

Measurement of nasal mucociliary clearance

G M CORBO,* A FORESI,* P BONFITTO,† A MUGNANO,* N AGABITI,† AND P J COLE‡

*Centro Auxologico Italiano, Divisione di Pneumologia Pediatrica, Milan, †Istituto di Clinica Medica, Servizio di Fisiopatologia Respiratoria, Università Cattolica del S Cuore, Rome, Italy, and ‡Host Defence Unit, Cardiothoracic Institute, Brompton Hospital, London

SUMMARY The saccharin test was carried out in a randomly selected sample of schoolchildren (142 boys and 153 girls, age range 11-14 years) to determine the variability and reproducibility of the test and to assess whether it could be used as a screening test for nasal mucociliary clearance. Nasal mucociliary clearance times were analysed according to clinical history (asthma, rhinitis, asthma with rhinitis, and acute upper respiratory tract infections), laboratory findings (positive skin test responses and bronchial hyper-reactivity assessed by methacholine challenge), and parental smoking. Nasal mucociliary clearance times showed a narrow coefficient of repeatability (six minutes) in 50 subjects and there was substantial agreement between the two tests. Nasal mucociliary clearance times were less than 40 minutes in all the children. Normal children had nasal mucociliary clearance times of less than 24 minutes while significantly impaired nasal mucociliary clearance was detected in those with positive skin reactions and a positive response to methacholine challenge. We were unable to show that passive smoking had any consistent effect on nasal mucociliary clearance.

We suggest that a time response between 30-60 minutes should be checked again at least two weeks later, and that children in whom repeated saccharin tests show a nasal mucociliary clearance of greater than 30 minutes should have ciliary beat frequency estimated.

Mucociliary clearance is a key defence mechanism in human upper and lower airways, and its impairment, both acquired¹ and genetically determined,²⁻³ predisposes to chronic infection of the nose, paranasal sinuses, and respiratory tree. The age of onset of clinical symptoms is usually early but diagnosis is often late, because symptoms are not specific (for example, cough and sputum) and techniques for measurement of mucociliary clearance are time consuming and too expensive for routine screening. There is, however, evidence that mucociliary clearance occurs in the trachea and main bronchi at a similar rate as in the nose and so several simple methods have been developed to measure the nasal mucociliary clearance.⁴

The saccharin test is inexpensive and simple to perform, and its results are similar to those obtained using a radioactively labelled particle.⁵ Recently it has been proposed as a screening test to detect abnormal mucociliary clearance.⁶ We have used the test to measure nasal mucociliary clearance in a random sample of schoolchildren. The purpose of the present study was to establish the normal range of results and reproducibility of the test in children,

and to determine whether acute upper respiratory tract infection (common cold), allergic diseases such as bronchial asthma and rhinitis, clinical bronchial hyper-reactivity and atopy, and exposure to passive smoking, prolonged the nasal mucociliary clearance.

Subjects and methods

SUBJECTS

The study was conducted in Verbania in northern Italy. Two hundred and ninety five schoolchildren, 142 boys and 153 girls, age range 11-14 years, were studied. The group was a 20% sample stratified for age and sex and randomly selected from secondary school records.

Information about respiratory diseases such as asthma and rhinitis was obtained from a questionnaire that was completed by the parents. The smoking habits of mothers and fathers were also recorded. The questionnaire incorporated the American Thoracic Society children's questionnaire⁹ expanded to include more detailed questions on allergic diseases. A diary card to complete daily was

given to the parents two weeks before the examination, and children who had a common cold during the seven days before the test were defined as having an acute upper respiratory tract infection.

SACCHARIN TEST

The saccharin test was carried out by the method first described by Andersen *et al*⁷ and modified by Rutland and Cole.⁸ A particle 1 mm in diameter was placed under direct vision on the inferior nasal turbinate 1 cm from its anterior end. The child was instructed not to sniff, sneeze, or cough during the test and to report a taste as soon as it was noted. Subjects who had severe watery rhinorrhoea were excluded. The time to the initial perception of a sweet taste was recorded in minutes. If no taste was apparent after 60 minutes the test was stopped and the ability of the subject to taste saccharin placed directly on the tongue was verified. In 50 children (25 boys and 25 girls) the test was repeated in the same nostril the following day to assess its reproducibility.

ATOPIC STATE AND BRONCHIAL RESPONSIVENESS

Atopic state was assessed by performing skin tests using five commonly inhaled allergens (grass, mugwort, birch, and parietaria, and house dust mite). The details of the skin test technique and the allergen extracts have previously been published.¹⁰ Wheal reactions were measured by a planimeter and a wheal area of 3 mm² or greater was recorded as a positive response. Bronchial responsiveness was evaluated by the methacholine test using a standard method.¹¹ Twelve concentrations (from 0.03 mg/ml up to 64 mg/ml) were given through a DeVilbiss 646 nebuliser attached to a dosimeter (Mefar). A reduction in forced expiratory volume in one second (FEV₁) of 20% or more was selected to categorise subjects into responders (in whom the provocative concentration of methacholine needed to reduce the FEV₁ by 20% was less than 64 mg/ml), and non-responders (in whom a dose of 64 mg/ml of methacholine failed to reduce the FEV₁ by 20%).

STATISTICAL ANALYSIS

Having assessed data from the questionnaires, diary cards, skin test reactions, and the bronchial response to methacholine we divided the sample into eight groups: patients with asthma alone; patients with rhinitis alone; those with asthma and rhinitis; those who had an acute upper respiratory tract infection within the last week; those who had positive reactions to skin tests alone; those who had a positive response to the methacholine challenge alone; those who had positive reactions to both the skin tests and the methacholine challenge; and normal subjects.

Comparisons were made between each of the first seven groups and the normal group separately using the Mann-Whitney U test. Lastly, to assess the effect of parental smoking, the children were grouped by the number of parents who currently smoked. Children whose parents had never smoked were compared separately with those who had one parent or both parents who were currently smokers, also using the Mann-Whitney U test.

Reproducibility was assessed by calculating the difference in nasal mucociliary clearance time between the tests on successive days in each subject and then the mean and standard deviation (SD) of the differences in the overall sample. The 95% coefficient of reproducibility is given as two SD.¹² The interclass correlation coefficient (Ri) was also computed.¹³

Results

In 36 subjects the test was not carried out either because of rhinorrhoea (n=12) or because the child was unwilling (n=24). The median nasal mucociliary clearance as measured by the saccharin test in the remaining 259 children was eight minutes (range 1–40) (fig 1). The difference between the measurements on successive days with respect to the mean in each subject is shown in fig 2. The mean value of the differences (–0.26) was not significantly different from zero.

The computed coefficient of reproducibility was

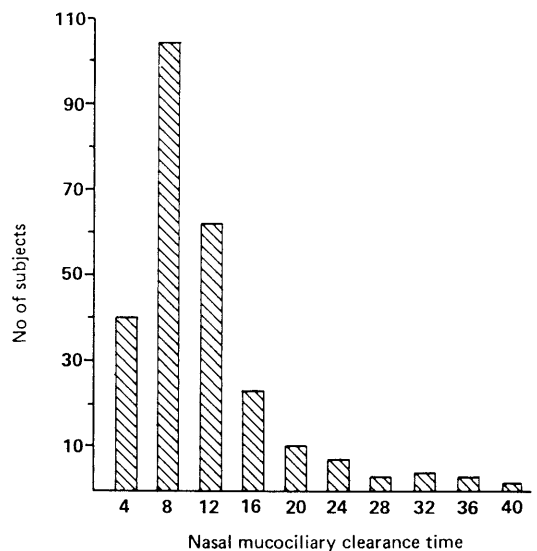


Fig 1 Nasal mucociliary clearance time measured by the saccharin test in 259 children.

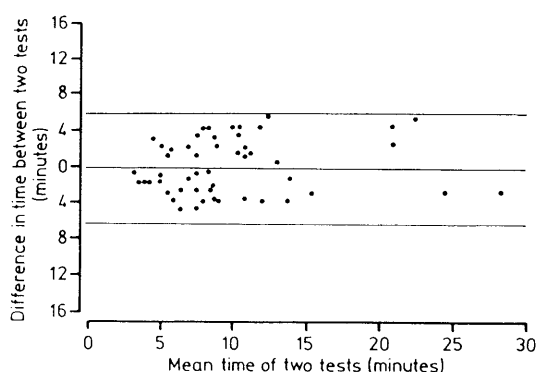


Fig 2 Mean nasal mucociliary clearance time of two tests plotted against the difference in time between the two tests. Horizontal lines indicate mean and two standard deviations above and below.

six minutes (2SD).¹³ The agreement between the two tests was substantial ($R_i=0.80$) according to the guidelines of Landis and Kock.¹⁴ When the data from the questionnaire were analysed six children were reported to have asthma without rhinitis, 25 had rhinitis alone, and 10 had both asthma and rhinitis. Forty children had positive reactions to skin tests and did not respond to the methacholine challenge. A reduction in FEV_1 of more than 20% after the methacholine challenge was detected in 20 subjects who did not react to the skin tests, and both positive reactions to skin tests and bronchial responses to the methacholine challenge were found in 20 children (table).

Normal subjects had nasal mucociliary clearance times of less than 24 minutes, while longer times were detected in some subjects in other groups. There were no significant differences between normal children compared with children with asthma, children with rhinitis, children with both asthma and

rhinitis, those with positive skin test reactions, those who responded to the methacholine challenge or children with acute upper respiratory infections. Children with colds had longer nasal mucociliary clearance times than normal children though the difference was not significant ($p=0.09$). A significant reduction in nasal mucociliary clearance was, however, found in those with positive reactions to skin tests and a positive response to the methacholine challenge ($z=2.404$, $p=0.01469$).

Ninety eight subjects (38%) had one parent currently smoking, 51 (20%) had both parents currently smoking, and 110 (47%) had parents who had never smoked. There was no significant difference between children whose parents never smoked and children whose parents currently smoked.

Discussion

The results of our study confirm those of Stanley *et al*⁶ that the saccharin test is an useful screening technique for measuring nasal mucociliary clearance as it is inexpensive, simple to do, and reproducible, and we have extended their results in adults to children. The nasal mucociliary clearance times found in normal children did not differ from those found by Andersen and Proctor⁴ who considered that any time in excess of 30 minutes was abnormal; we did not find any result in excess of 24 minutes.

The test showed a rather narrow coefficient of reproducibility (six minutes, figure) and the mean value of the differences (-0.26) was not significantly different from zero, which means that the first measurement is not affecting the second and the difference does not vary in any systematic way over the range of measurements. The agreement between the two tests was substantial ($R_i=0.80$).

Nasal mucociliary clearance depends on two principal components, the physiochemical qualities and quantity of the mucus, and the properties of the

Table Nasal mucociliary clearance times in each group measured by the saccharin test

Clinical group	Total No	Median (range) clearance time (minutes)
Normal subjects	98	8 (1-24)
Patients with asthma alone	6	9 (3-36)
Patients with rhinitis alone	25	8 (2-36)
Patients with asthma and rhinitis	10	9 (3-32)
Patients with upper respiratory tract infections	40	9 (1-40)
Patients with positive skin responses alone	40	8 (2-35)
Patients with positive response to methacholine challenge alone	20	7 (2-36)
Patients with positive skin responses and positive response to methacholine challenge	20	10 (3-40)

cilia that propel it (for example, beat frequency and coordination); these are affected by disease. We did, however, find a significantly impaired nasal mucociliary clearance only in children with both positive skin reactions, and a positive response to the methacholine challenge. At present data on mucociliary clearance in allergic diseases are controversial.^{15 16} Abnormal mucociliary clearance was seen in patients with bronchial asthma,¹⁷ and abnormal cilia were found in subjects with perennial rhinitis.¹⁸ Impairment of mucociliary clearance in patients with asthma seems to be the result of qualitative^{19 20} and quantitative²¹ alterations in respiratory secretions rather than differences in ciliary beating.²² As these changes are transient, the saccharin test may fail to show impairment of nasal mucociliary clearance depending on the time that has elapsed since the exposure to the allergen. We found a prolonged nasal mucociliary clearance time only in subjects with both skin reactivity to allergen extracts and increased bronchial response to methacholine.

Acute upper respiratory tract infections may reduce the nasal mucociliary clearance by direct damage to the cilia and change in the rheological properties of the nasal secretions.²³⁻²⁵

Tobacco smoke has a ciliostatic effect²⁶ and changes the viscoelastic properties of mucus.^{27 28} Recently nasal mucociliary clearance time in smokers has been found to be longer than in non-smokers.²⁹ In the same study the route of exhalation was shown to be crucial, as smokers who regularly exhaled through their noses had a longer mean nasal mucociliary clearance time than smokers who did not regularly exhale by this route. We have not shown any consistent effect of passive smoking on nasal mucociliary clearance, however, probably because the effect is cumulative,²⁹ and depends on the degree and the duration of exposure (that is, the current amount of parental smoking of cigarettes, number of parental smokers during the child's lifetime, and the duration of parental smoking during child's lifetime).

In conclusion, we have shown that the saccharin test can be used on a large sample to study nasal mucociliary clearance in childhood because it is simple to carry out and the results have a good coefficient of repeatability. We have confirmed that 30 minutes is the cut off point that discriminates normal subjects from subjects with impaired nasal mucociliary clearance. Patients with positive responses to skin tests and increased bronchial reactivity showed a significantly prolonged nasal mucociliary clearance time. We failed to show any differences between the normal group and the other groups, because the range in the results in the

various groups was so wide that it was difficult to pick up small differences.

Nevertheless, a time response of more than 30 minutes should be checked again at least two weeks later in case it is due to an acute upper respiratory infection. Lastly, children who have repeated saccharin tests in which they take longer than 30 minutes to respond when they do not have an acute infection need further investigation.

We thank I Annesi and F Kauffmann for their comments and suggestions, and M Lebowitz for his advice.

References

- 1 Afzelius BA, Camner P, Mossberg B. Acquired ciliary defects compared to those seen in the immotile-cilia syndrome. *Eur J Respir Dis* 1983;**64**(suppl 127):5-10.
- 2 Sleigh MA. Primary ciliary dyskinesia. *Lancet* 1981;ii:476.
- 3 Eliasson R, Mossberg B, Camner P, Afzelius BA. The immotile cilia syndrome. A congenital abnormality as an etiologic factor in chronic airway infections and male sterility. *N Engl J Med* 1977;**297**:1-7.
- 4 Andersen I, Proctor DF. Measurement of nasal mucociliary clearance. *Eur J Respir Dis* 1983;**64** (suppl 127):37-40.
- 5 Andersen IB, Camner P, Jensen PL, Philipson K, Proctor DF. Nasal clearance in monozygotic twins. *Am Rev Respir Dis* 1974;**110**:301-5.
- 6 Stanley P, MacWilliam L, Greenstone M, Mackay I, Cole P. Efficacy of a saccharin test for screening to detect abnormal mucociliary clearance. *Br J Dis Chest* 1984;**78**:62-5.
- 7 Andersen I, Camner P, Jensen PL, Philipson K, Proctor DF. A comparison nasal and tracheobronchial clearance. *Arch Environ Health* 1974;**29**:290-3.
- 8 Rutland J, Cole PJ. Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis. *Thorax* 1981;**36**:654-8.
- 9 Ferris BG. Epidemiology standardization project: children's questionnaire. *Am Rev Respir Dis* 1978;**118**(suppl):36-53.
- 10 Corbo GM, Foresi A, Verga AE, Mattoli S, Polidori G, Ciappi G. Allergy indices based on allergen dose-response curve in a randomly selected sample of schoolchildren. *Allergy* 1987;**42**:230-7.
- 11 Ryan G, Dolovich MB, Roberts RS, et al. Standardization of inhalation provocation tests: two techniques of aerosol generation and inhalation compared. *Am Rev Respir Dis* 1981;**123**:195-9.
- 12 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-10.
- 13 Kramer MS, Feinstein AR. Clinical biostatistics. LIV. The biostatistics of concordance. *Clin Pharmacol Ther* 1981;**29**:111-23.
- 14 Landis RJ, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;**33**:159-74.
- 15 Wanner A. Allergic mucociliary dysfunction. *J Allergy Clin Immunol* 1983;**72**:347-50.
- 16 Wasserman SJ. Ciliary function and disease. *J Allergy Clin Immunol* 1984;**73**:17-19.
- 17 Mezey RJ, Cohn MA, Fernandez EJ, Januskiewicz AJ, Wanner A. Mucociliary transport in allergic patients with antigen-induced bronchospasm. *Am Rev Respir Dis* 1978;**118**:677-84.
- 18 Wihl JA, Mygind N. Studies on the allergen-challenged human nasal mucosa. *Acta Otolaryngol* 1977;**84**:281-6.
- 19 Dulfano MJ, Luk CK. Sputum and ciliary inhibition in asthma. *Thorax* 1982;**37**:646-51.
- 20 Shelhamer JH, Marom Z, Kaliner M. Immunologic and

- neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *J Clin Invest* 1980;**66**:1400-8.
- ²¹ Yamatake Y, Sasagawa S, Yanaura S, Kobayashi N. Allergy induced asthma with *Ascaris suum* administration to dogs. *Jpn J Pharmacol* 1977;**27**:285-93.
- ²² Greenstone M, Cooper P, Warner J, Cole PJ. Effect of acute antigenic challenge on nasal ciliary beat frequency. *Eur J Respir Dis* 1983;**64**(suppl 128):449-50.
- ²³ Sakakura Y. Changes of mucociliary function during colds. *Eur J Respir Dis* 1983;**64**(suppl 128):348-54.
- ²⁴ Carson JL, Collier AM, Hu SS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. *N Engl J Med* 1985;**312**:463-8.
- ²⁵ Wilson R, Alton E, Rutman A, *et al*. Upper respiratory tract viral infection and mucociliary clearance. *Eur J Respir Dis* 1987;**70**:272-9.
- ²⁶ Kaminski EJ, Fancher OE, Calandra JC. In vivo studies of the ciliastatic effects of tobacco smoke. Absorption of ciliastatic components by wet surfaces. *Arch Environ Health* 1968;**16**:188-93.
- ²⁷ Jeffery PK, Reid LM. The effect of tobacco smoke, with and without phenylmethyloxadiazole (PMO), on rat bronchial epithelium: a light and electron microscopic study. *J Pathol* 1981;**133**:341-59.
- ²⁸ Kollerstrom N, Lord PW, Whimster WF. A difference in composition of bronchial mucus between smokers and non-smokers. *Thorax* 1977;**32**:155-9.
- ²⁹ Stanley PJ, Wilson R, Greenstone MA, MacWilliam L, Cole PJ. Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. *Thorax* 1986;**41**:519-23.

Correspondence and requests for reprints to Dr G M Corbo, Divisione di Pneumologia Pediatrica, Centro Auxologico Italiano, 28044 Verbania-Intra(No), Italy.

Accepted 11 October 1988