiveness to methacholine. *Eur Respir J* 1990;**3**:867-71.
  - 17 Connolly MJ, Kelly CA, Walters EH, Hendrick DJ. An assessment of methacholine inhalation tests in elderly asthmatics. *Age and Ageing* 1988;**17**:123-8.
  - 18 Beckett WS, McConnell WF, Wong ND. Tolerance to methacholine inhalation challenge in nonasthmatic subjects. *Am Rev Respir Dis* 1988;**137**:1499-501.
  - 19 Beach JR, Young CL, Harkawat R, Stenton SC, Connolly MJ, Walters EH, *et al*. Is there refractoriness to methacholine provoked bronchoconstriction 24 hours after a measurement of PD20 methacholine? *Am Rev Respir Dis* 1991;**143**:A410.