Specific Approach on Dose-Response Curves to Inhaled Carbachol Assessed by the Interruption Technique in Children¹

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ABSTRACT. To determine significant thresholds obtained by bronchial challenge tests in children for assessment of bronchial reactivity, changes of respiratory resistance (R_{int}) induced by inhaled carbachol were evaluated in 20 asthmatic children (10 boys and 10 girls, mean age 9.1 ± 2.8 y, range 5 to 15 y) and 20 healthy control children (10 boys and 10 girls, mean age 12.1 ± 2.6 y, range 8 to 16 y). Baseline lung function was assessed by whole body plethysmography. The carbachol challenge test was performed by the interruption technique. The objectives were to search for the most predictable provocation dose (PD) according to statistical and practical considerations. The statistical argument is given by the condition that the target PD must definitively be out of the measurement error of baseline values. From the practical point of view, the object is to provoke the subjects the least amount possible. As thresholds of bronchial reactivity, the provocation doses inducing a 50%, 65%, 75%, or 100% increase in baseline R_{int} (PD_{50} , PD_{65} , PD_{75} , PD_{100}) were computed by a 4th power polynomial function analysis including data points of the entire dose-response curve. In comparison, a new threshold, the "variance based" provocation dose (PD_{vb}), was defined as threshold to be definitively above the range of the baseline R_{int} variation before initiating challenge [$PD_{vb} > \text{mean} + 2 \text{ SD range of } R_{int}(0)$]. Although the PD_{50} , PD_{65} , PD_{75} , and PD_{100} can be adequately computed by the 4th power function from the whole dose-response curve, comparison between the different provocation thresholds revealed that PD_{vb} gives the best values to differentiate between healthy and asthmatic children ($p < 0.002$). Best cumulative sensitivity and specificity were obtained for PD_{vb} and even at a lower provocation level (95% true-negatives at 300 μg ; 95% true-positives within the range up to 780 μg of carbachol), whereas these criteria for PD_{50} to PD_{100} are only reached at much higher doses. Therefore, PD_{vb} features a mode to determine bronchial reactivity with improved sensitivity and specificity at lowest provocation dose levels. Other advantages are a shorter duration of testing, less occurrence of symptoms and a simpler mathematical approach. (*Pediatr Res* 34: 478–484, 1993)

Abbreviations

BR, bronchial reactivity

FEV₁, forced expiratory volume in 1 s

MEF₅₀, maximal expiratory flow at 50% of vital capacity

$PD_{50/65/75/100}$, provocation dose (μg carbachol) increasing R_{int} from baseline (100%) to 150, 165, 175, or 200%

PD_{vb} , variance-based provocation dose; threshold significantly above baseline values [$R_{int}(0)$]

R_{aw} , airway resistance, plethysmographically determined

R_{int} , respiratory resistance, measured by the interruption method

$R_{int}(0)$, baseline respiratory resistance, measured by the interruption method

s, sensitivity

f, specificity

TGV, thoracic gas volume (end-expiratory resting level, plethysmographically determined)

The procedure of inhalative bronchial challenge tests and its accuracy to assess the degree of BR in a healthy and asthmatic adult population is well standardized and documented (1–6). It has also become a valuable test in children (7–20). Although the bronchoconstrictor responses to physical or natural challenges of exercise, hyperventilation of cold, dry air, and hypo- or hyperosmolar stimuli seem to be more sensitive for asthma but less specific in detecting nonspecific reactivity in nonasthmatic subjects (21, 22), the most common way to detect BR in children is the inhalation of an agent such as histamine or acetylcholine derivatives that induce bronchoconstriction. However, despite the usefulness of a pharmacologic test to define the clinical conditions of asthma (6), concluding that a provocation causing a 20% fall in FEV₁ supports the clinical diagnosis of asthma currently present in children, some technical conditions should be observed. Forced breathing maneuvers are highly cooperation dependent, which may cause problems in young children. In addition, such test procedures do not allow the reaction of the bronchial system to be followed breath by breath and thus do not fulfill the requirements of safety for routine use in children. Finally, the observation that children have more responsive airways than adults (10, 11) has to be taken into account. There exists no satisfying standardization of the most suitable test procedure to ensure safety, to have the least dependency on the child's cooperation, and to offer more convincing data for accurate test f and s.

This article reports on a new, safe technique to follow bronchoconstrictor response to carbachol chloride, delivered in standardized quantities, measuring R_{int} changes breath by breath. For the analysis of the dose-response curve, different curve-fitting models were used to compute the cumulative doses of carbachol that had been inhaled during subsequent provocation levels in each patient. According to the increase of R_{int} (150%, 165%, 175%, and 200% of the baseline value) four response levels (PD_{50} , PD_{65} , PD_{75} , and PD_{100}) were empirically selected and defined. The question was posed whether such a PD in its standard form represents significant provocation thresholds and whether another analytic treatment of the dose-response curve

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would better reflect BR in healthy and asthmatic children, taking into account practicability, safety, and accuracy of the test for the pediatric population.

MATERIALS AND METHODS

Healthy subjects. Twenty healthy children (10 boys and 10 girls, mean age 12.1 ± 2.6 y, range 8 to 16 y) investigated for other purposes (18) represent the control subjects. Standardized case histories and physical examinations were taken by trained pediatricians and, in case of doubts, histories were verified with the family doctor. The criteria for inclusion into the study were that the child was healthy, especially free from respiratory symptoms, and showed no signs of viral infections or vaccinations during the last 6 wk before the test. Atopic diseases were excluded by determination of normal serum IgE (Pharmacia) and absence of positive radioallergosorbent tests (Phadiatop Kabi Pharmacia, Stockholm, Sweden) for grass pollen, house dust mites, and animals.

Asthmatic children. Twenty asthmatic children with exogenous allergic asthma (10 boys and 10 girls, mean age 9.1 ± 2.8 y, range 5 to 15 y) seen at the Paediatric Pulmonary Out-patient Clinic of the University Children's Hospital in Berne, Switzerland, were randomly selected as study patients. In each patient, asthma was diagnosed by a history of recurrent attacks with wheezing, elevated IgE levels (paper radioimmunosorbent test), positive specific IgE (radioallergosorbent) for one or more allergens, and the documentation of airway obstruction (R_{aw} , FEV_1 , and MEF_{50}), which was reversible with sympathomimetics (2, 4–6). At the time of testing, the patients were in a symptom-free interval (no signs of asthma clinically detectable) and the last acute asthma episode was at least 2 wk before the examination. Bronchodilator treatment was withheld 12 h before, inhalative steroids or disodium cromoglycate 24 h before the test. None of the patients were on xanthine treatment, systemic steroids, or oral β -2-agonists.

All parents gave informed consent for the examinations and investigations, and the study was approved by the local ethical committee.

Lung function tests. After the medical interview and a physical examination, pulmonary function tests were performed. The functional abnormalities were evaluated by whole-body plethysmography (16, 23, 24), using an air conditioned, constant volume, variable pressure whole-body plethysmograph (Jaeger, Würzburg, Germany). TGV at resting level was taken as an estimate of the degree of pulmonary hyperinflation, and R_{aw} as an estimate of the degree of bronchial obstruction. The R_{aw} was determined at half inspiratory flow, the subject rebreathing from a Douglas bag under body temperature pressure saturated conditions. For TGV, static respiratory efforts against the shutter were performed at low frequency and TGV was calculated by the Boyle's law technique. TGV values were corrected for resting expiratory levels of the spirogram. The instrumental dead space of the mouthpiece and the estimated stomach gas volume were subtracted. Based on TGV at end-expiratory resting level, static lung volumes, the total lung capacity, the inspiratory vital capacity, and the residual volume were obtained. R_{aw} was measured during calm breathing (no panting) of air from a Douglas bag. Flow-volume curves gave the FEV_1 and the MEF_{50} . Lung function data were expressed as a percentage of predicted values (25).

Bronchial challenge test. Inhalative provocation tests were performed with the Bronchoscreen (Jaeger), a computerized apparatus with a combined nebulizer-shutter head measuring with each breath changes in the R_{int} . During calm breathing, this technique, originally developed by van Neergaard and Wirz (26) and further improved by Veuilleumier (27) and others (28–31), measures R_{int} after interruption of the airflow during 100 ms, from the "opening limb" of the interruption curve (29), during each expiration. R_{int} was determined using the formula $R_{int} = (P_{alv}/P_{ao} - 1)R_{ref}$, and the pressure in the alveolar compartment (P_{alv}) was obtained indirectly by measuring the mouth pressure

after closing the shutter. P_{ao} was the pressure measured during free flow at pressure equilibrium (28). R_{ref} was a constant known serial resistance. With the subject seated, noseclip in place, the subject breathes air-conditioned ambient air to grow accustomed to the apparatus. Baseline measurements of $R_{int}(0)$ were performed by inhaling 10 to 20 breaths of physiologic NaCl solution (0.9%) from the built-in dosimeter. Carbachol chloride in saline buffered to pH 7.0 with sodium bicarbonate (Merck Inc., Darmstadt, Germany) was used in a low concentration solution of 1.25 mg/mL during the first 10 breaths, then in a higher concentration of 2.5 mg/mL until the end of the test procedure. This protocol was used for safety reasons to immediately detect children reacting to very low doses of carbachol. Generation time for aerosolization was 0.5 s. Multiple nebulizer output measurements revealed a mean output of 44.1 (SD 5.5) mg of solution per 10 breaths, and 23.1 (SD 1.7) mg per five breaths. Mean values of R_{int} from five breathing cycles were calculated in relation to the cumulative dose at each level of carbachol. Special care was taken to increase the dose gradually, to achieve progressive small changes in R_{int} . Using this procedure, a dose-response curve was obtained and the provocation procedure was terminated when an increase of $R_{int}(0)$ to 185% was reached. Thereafter, inhalation was switched to a bronchodilator.

The analytical approach to assess BR in healthy and asthmatic children from the provocation dose-response curves consisted in the computation of the provocation dose at 50%, 65%, 75%, and 100% increase of $R_{int}(0)$ (PD_{50} , PD_{65} , PD_{75} , and PD_{100}).

Fitting model. Starting at $R_{int}(0)$, the data were calculated as percent change from the postsaline value of R_{int} against the cumulative dose of carbachol (Fig. 1). From previous experiences, the best fit of the dose-response curve was found by a 4th power function using the more general function derived from Charpin's equation (32): $y = \alpha + \beta x + \gamma x^n$ ($\alpha, \beta, \gamma > 0$), where the exponent n was assigned values of 2, 4, and 6. In this function, α reflects the constant as intercept, βx the initial linear part, and γx^n the curvilinear part of the dose-response curve. The average coefficient of determination r^2 for the 4th power function showed a slightly although insignificantly better fit (0.85 ± 0.11) compared with the 6th power function (0.82 ± 0.21). In asthmatic children having high BR, the exponential part of the dose-response curve must subsequently be weighted in the choice of analytic treatment, whereas in healthy children showing flat, less curvilinear dose-response curves, a more linear fit would be preferable. Using this procedure, the exact interpolation for any measured increase of R_{int} could be calculated until a dose of 1200 μ g of carbachol as the highest dose was administered. According to the increase of R_{int} to 150, 165, 175, and 200% of $R_{int}(0)$, the provocation doses PD_{50} , PD_{65} , PD_{75} , and PD_{100} were calculated.

Alternative PD calculation. Having these empirically defined thresholds, not really linked to a biologic phenomenon, and

Fig. 1. Schematic representation of dose-response curves after carbachol challenge in an asthmatic and a healthy child. Dotted areas, range of single values at corresponding level.

based on the statistical consideration that a bronchial challenge test has been considered positive when R_{int} significantly increases in relation to $R_{int}(0)$, the so-called "variance-based" provocation dose (PD_{vb}) was defined. PD_{vb} represents the threshold where R_{int} is significantly higher than the individual mean $+2$ SD of a particular subject at baseline. The validity of these computed provocation thresholds was double-checked by a two-tailed t test looking at the significance of the distribution of R_{int} measurements on the different provocation levels in relation to the variation of $R_{int}(0)$ data before the challenge test (Fig. 1). By double-checking the provocation thresholds achieved stepwise using a t test, the changing variance and thus distribution of data points of each provocation level with respect to that at baseline are taken into account.

Statistical methods. Data were analyzed on a Macintosh computer with the statistical package SYSTAT 5.2. Relationships were explored using the χ^2 analysis of contingency tables, the Kruskal Wallis test for nonparametric variables, and multiple linear regression analysis. s is defined as the capacity of a test to positively identify those persons with the disease, and f is defined as the capacity of a test to exclude as healthy those subjects who do not have the disease. Therefore, s was calculated as the ratio of the true-positive to the sum of the true-positive plus false-negatives for the asthmatic population and f as the ratio of the true-negatives and the sum of the true-negatives plus false-positives in the healthy population.

RESULTS

Age, sex, and $R_{int}(0)$ of the 20 healthy and 20 asthmatic children are summarized in Table 1. Baseline values of lung function measured in the asthmatic children before the challenge tests are given in Table 2. Both collectives of randomly selected individuals reflect weighed means of corresponding populations. Seven asthmatic children showed lung function within the range of normal, whereas three presented with pulmonary hyperinflation ($TGV > 130\%$ of predicted value), 10 with bronchial obstruction of more central airways ($R_{aw} > 130\%$ of predicted value), and nine with obstruction of more peripheral airways ($MEF_{50} < 80\%$ of predicted value), seven of them showing combined functional abnormalities. In addition, the individual values for PD_{vb} and PD_{50} – PD_{100} are given (Table 1). Whereas for PD_{vb} all doses fell within the range of measured values (to $1200 \mu\text{g}$ of carbachol) in healthy and asthmatic subjects, an increasing number of subjects (two of 20 to 12 of 20 depending on threshold and/or collective concerned) showed values higher than $1200 \mu\text{g}$ of carbachol and thus would require extrapolation data.

In Figure 2, the five thresholds PD_{vb} , PD_{50} , PD_{65} , PD_{75} , and PD_{100} are given. All PD calculated on the basis of the 4th power model showed wide variations, especially in healthy children. Therefore, in comparing data distribution according to Kruskal Wallis, no systematic difference between healthy and asthmatic children could be found. In contrast, PD_{vb} , having for both healthy and asthmatic children a much lower mean than PD_{50} ($656.8 \pm 292.7 \mu\text{g}$ of carbachol for healthy children; $376.5 \pm 265.2 \mu\text{g}$ of carbachol in asthmatics), showed less variation and therefore differentiates significantly between health and disease ($p < 0.002$). Table 3 shows for each of the four computed PD (PD_{50} , PD_{65} , PD_{75} , and PD_{100}) the proportion of threshold values that were higher than the mean $R_{int}(0) + 2$ SD; this was taken as a measure of the accuracy of the PD. Although in asthmatics 100% accuracy can be achieved for PD_{65} , PD_{75} , and PD_{100} , in healthy subjects even the PD_{100} reached only 95% accuracy. By definition, the accuracy of PD_{vb} is 100%. The discrepancy between both methods is obviously caused by the fact that the method of assessing PD_{vb} takes full account of the baseline variability. In contrast, the other PD (PD_{50} , PD_{65} , PD_{75} , and PD_{100}) can give spurious values when variability is proportionally large, an event most likely to occur if $R_{int}(0)$ is low, or when very

hyperreactive subjects (steep exponential dose-response curves) are tested.

In Figure 3, f and s for all five PD are synoptically presented. It can be seen that f and s for PD_{vb} both are the most left positioned (lower dose levels). In addition, the 95% true-negative level for the f of PD_{vb} is reached already at $300 \mu\text{g}$ of carbachol and the 95% true-positive level of s of PD_{vb} is up to $780 \mu\text{g}$ of carbachol, whereas these criteria for PD_{50} to PD_{100} are at much higher provocation doses. Therefore, f and s for PD_{vb} are substantially better than for PD_{50} , PD_{65} , PD_{75} , and PD_{100} . One analytic mathematical approach to establish suitability of a test is to depict the crossing point of the declining f curve with the increasing s curve of cumulative percent response (Fig. 3, middle). It can be shown that the crossing point for the PD_{vb} is already reached at $480 \mu\text{g}$ of carbachol giving an equalized f and s of 70%. Therefore, PD_{vb} features a mode to determine BR with improved f and s at the lowest provocation dose levels.

DISCUSSION

The objectives of the present study were first to search for a convenient fitting model for dose-response curves of inhalative bronchial challenge tests to obtain accurate provocation thresholds in children. Second, this study intended to identify the best predictable target PD within a lower and upper limit of the dose scale. The former is given by the statistical argument that the target PD must definitively be out of the measurement error of baseline values, and the latter is given by the practical aim to minimize induced airway obstruction. This allows comparison of how well the different provocation thresholds differentiate between health and disease. The question was asked whether an alternative simple calculation of a significant PD would not result in a test procedure that is free from a complex mathematical model, can be applied on-line, and shortens the duration of the test. In this context, the PD_{vb} was elaborated. It is defined as the dose corresponding to the first significant increase of flow resistance in relation to the variability of baseline resistance.

It was not the aim of this study to find a mathematical expression that fits all the data points perfectly, or to predict whether the curves are exponential or sigmoid in shape, but to find a mathematical expression that would allow simple computation of different provocation thresholds. Another purpose was to explore differences in constants between normal and asthmatic children; therefore, the mathematical model was chosen for both subject groups. In healthy children, it seems likely that the data points describe a sigmoid function. In asthmatic children, this depends on whether the test procedure was discontinued because of a dramatic exponential increase in airflow resistance. The constants obtained in a previous study (33, 34) have not shown any significance in clinical terms, either for β , indicating the position of the curve, or for γ as index of the slope of the dose-response curve.

The validity of our computed curve fits was analyzed by looking at the significance of the distribution of R_{int} measurements for the different provocation levels in relation to the variation of $R_{int}(0)$ data before the challenge test. As defined in the present study, PD_{vb} is the provocative dose at which respiratory resistance is statistically significantly increased, i.e. outside the range of baseline values (mean ± 2 SD). It is of interest to have the test standardized to achieve a provocation level that is sufficiently high [out of the 2 SD range of variability of $R_{int}(0)$], but not so high that the patient feels discomfort. Apart from biologic considerations, statistical arguments have an important role to play in establishing a provocation threshold. Especially in children, lung function parameters vary considerably, and with respect to the dose-response curve during bronchial provocation, it must be assumed that variation predominantly in the exponential part of the curve is substantial. By that it can be explained that accuracy, s , and f are better for PD_{vb} . While calculating PD_{vb} , a direct comparison between the range of individual single

Table 1. Age and sex distribution, $R_{int}(0)$, and computed PD obtained in 20 healthy and 20 asthmatic children*

Subject no.	Age (y)	Sex	$R_{int}(0)$ [kPa/(L/s)]	PD _{vb}	PD ₅₀	PD ₆₅	PD ₇₅	PD ₁₀₀
Healthy children								
1	8	M	0.54	450	533	564	581	619
2	8	M	0.42	1065	883	996	1058	1183
3	9	F	0.58	1060	1174	>1200	>1200	>1200
4	9	F	0.47	1200	>1200	>1200	>1200	>1200
5	10	M	0.42	430	452	497	523	574
6	10	M	0.41	430	617	732	795	924
7	10	M	0.35	685	618	654	675	720
8	10	M	0.39	810	1019	1084	1121	>1200
9	12	M	0.32	560	1000	1172	>1200	>1200
10	12	M	0.48	1060	1194	>1200	>1200	>1200
11	13	F	0.29	810	631	675	700	754
12	13	F	0.45	430	930	1047	1110	>1200
13	13	M	0.29	560	1051	1190	>1200	>1200
14	14	F	0.38	310	456	504	531	585
15	14	M	0.20	290	493	641	739	986
16	15	M	0.30	560	513	569	600	664
17	15	M	0.31	430	960	>1200	>1200	>1200
18	15	M	0.45	1065	>1200	>1200	>1200	>1200
19	15	F	0.23	570	>1200	>1200	>1200	>1200
20	16	F	0.29	360	673	857	972	>1200
Asthmatic children								
1	5	F	0.45	320	302	321	332	356
2	6	F	0.45	60	207	270	311	415
3	6	F	0.65	310	525	601	642	725
4	7	F	0.45	60	732	951	1098	>1200
5	7	M	0.60	370	1004	1112	1171	>1200
6	7	F	0.92	60	1034	1107	1149	>1200
7	8	F	0.62	560	1036	1124	1175	>1200
8	8	M	0.54	60	216	281	324	432
9	8	M	0.75	180	1051	1153	>1200	>1200
10	8	F	0.52	310	1098	1173	>1200	>1200
11	9	F	0.34	560	1093	1181	>1200	>1200
12	9	F	0.50	810	>1200	>1200	>1200	>1200
13	9	F	0.25	310	>1200	>1200	>1200	>1200
14	10	M	0.39	240	298	315	326	348
15	10	M	0.57	790	1069	1157	>1200	>1200
16	11	M	0.43	560	1049	1124	1166	>1200
17	11	M	0.43	430	560	686	759	908
18	14	M	0.34	310	273	314	337	382
19	14	M	0.49	240	277	318	341	386
20	15	M	0.46	990	1037	1132	1167	>1200

* PD expressed in μg of carbachol. PD >1200 means data points were outside the range of measured R_{int} .

measurements on each consecutive dose level with the range of initially measured baseline values is computed by t test statistics. This approach is independent from any assumptions about the underlying shape of the dose-response curve and therefore generally applicable. By taking PD_{vb} as target PD, better s and f are achieved, even at lower dose of the challenging agent. Another desirable feature of any analytical procedure is that it helps limit the test time to a minimum while producing accurate results. We would speculate that, based on the data obtained by the new calculation of PD_{vb}, discrimination between healthy and asthmatic children could be improved.

Limits of methods. The interruption technique to measure R_{int} as an estimate of respiratory resistance has not been widely used, partly because the equipment is expensive and partly because of theoretical reservations regarding the validity of the method. Recent theoretical analyses by Bates *et al.* (35, 36) suggest that the method is essentially valid, although upper airway compliance may cause R_{int} to underestimate the true value of R_{aw} . Upper airway compliance can be minimized by supporting the cheeks and pharynx. Experimental work in dogs by Bates *et al.* (37) shows that in open-chested dogs R_{int} closely approximates R_{aw} even in the presence of parallel heterogeneity. In closed-

chested dogs, R_{int} is strongly correlated with airway resistance even in the presence of bronchoconstriction but includes a contribution from the chest wall and therefore tends to exceed R_{aw} (38, 39). More recently, close correlation was found comparing R_{int} and R_{aw} in 43 adult patients (40). Changes in R_{int} after use of a bronchodilator were similar to those in R_{aw} , and the comparison of the average percentage change was within 2%. Acceptability was also checked in 10 3-y-old asthmatics showing an increase of R_{int} after methacholine and a decrease after salbutamol (39). In view of this recent theoretical and practical work and the advantages of the method, this technique provides an instantaneous, breath by breath measurement of respiratory resistance that can be successfully used in a pediatric population.

The form of the algorithm used to extrapolate mouth pressure to obtain R_{int} requires further evaluation. Bates *et al.* have suggested a more sophisticated exponential procedure (36). In cases where pulmonary hyperinflation is present and thus the site of resistance lies upstream (on the alveolar side) of a compliant compartment, true airway resistance is underestimated (31). Therefore, we already proposed to apply an entire resistance-capacitance-inertance model to obtain a more reliable estimation of R_{int} (31).

Table 2. Lung function data of 20 asthmatic children, measured before carbachol challenge test*

Subject	TLC	TGV	RV	R _{aw}	FEV ₁	MEF ₅₀
1	103	106	129	62	74	64
2	101	106	70	118	91	64
3	97	90	100	184	85	67
4	92	144	78	64	122	116
5	113	109	145	135	104	106
6	80	85	94	149	75	75
7	101	77	56	122	91	132
8	102	85	57	103	98	83
9	118	131	137	150	84	70
10	89	98	122	142	78	71
11	103	74	87	125	114	98
12	87	91	61	112	107	106
13	88	74	75	67	93	113
14	124	137	148	141	89	72
15	92	100	102	133	80	100
16	88	81	68	171	90	73
17	87	80	103	107	93	106
18	94	125	73	128	92	85
19	82	83	97	172	79	77
20	95	81	88	148	100	110

* TLC, total lung capacity; RV, residual volume. Data are % of predicted values (25).

Fig. 2. Means and SD of PD obtained in 20 healthy and 20 asthmatic children.

Table 3. Accuracy of PD computed according to nonlinear model from complete dose response curves of 20 healthy and 20 asthmatic children tested by carbachol challenge test*

Thresholds from 4th power function	PD ₅₀	PD ₆₅	PD ₇₅	PD ₁₀₀
Healthy subjects (n = 20)				
PD(x) > R _{int} (0) + 2 SD (above baseline range)	16	17	17	19
PD(x) < R _{int} (0) + 2 SD (within baseline range)	4	3	3	1
Subjects accurately measured (%)	80	85	85	95
Asthmatic children (n = 20)				
PD(x) > R _{int} (0) + 2 SD (above baseline range)	18	20	20	20
PD(x) < R _{int} (0) + 2 SD (within baseline range)	2	0	0	0
Patients accurately measured (%)	90	100	100	100

* PD(x) = either PD₅₀, PD₆₅, PD₇₅, or PD₁₀₀.

There is increasing concern that the end-expiratory resting level may change during the provocation procedure. In consequence, the dose-response curve of a bronchial challenge representing changes in airway mechanics may be significantly influ-

enced by changes in volume history. Because of the difficult technical approach, we have not been able to verify this point in the present study. However, in another study we have been able to demonstrate the influence of the kind of functional abnormality (pulmonary hyperinflation, bronchial obstruction, or mixed type) as a confounding factor for the detection of bronchial hyperreactivity in asthmatic children (33). In addition, potential changes in end-expiratory resting level during a bronchial challenge may influence the exact detection of the degree of bronchial obstruction.

Reproducibility and ethical considerations. Carbachol is highly cumulative in its action. The pharmacologic property precludes repeating the test at short intervals, so that the reproducibility of the PD_{vb} can be established in stable asthmatics in whom measurements are repeated on different days; this did not form part of the protocol and needs to be established in future studies. In view of the cumulative effects, we also recommend that the test be terminated by administration of a β -2-agonist, so that respiratory resistance is restored at a lower level. It follows that, for a repetition of a bronchial challenge, not only the previous challenging agent but in parallel also the interaction of the β -2-stimulator have to be considered. Each second test will unavoidably be influenced by the preceding test. When the second test is postponed until the next day, the patient, especially the asthmatic child, will naturally be in a different condition with respect to lung function. Therefore, it is extremely difficult to achieve identical baseline conditions. In consequence, it was deemed unethical to perform such repeated tests in children. Moreover, it is of utmost interest and of an urgent need to have clear recommendations for such research in pediatrics.

Practical aspects: alternative techniques. In an older age range of children, measurements using forced breathing maneuvers and taking the FEV₁ as a target parameter to estimate the test-induced bronchial obstruction can be considered the gold standard. It was not a formed part of the present study and because of the age range of the study subjects (5–15 y) it was not possible to compare the interruption technique with the technique using forced breathing maneuvers. However, such comparison was made in another group of older asthmatic children comparing R_{int} with FEV₁ and MEF₅₀ at different provocation levels (unpublished data). In only 56% did R_{int} behave similarly to FEV₁. The correlation largely depends on whether asthmatic children with pulmonary hyperinflation, bronchial obstruction, or both were investigated. The only other technique that does not involve a high degree of active cooperation is the forced oscillation technique (41, 42). However, this technique involves the patient maintaining a patent upper airway for several seconds. This was particularly difficult for young children. R_{int} is measured during spontaneous airflow when momentary closure of the glottis does not occur. The advantages of calculating PD_{vb} and its application to assessing BR are safety and accuracy. For practical purposes, there is no need to have a sophisticated curve-fitting model, which is time-consuming and does not reflect a biologic model applicable to both patients and healthy subjects. In addition, extrapolation must be avoided. For the present study, the number of PD that has to be judged "inaccurately assessed" can be obtained from Table 1 and Figure 2 (PD_x > 1200 μ g of carbachol). The new technique features the additional advantage that the test procedure can be consistently shortened in time, because a much lower dose needs to be administered for even better *f* and *s*. This latter point is also an argument for safety. We do not yet have sufficient experience with applying this technique to children aged 1 to 5 y, and so far, comparison with the technique previously worked out by Godfrey and coworkers (43–45) cannot be made. By this latter technique, lung sounds are recorded at the tracheal level for 1 min after nebulization of a bronchoconstricting drug and the concentration causing "wheeze" is taken as the target parameter. The validity of our test procedure and calculation of PD_{vb} will be evaluated in much larger collectives, using the present algorithm for measurements of respiratory system resistance.

Fig. 3. Cumulative percent decline of f and cumulative percent increase of s for PD levels, obtained by bronchial challenge tests with carbachol in 20 healthy and 20 asthmatic children.

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Announcement

Paediatric Week Holland

The Paediatric Week Holland will be organized in Rotterdam, The Netherlands from June 29 to July 6, 1994. From July 3 through July 6, a combined meeting will be held of the European Society for Paediatric Research, the European Paediatric Respiratory Society, the European Respiratory Society-Paediatric Assembly, the European Society of Paediatric Allergy/Clinical Immunology, the European Society for Paediatric Infectious Diseases, the European Society of Paediatric Intensive Care, and the European Society for Paediatric Hematology and Immunology. From June 29 through July 2, the British Association of Paediatric Surgeons will meet, and the Dutch Society of Psychosocial Consultation and Paediatrics will meet from July 3 through July 5. Postgraduate courses will be organized in the overlapping weekend. *For further information, please contact:* Pieter J. J. Sauer, M.D., % Erasmus University Rotterdam, P.O. Box 1738, NL-3000 DR Rotterdam, The Netherlands.