Estimation and repeatability of the response to inhaled histamine in a community survey

S CHINN, J R BRITTON, P G J BURNEY, A E TATTERSFIELD, A O PAPACOSTA

From the Department of Community Medicine, United Medical and Dental Schools, St Thomas's Campus, London; and the Respiratory Medicine Unit, City Hospital, Nottingham

ABSTRACT Epidemiological problems arising from the absence of an agreed definition of asthma have led to the use of bronchial reactivity tests in community surveys of asthma prevalence. Since only a minority of the general population will develop bronchoconstriction in response to the dose of histamine considered acceptable for use in the community it is important to make maximum use of the data available. Several methods for summarising the information in the dose-response curve obtained from a histamine challenge test have been compared. A standardised histamine challenge test was administered to 797 subjects selected from two communities, and a repeat test to 106 subjects. The test was well accepted. For most subjects FEV_1 rose initially after administration of histamine (median rise 100 ml), so maximum FEV_1 was used as the baseline from which the 20% fall to achieve a PD_{20} was calculated. In order to use all the data rather than just two points on the FEV_1-log dose graph, PD_{20} was estimated by means of curve fitting, and the values were compared with PD_{20} from linear interpolation. An exponential curve was found to fit the data well. Extrapolation from the maximum dose of 4 μmol up to 8 μmol was allowed in the estimation of PD_{20} by both methods. The curve fitting method gave slightly more reproducible PD_{20} values than did linear interpolation, and also gave more estimates in the range 0·03–8 μmol. The repeatability of PD_{20} compared well with that of asthmatic subjects tested in a clinical environment. Curve fitting has an advantage over linear interpolation in large community studies, for which analysis of data by computer is essential.

Introduction

Most patients with asthma show bronchial hyperreactivity to a variety of non-allergenic stimuli,[1] although the precise relationship between reactivity and asthma is still debated. In general, hyperreactivity is related to the severity of asthma, as judged by symptoms,[2] requirement for treatment,[3] and diurnal variation in peak expiratory flow.[4] Various guidelines have recently been suggested for the standardisation of bronchial challenge tests.[5] [6]

The epidemiological problems arising from the absence of any agreed definition of asthma have led to interest in the possibility of using tests of bronchial reactivity in community surveys. In this situation the test needs to be quick and simple and be acceptable to

Address for reprint requests: Miss S Chinn, Department of Community Medicine, St Thomas's Hospital Medical School, London SE1 7EH.

the general population, and to give reproducible results under field conditions. It is essential to have a standardised protocol to be used for all subjects. Yan et al[6] have developed a method for this purpose, giving increasing concentrations of histamine from hand held deVilbiss nebulisers and measuring the response as the dose of histamine estimated by interpolation to cause a 20% fall (PD_{20}) in one second forced expiratory volume (FEV_1). In a pilot study Britton et al[7] showed Yan's method to be quicker and at least as reproducible as two alternative tests, and this was therefore the method chosen for the community survey.

Measurement of bronchial reactivity in epidemiological studies is subject to certain restraints. Few of the population will have had any previous experience of forced expiratory manoeuvres. Since the general population may be less tolerant of side effects than subjects in the laboratory, the maximum dose of histamine that can be given is lower, and a smaller proportion of subjects will have a PD_{20} that can be esti-

mated. Hence it is important to obtain the maximum information from the data that are obtained.

In the main study Yan's method of histamine challenge was used to measure PD_{20} in 797 subjects as part of a community survey of asthma prevalence in two areas of southern England.[8] Repeat studies were performed in 106 subjects 1–14 days after the first test. In the study reported in this paper we set out to establish the best way to analyse histamine challenge dose-response data obtained in an epidemiological setting by determining: (1) the proportion of subjects for whom a PD_{20} value was obtained by fitting dose-response curves compared with the use of linear interpolation; (2) the repeatability of PD_{20} values for the two methods; (3) whether PD_{20} should be estimated as a 20% fall from the post-saline FEV_1 or the maximum FEV_1.

PD_{10} was estimated and assessed in the same way as PD_{20}. The fact that more subjects would have a PD_{10} than a PD_{20} value of $\leqslant 8$ µmol could favour the use of PD_{10} in a community survey if the results are as repeatable.

Methods

SUBJECTS

A questionnaire about symptoms of asthma was administered to all subjects aged 18–64 years in two villages in Hampshire and a market town in Dorset as part of a study of asthma prevalence. After completion of the questionnaire 1325 subjects were asked to attend for a histamine challenge test at their general practitioner's surgery or health clinic. The subjects consisted of two groups, a 20% random sample (855) of the 4277 subjects aged 18–64 years who returned a completed questionnaire and all remaining subjects (470) who answered "Yes" to the question "Have you had wheezing or whistling in your chest at any time in the last 12 months?" In this paper no distinction is made between the two groups of subjects; the prevalence of hyperreactivity and its relation to smoking history and skin sensitivity as estimated from the random sample has been reported[8] and the relation of hyperreactivity to associated symptoms will be reported later.

Subjects who had taken theophyllines or antihistamines in the previous 24 hours or a bronchodilator in the last six hours were asked to return later after omitting this treatment. Ethical approval was obtained from the local ethical committees and all subjects had the test explained and signed a consent form before the test was carried out.

A total of 170 subjects were invited to return for a second histamine challenge test. These consisted of a 10% random sample of all subjects in one area and in addition all subjects whose FEV_1 had fallen by 20%

in the initial test in both areas, plus a few subjects whose fall in FEV_1 approached 20%. The decision to adminster the second test by the same or a different person was taken at random.

MEASUREMENTS

Height was measured and predicted FEV_1 calculated as recommended by Cotes *et al*.[9] FEV_1 and forced vital capacity were measured with a dry spirometer (Vitalograph, Buckingham, England). Initial FEV_1 was recorded as the maximum of three consecutive measurements that agreed to within 5%. Subjects whose initial FEV_1 was less than 60% of the predicted value were not challenged with histamine.

Other details of the histamine challenge test were as described by Yan *et al*,[6] with doubling doses of from 0·03 to 4 µmol administered to subjects with a history of wheezing or whose post-saline FEV_1 was less than 90% predicted. All other subjects were given 0·06 µmol histamine followed by quadrupling doses of histamine until their FEV_1 had fallen by at least 10%, when the challenge regimen was changed to the slower schedule. The test was stopped when the FEV_1 had fallen by 20% or more from the post-saline value or the 4·0 µmol dose had been given, or at the subject's request.

ESTIMATION OF PD_{20}

PD_{20} FEV_1 was estimated by linear interpolation on the basis of data from the last two doses administered,[10] with extrapolation to one doubling dose (8 µmol) beyond the maximum administered, following Cockcroft *et al*.[11] PD_{20} was estimated by using all the available data and fitting the exponential curve

$$\log_e(c - y) = a + bx, \qquad \text{(Curve 1)}$$

where y is FEV_1, x is $\log_{10}$ (dose), c represents mean FEV_1 before it is affected by histamine, b is a "slope" parameter, and a is a curve position parameter. Since Woolcock *et al*[12] fitted a logistic curve, we also fitted

$$\log_e (c - y)/y = a + bx. \qquad \text{(Curve 2)}$$

Extrapolation to a dose of 8 µmol was allowed for both curves as with the linear interpolation method. Curves were fitted provided that two or more doses of histamine were administered—that is, they were fitted to all sets of data for which PD_{20} values were obtained by linear interpolation. When only two doses of histamine were given the post-saline FEV_1 was used as the estimate of c. Details are given in the appendix.

Most subjects showed a small increase in FEV_1 after the low dose of histamine, so we also calculated the estimates described above as the dose producing a 20% fall from maximum FEV_1. Linear interpolation uses the doses either side of the estimate. Occasionally both the last two doses administered produced a
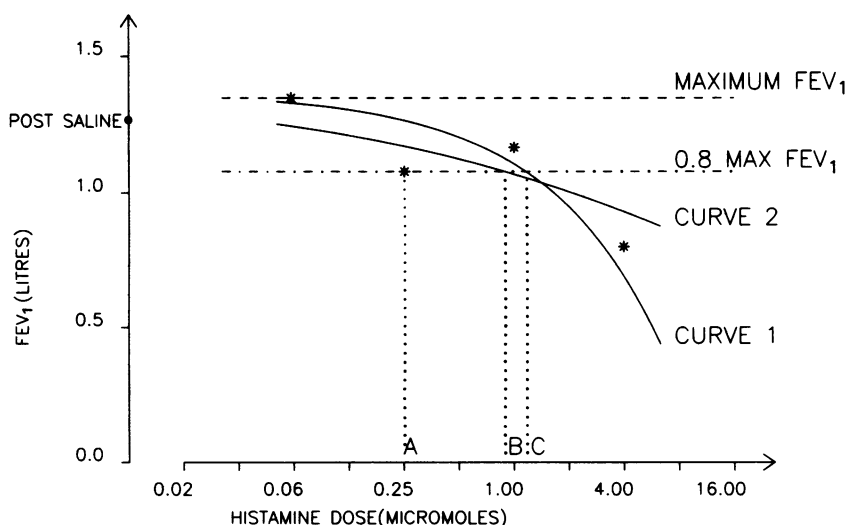
Fig 1   *Curves fitted to data for a subject showing more variability in FEV_1 than the average, illustrating the desirability of using all the data rather than just two points. The post-saline FEV_1 (●) and FEV_1 (*) after doses 0·06, 0·25, 1·0, and 4·0 μmol histamine are shown. The line – – – – – denotes maximum FEV_1 and –·–·–·– 80% of maximum FEV_1. The histamine dose causing a 20% fall in FEV_1 (PD_{20}) is estimated as A: 0·25 (linear interpolation); B: 0·89 (curve 2); and C: 1·17 μmol (curve 1).*

greater than a 20% fall from maximum FEV_1 and in this case the two appropriate earlier doses were used for the estimate. This corresponds to the procedure that would be followed if the test were terminated when a 20% fall from *maximum* FEV_1 was reached. An example of each curve fitted to data for one subject is shown in figure 1, for whom the second administered dose of histamine produced a 20% fall from FEV_1 maximum.

The methods of estimating PD_{20} were compared according to the number of subjects who achieved a PD_{20} and the repeatability of the measurements.

PD_{10} was estimated, by linear interpolation and from the same curves, in a similar way to PD_{20}.

REPEATABILITY
The mean and standard deviation of the difference between the estimates of PD_{20} for repeat tests were calculated for the subjects tested twice. Repeatability was also calculated for PD_{10}, post-saline FEV_1, and maximum FEV_1. The within subject standard deviation was calculated by dividing the standard deviation of the difference of two repeat estimates by the square root of 2.[13] A dimensionless measure of repeatability, intraclass correlation coefficient,[14] was also calculated.

Results

Of 1325 subjects invited to undergo a histamine chal-

lenge test, 834 (63%) agreed. Thirty seven subjects were not given histamine, four owing to difficulty in complying with instructions and 33 because the initial or post-saline FEV_1 was less than 60% predicted. Thus 797 subjects were given at least one dose of histamine at the first test, including four subjects with an initial FEV_1 less than 60% predicted who were given histamine in error; of these, 512 (64·2%) were randomly selected.

Most subjects (74·4%) increased their FEV_1 after the post-saline measurement, 83 (10·4%) having their maximum recorded FEV_1 after the final 4 μmol dose of histamine. The increase in FEV_1 over the post-saline FEV_1 ranged up to 0·9 litre in absolute terms (a 30% increase), although the median increase was only 100 ml.

Four subjects were given just one dose of histamine, of whom three had a 20% fall in FEV_1 after that dose and so did not have a PD_{20} that could be estimated. Each method of estimation of PD_{20} was applied to the data for the remaining 793 subjects. The residual standard deviation about the curves was less than 0·21 for 88·1% of subjects for curve 1 and 86·0% for curve 2; the mean residual standard deviation was 0·151 for each curve. The larger residual standard deviations were found to be due to random variation in the FEV_1 rather than systematic deviations from the fitted curves. This is illustrated in the example in figure 1, where the residual standard deviations about the curves were 0·23 and 0·21 l.

NUMBERS OF SUBJECTS WITH PD_{10} AND PD_{20} VALUES

The number of estimates of PD_{20} is shown in table 1, and varies according to method from 20·2% to 26·2% of the 793 subjects. Curve fitting produced more estimates in the range 0·03—8 μmol than did linear interpolation, the difference being between 28 and 38 subjects. Use of the maximum FEV_1 as baseline rather than the post-saline FEV_1 increased the number of estimates, since a 20% fall was achieved at greater absolute FEV_1. The numbers achieving a 20% fall from the maximum FEV_1, when calculated by linear interpolation and by fitting curves 1 and 2, were 173, 208, and 201 respectively, whereas the corresponding numbers achieving a 10% fall were 292, 324, and 319, about 50% more.

REPEATABILITY

A total of 112 (66%) of the 170 subjects invited presented for retest. These included 19 (48%) of the 40 randomly selected subjects and 93 (72%) of the 130 who had a fall of 20%, or nearly 20%, in FEV_1 at the first test. Six subjects were not given histamine on the second occasion, five because of recurrent wheezing after the first test, and one because of error in calculating the initial FEV_1 as being less than 60% predicted. Two subjects had a 20% fall in FEV_1 after one dose of histamine, so that a second PD_{20} could not be estimated. Duplicate estimates of PD_{20} were therefore available in 104 subjects.

The repeatability of log PD_{20} values obtained by each method is given in table 2 as the standard deviation of the differences between test and retest values. This is given for all 104 subjects, for the 90 subjects with at least one estimate less than or equal to 8 μmol, and for the 64 subjects with all estimates less than 8 μmol. PD_{20} values estimated by curve fitting were a little more repeatable (had a smaller standard deviation) than those for linear interpolation, and PD_{20} values from curve 1 were slightly more repeatable than those from curve 2 (columns one and two). The repeatability of the three methods was similar when restricted to the 64 subjects with no estimate greater than 8 μmol. The number with a PD_{20} of 8 μmol or less on both occasions varied from 68 for linear interpolation, with post-saline FEV_1 as baseline, to 73 for curve 1. Figure 2 shows the relation between curve 1 PD_{20} for the second test to that for the first, on the basis of fall from maximum FEV_1. The choice of baseline had little influence on the repeatability of the estimated PD_{20}.

The post-saline FEV_1 was slightly less repeatable than maximum FEV_1. Table 3 also shows the repeatability of PD_{10} estimates from curve 1, with FEV_1

Table 1 *Numbers of censored and estimated PD_{20} values obtained by methods of linear interpolation and curve fitting for the 793 subjects given at least two doses of histamine*

Method	Censored <0·03 μmol	Estimated	Censored >8 μmol
(a) PD_{20} is dose producing 20% fall in FEV_1 from post-saline FEV_1			
Linear interpolation	1	160	632
Curve 1	2	198	593
Curve 2	2	190	601
(b) PD_{20} is dose producing 20% fall in FEV_1 from maximum FEV_1			
Linear interpolation	1	173	619
Curve 1	2	208	583
Curve 2	2	201	590

PD_{20}—histamine dose (μmol) causing 20% fall in FEV_1.

Table 2 *Repeatability of log_{10} PD_{20} (values greater than log 8 set to log 8 where appropriate)*

	Difference between 2nd and 1st test (log-scale)					
	All subjects tested twice (n = 104)		Subjects with at least one value ≤8 μmol (n = 90)		Subjects with all values ≤8 μmol (n = 64)	
	Mean	SD	Mean	SD	Mean	SD
(a) PD_{20} is dose producing 20% fall in FEV_1 from post-saline FEV_1						
Linear interpolation	−0·04	0·40	−0·04	0·43	−0·07	0·40
Curve 1	−0·03	0·36	−0·03	0·38	−0·07	0·40
Curve 2	−0·02	0·39	−0·02	0·42	−0·06	0·39
(b) PD_{20} is dose producing 20% fall in FEV_1 from maximum FEV_1						
Linear interpolation	−0·03	0·39	−0·03	0·42	−0·05	0·41
Curve 1	−0·02	0·36	−0·03	0·39	−0·06	0·40
Curve 2	−0·01	0·39	−0·01	0·42	−0·05	0·39

PD_{20}—histamine dose (μmol) causing 20% fall in FEV_1.

Table 3 *Repeatability of FEV$_1$ and curve parameters*

|	*n*	Difference between 2nd and 1st test		*Within subject SD*	*Between subject SD*	*Intraclass correlation coefficient*
		Mean	*SD*			
FEV$_1$ post-saline (litres)	107	0·01	0·42	0·30	0·98	0·91
FEV$_1$ maximum (litres)	106	0·02	0·40	0·28	1·07	0·94
log$_{10}$ (PD$_{20}$) (estimated as dose producing 20% fall from FEV$_1$ maximum, on the basis of curve 1)	90	−0·03	0·39	0·27	0·56	0·81
log$_{10}$ (PD$_{10}$) (estimated as dose producing 10% fall from FEV$_1$ maximum, on the basis of curve 1)	90	0·01	0·46	0·33	0·50	0·70
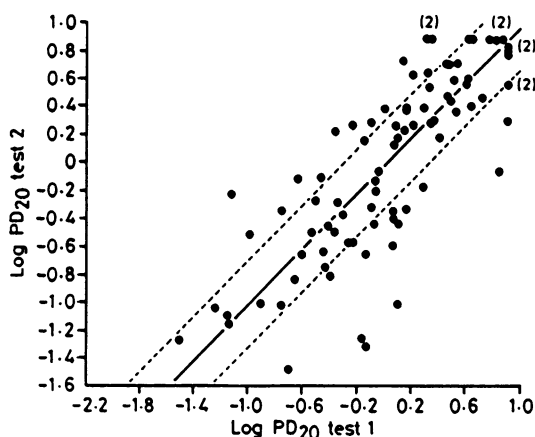
Fig 2 *Relation between (log) PD$_{20}$ (histamine dose (μmol) causing 20% fall in FEV$_1$) at the second test to (log) PD$_{20}$ at the first test, as estimated from curve 1 with maximum FEV$_1$ as baseline. The line —— is the line of identity and the lines − − − − denote a difference of one doubling dose of histamine.*

maximum as baseline, compared with that of PD$_{20}$ in the same 90 subjects, whose data are given in column 2 of table 2. The intraclass correlation coefficient was less than that for PD$_{20}$ in these subjects. Estimates of repeatability of PD$_{10}$ from curve 2, and estimates based on post-saline FEV$_1$, were similar. PD$_{10}$ estimated by linear interpolation was less repeatable, the difference in repeatability being similar to that of PD$_{20}$ (data available but not shown).

Repeatability was affected to a small extent by differences in time of day of measurement, the time between tests (range 1–14 days), and the person performing the test. Data were available for the 27 subjects retested one day later, at the same time of day to within one hour, and by the same tester. If we excluded two subjects with a PD$_{20}$ greater than 8 μmol on both occasions, the within subject standard deviation of curve 1 PD$_{20}$ was reduced from 0·27 (table 3) to 0·18.

Discussion

Studies on asthma prevalence have been greatly hampered by the absence of an agreed definition of asthma. Measurement of bronchial responsiveness provides an objective test, and since it is an important marker of asthma there has been interest recently in exploring the role of bronchial reactivity measurement in the assessment of asthma prevalence in the community.

Measurement of bronchial reactivity in large surveys poses different problems from measurements in the laboratory. The incidence of side effects considered acceptable in a community may be lower than for subjects in the laboratory, so a lower maximum dose of histamine has to be given. We found in a pilot study that the test described by Yan *et al*[6] was feasible in inexperienced subjects and that 4 μmol histamine was the highest dose that was generally well tolerated. With this low maximum dose of histamine fewer subjects will develop bronchoconstriction, so it is important to estimate the response by a method that makes maximum use of the information available.

PD$_{20}$ values have usually been obtained by linear interpolation between the response to the last two doses of histamine, with extrapolation up to one further doubling concentration of histamine by some workers.[4 11] This technique is simple and gives an immediate answer. Fitting a curve to the data is more complicated but, when the data are of necessity analysed by computer, curve fitting is no more difficult than linear interpolation. Since we needed to analyse a large number of histamine dose-response curves we decided to compare the linear interpolation method with two curve fitting models, extrapolating up to one doubling dose (8 μmol) for each method to increase the number of estimates. We did not include in this comparison estimates of PD$_{20}$ if only one dose of histamine was given, as the published method of estimation requires linear interpolation between post-saline FEV$_1$ and post-histamine FEV$_1$ at the first dose on a linear scale[10] and is inappropriate when linear

interpolation is otherwise carried out on a log-dose scale (post-saline "dose" = 0, and log (0) = −∞).

We looked at two curves, both of which are described by the minimum three parameters, upper asymptote, slope, and position. Both curves appear to be preferable to the quadratic equation recommended by Neijen's[15] which is less flexible in shape and has no slope parameter. Curve 1 was the simplest such curve. Curve 2 was chosen because Woolcock *et al*[12] had fitted a logistic curve to their data. The repeatability of PD_{20} values from curve 2 was less good than that of values from curve 1.

On the basis of fall from maximum FEV_1, a PD_{20} value was obtained in 173 subjects by the linear interpolation method. Curve fitting increased the number of subjects with an estimated PD_{20} value by 35 and 28 subjects respectively for curves 1 and 2. The lower number from linear interpolation occurred in part because an extrapolated value was obtained only if the FEV_1 after the final dose was less than the previous FEV_1, whereas curve fitting provided an estimate whether or not this was the case. In the subjects tested twice there were correspondingly more estimates by curve fitting than by linear interpolation, which led to more subjects with one or both estimates less than 8 μmol. The repeatability of PD_{20} values obtained by fitting curve 1 was better than that of values obtained by linear interpolation, as shown by column 2 of table 2. Column 3, which contains results only for subjects with *all* estimates less than 8 μmol, apparently shows greater repeatability for linear interpolation and curve 2 estimates than does column 2 and lower repeatability for curve 1 estimates. This is because some of the subjects who are included in column 2 but not in column 3 had much more discrepant values derived from linear interpolation and curve 2 than from curve 1, and column 3 is included only to demonstrate this point. Selective exclusion of subjects with values above 8 μmol for each method would be even more misleading as different subjects would be included for each method.

With any measurement having an upper limit above which values cannot be obtained there is no ideal solution to the problem of estimating repeatability. Methods can be properly compared only when carried out on the same or comparable subjects, and even then care must be taken not to exclude subjects more variable by one method than another. There is also the problem of whether the between subject variation used in the calculation of the intraclass correlation coefficient should be estimated from subjects tested twice or from a sample more representative of the population. PD_{10} had a greater within subject variation and greater between subject variation than PD_{20} in the 90 subjects; the intraclass correlation coefficient showed PD_{10} to be relatively less repeatable than

PD_{20}. Judged against variation in all 793 subjects studied, PD_{10} and PD_{20} had similar repeatability. We have preferred to use PD_{20} since this has been used more often by others.

Other measures of reactivity that have been used elsewhere include threshold dose,[16] which has been shown to be less repeatable[17] than PD_{20} and to add little information.

Most subjects (75%) increased their FEV_1 after the post-saline measurement. In most the increase was less than 100 ml, which is within the 200 ml 95% range that would be expected in subjects unfamiliar with a spirometer.[17] If we assume that the highest value is closer to the true FEV_1 it is appropriate to take the maximum FEV_1 as a baseline rather than the post-saline FEV_1. This decision is supported by the slightly smaller intraclass correlation coefficient of the maximum FEV_1 than of the post-saline FEV_1 (table 3). The difference, however, between the results from the two methods is trivial, the PD_{20} derived from the maximum FEV_1 being on average only 0·008 log μmol (equivalent to 0·03 doubling doses) less than that derived from the post-saline FEV_1 (standard deviation of differences 0·036 log μmol).

The repeatability of PD_{20} in this study, as estimated from curve 1, was similar to that found in laboratory based studies. Dehaut *et al*[18] reported a 95% single determination "confidence interval" (strictly speaking a range, not a confidence interval) of 1·59 doubling concentrations; our within subject standard deviation of 0·27 gives a corresponding figure of 1·79 doubling doses. When our calculation was restricted to data tested under conditions more like those that would be imposed in a laboratory setting the within subject standard deviation was 0·18, equivalent to a 95% single determination interval of 1·2 doubling doses. Other laboratory studies have found better agreement than Dehaut between duplicate measurements, in trained and selected subjects; but these results should not be compared with our findings. An epidemiological study of a community population must include all respondents irrespective of technique if bias is to be avoided, and this will inevitably decrease repeatability of the method used.

Variability in FEV_1 makes linear interpolation from just two measurements unreliable and also reduces the number of estimates; the FEV_1 at 4 μmol can, by chance, be greater than the previous value; this renders linear extrapolation to a PD_{20} impossible whereas curve fitting will give an estimate. We did not obtain a direct estimate of the short term repeatability of FEV_1, but the residual standard deviation about curve 1 was 0·15 l, well within the range of 0·1–0·3 l for different subgroups quoted by Tweeddale *et al*[18] for repeat FEV_1 measurements in inexperienced subjects. Extrapolation using curve 1 increased the number of

PD_{20} values by about 20% without a reduction in repeatability (data available but not included). Extrapolation to one doubling dose beyond the maximum administered by fitting a curve to all the data is therefore justified and desirable in epidemiological surveys in which the maximum dose of histamine is low. In clinical studies, where larger doses can be given and where ease of computation may be important, interpolation between the last two points gives satisfactory results.

Appendix: Fitting the curves

Curve 1 is an exponential curve with a "slope" *(b)* and an upper asymptote *(c)* provided that *b* is positive.

Curve 2 is a logistic (sigmoid) curve with an upper and a lower asymptote. The lower asymptote was fixed at zero FEV_1 as doses of histamine were too low for FEV_1 to reach the asymptote. (Woolcock *et al*,[12] who gave higher doses, attempted to estimate the lower asymptote, but fixed the upper asymptote at the post-saline FEV_1.)

Each curve was fitted iteratively by a two step procedure, with the post-saline FEV_1 as the initial estimate of parameter *c*. Step 1 estimates *a* and *b*. Given *a* and *b*, step 2 estimates *c* to give the minimum residual sum of squares on the FEV_1 scale. Iteration was continued until there was a reduction in the residual sum of squares from the previous iteration and:

    (i) *c* changed by less than 0·01;
or (ii) *c* changed by less than 0·05 and number of iterations >10;
or (iii) number of iterations >50.

CURVE 1 $y = c - \exp(a + bx)$
Step 1 Estimate *a* and *b* by regression of $\log_e (c - y)$ on *x*. If some values of FEV_1 *(y)* are greater than or equal to *c*, only data for values of *x* greater than the highest *x* for which $y > c$ are used.
Step 2 Estimate $c = (\Sigma y + \Sigma\exp(a + bx))/n$, where *n* is total number of doses of histamine.

CURVE 2 $y = c/(1 + \exp(a + bx))$
Step 1 Estimate *a* and *b* by regression of $\log_e ((c - y)/y)$ on *x*. Omit data as for curve 1.
Step 2 Estimate $c = \Sigma yz/z^2$, where $z = 1/(1 + \exp(a + bx))$.

Because minimisation in step 1 is on the log scale and that in step 2 on the original scale, non-convergence can occur. Omission of values in step 1 can also cause this, and the second and third criteria for stopping iteration were to cope with the few data sets for which this occurred. As rising curves were of no interest, *b* was set to zero if a value less than zero resulted from step 1.

Parameter *c* is the asymptote of the curve unless *b* is zero, when the "curve" is a straight line with ordinate $c - e^a$ for curve 1 or $c/(1 + e^a)$ for curve 2. The algorithms are approximate in that in some cases the minimum residual sum of squares will not be found—generally when the curve fits the data poorly. The computing time required for algorithms guaranteeing the best fit for each curve to each of the data sets, which totalled 897, could not be justified. The algorithms failed to converge for less than 5% of data sets.

The program to fit the curves was written in FORTRAN for a minicomputer. The calculations for fitting curve 1 could be carried out on a microcomputer, or even on a sophisticated programmable calculator provided that sufficient data stores as well as program steps are available.

References

1 Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. State of the art bronchial hyperreactivity. *Am Rev Respiratory Dis* 1980;**121**:389–411.
2 Laitinen LAI. Histamine and metacholine challenge in the testing of bronchial reactivity. *Scand J Respir Dis* 1974;suppl 86.
3 Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and metacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* 1981;**36**:575–9.
4 Ryan G, Latimer KM, Dolovitch J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. *Thorax* 1982;**37**:423–9.
5 Eiser NM, Kerrebijn KF, Quanjer PH. Guidelines for standardisation of bronchial challenges with (non-specific) bronchoconstricting agents. *Bull Eur Physiopathol Respir* 1983;**19**:495–514.
6 Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;**38**:760–5.
7 Britton JR, Mortagy A, Tattersfield AF. Histamine challenge testing: comparison of three methods. *Thorax* 1986;**41**:128–32.
8 Burney PGJ, Britton JR, Chinn S, *et al*. The descriptive epidemiology of bronchial reactivity in an adult population: results from a community study. *Thorax* 1987;**42**:38–44.
9 Cotes JE. *Lung function: assessment and measurement.* 4th ed. Oxford: Blackwell Scientific Publications, 1979:369.
10 Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and metacholine. *Thorax* 1978;**33**:705–10.
11 Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE.

Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977;7:503–13.

12 Woolcock AJ, Salome CM, Yan K. The shape of the dose-response curve to histamine in asthmatic and normal subjects. *Am Rev Respir Dis* 1984;**130**:71–5.

13 Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. *Statistician* 1983;**32**:307–17.

14 Fisher RA. *Statistical methods for research workers.* 14th ed. Edinburgh: Oliver and Boyd, 1970:225.

15 Neijens HJ, Hofkamp M, Degenhart HJ, Kerrebijn KF. Bronchial responsiveness as a function of inhaled histamine and the methods of measurement. *Bull Eur Physiopathol Respir* 1982;**18**:427–38.

16 Cockcroft DW, Berscheid BS, Murdock KY. Measurement of responsiveness to inhaled histamine using FEV_1: comparison of PC_{20} and threshold. *Thorax* 1983;**38**:523–6.

17 Tweeddale PM, Merchant S, Leslie M, Alexander F, McHardy GJR. Short term variability in FEV_1 relation to pretest activity, level of FEV_1, and smoking habits. *Thorax* 1984;**39**:928–32.

18 Dehaut P, Rachiell A, Martin RR, Malo JL. Histamine dose-response curves in asthma: reproducibility and sensitivity of different indices to assess response. *Thorax* 1983;**38**:516–22.