Eur Respir J 1999; 14: 328–334
Printed in UK – all rights reserved


European Respiratory Journal
ISSN 0903-1936

# Quantification of the dose of inhaled flour: relation with nonspecific bronchial and immunological reactivities

D. Choudat*, J.F. Fabriès**, J.C. Martin*, C. Villette+, F. Tabka*, J.F. Dessanges+, J. Dall Ava+, F. Conso*

*Quantification of the dose of inhaled flour: relation with nonspecific bronchial and immunological reactivities. D. Choudat, J.F. Fabriès, J.C. Martin, C. Villette, F. Tabka, J.F. Dessanges, J. Dall Ava, F. Conso. ©ERS Journals Ltd 1999.*

**ABSTRACT: The aim of this study was to investigate the relationship between specific bronchial reactivity and respective nonspecific bronchial and immunological reactivities.**

**Twenty-one patients underwent bronchial challenges with lactose and flour. The aerosol of particles was generated by a computer-controlled aerosolizer. Specific bronchial challenge results were expressed as the provocative dose of flour (PDf) that caused a 20% or 15% decrease in the forced expiratory volume in one second ($FEV_1$). For each subject, the decrease in $FEV_1$ observed during the challenge with flour was compared with the calculated lower limit of the 99.7% confidence interval for the lactose challenge. The subjects also underwent a nonspecific challenge with methacholine and a measurement of the specific immunoglobulin E against wheat.**

**The inhalation of lactose did not significantly affect $FEV_1$. Nine subjects had high reactivity to wheat flour with a $PDf_{20}$ <400 µg. Five subjects had intermediate reactivity: $FEV_1$ fell by <20% but by significantly more than that in the test with lactose. For 7 subjects, there was no significant change in $FEV_1$ for inhaled doses of flour over 1390 µg. The results for specific bronchial challenge were significantly correlated with those for the methacholine test ($p<0.02$). Positive skin tests and specific immunoglobulin E against wheat were observed more frequently in the high reactivity group.**

**Specific bronchial challenge can be performed safely to establish precise dose-response curves. The provocative dose of flour causing a 20% decrease in forced expiratory volume in one second is useful for evaluating the degree of specific reactivity but is not suitable in cases of intermediate reactivity in which comparison with the lactose test is necessary. Specific reactivity is probably a function of immunological and nonspecific bronchial reactivities.**
*Eur Respir J 1999; 14: 328–334.*

*Centre Hospitalier Universitaire Cochin, AP-HP-Université Paris, Paris, France. **Institut National de Recherche et de Sécurité (INRS), Vandoeuvre, France. +Hôpital Cochin, Service de Physiologie-Explorations fonctionnelles, Paris, France.

Correspondence: D. Choudat
Hôpital Cochin
Service de Pathologie Professionnelle
27 rue du Faubourg Saint-Jacques
75014 Paris
France
Fax: 33 142345094

Keywords: Occupational asthma
specific bronchial challenge
wheat flour

Received: September 3 1998
Accepted after revision April 1 1999

This study was supported by grants from Caisse Nationale d'Assurance Maladie des Travailleurs Salariés and Caisse Régionale d'Assurance Maladie d'Ile-de-France.

Wheat flour is the most common allergen responsible for occupational asthma in France and other European countries [1]. Specific bronchial challenge is useful for confirming the diagnosis of occupational asthma and for the accurate identification of the agent responsible for asthma [2–4]. CLOUTIER and coworkers [5–7] developed an exposure apparatus that generates airborne particles. This apparatus automatically controls the size and concentration of particles, resulting in a stable concentration of small airborne particles.

The amount of inhaled flour causing asthma symptoms may be very small in sensitized subjects, but no precise measurement of exposure is usually available. The apparatus make it possible to determine precisely the inhaled dose and to evaluate its effects on expiratory flow. The aim of this study was to investigate specific bronchial reactivity and its relation to nonspecific bronchial and immunological reactivities. Thus, the dose of flour inhaled and the changes in expiratory flow were measured among workers with occupational asthma.

## Method

### *Patients*

Twenty-one patients referred for assessment of occupational asthma caused by flour agreed to undergo a specific bronchial challenge. All reported work-related symptoms of asthma and underwent a standard check-up including a questionnaire, spirometry, methacholine test [8], skin-prick test to wheat and measurement of specific serum immunoglobulin E (IgE) (Cap System; Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Twenty-one challenges were performed with wheat flour. The challenge was not performed if the initial lung function was impaired relative to the European predicted value [8]. Bronchodilator and steroid treatments were stopped 24 h before the challenge. The subjects were outpatients and the challenges were performed on two separate days with inhalation periods beginning at 09.00.

### *Specific bronchial challenge*

Specific bronchial challenge was performed with an equipment analogous to that previously developed by CLOUTIER and coworkers [5–7]. The apparatus consisted of an aerosol generator (Medicana Inc., Saint Laurent, Canada), a particle-size selector, and a vertical inhalation chamber 95 cm high and 15 cm in diameter. The equipment was fully computerized, making it possible to calculate the cumulative dose of aerosol inhaled by the patient through a venturi system and a mouthpiece. This venturi was calibrated and was sensitive for measurements of instantaneous inspiratory flow rates, even below 20 $L \cdot min^{-1}$. It was fixed perpendicularly to the wall of the chamber at mid-height and was connected to a differential pressure transducer (Cole Parmer, Chicago, IL, USA) which yielded the instantaneous inspiratory flow rate signal after pressure to flow rate conversion.

The cumulative dose of aerosol inhaled was calculated from the inspiratory flow rate and the instantaneous aerosol concentration measured by a calibrated photometer (RAM-1, Monitoring Instruments for the Environment Inc., Bedford, MA, USA). The aerosol concentration was not required to be stable, because the two signals were integrated at a given time whatever the concentration. However, the concentration inside the inhalation chamber was usually maintained close to 3.0±0.2 $mg \cdot m^{-3}$ (mean±SD). The aerosol was generated from the original powdered material (wheat flour or lactose) by a compressed air ejector. The particle-size selector was a cyclone that eliminated coarse particles >15 µm in aerodynamic diameter. The influence of the mouthpiece on aerosol concentration and particle size distribution was checked and found to be low.

The patient was asked to breathe at tidal volume. Increasing the number and duration of periods of exposure increased the inhaled dose. The aerosol was inhaled over several periods. The duration of the inhalation period was increased in a step-wise manner. A maximum of nine periods was used (15 s, 30 s, 1 min, 2 min, 5 min, 15 min, 20 min, 30 min, and 30 min). Therefore, the cumulative duration of exposure was 15 s–104 min, according to specific bronchial reactivity. Each period was separated from the next by an interval of 10 min. During each inhalation period, the dose inhaled was continuously determined by integration of the instantaneous concentration and inspiratory flow rate. The cumulative inhaled dose from the beginning of the challenge was determined at the end of each period. Forced expiratory volume in one second ($FEV_1$), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured by flow-volume curves with an automated electronic spirometer (Autospiro AS 500; Minato, Medical Science Co., Osaka, Japan) 1–10 min after each lactose or flour inhalation period. The time-response curve was immediately drawn by computer (fig. 1a). The lower of the lung function values 1–10 min after each inhalation period and the cumulative dose since the beginning of the challenge were automatically plotted to establish dose-response curves (fig. 1b). If there was <20% change in $FEV_1$, the patient was subjected to another inhalation period. The test was stopped if $FEV_1$ decreased by 20% or after 104 min of exposure.

Lung function was measured until about 15.00. in the laboratory. For 24 h, the patient was given a peak flow meter (Mini-Wright®, Airmed, London, UK) or an electronic device that recorded the time (day, h, min), PEF and $FEV_1$ (One-Flow®; STI Plastics, Saint-Romans, France).

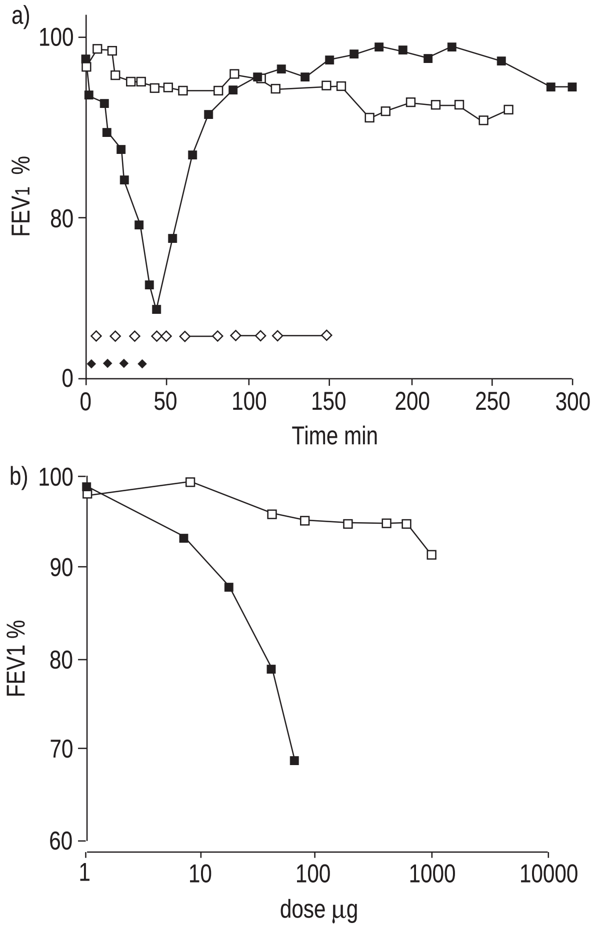


Fig. 1. – a) time-response curves showing changes in forced expiratory volume in one second ($FEV_1$) over time during the test with lactose and the test with wheat flour. □: $FEV_1$ lactose; ■: $FEV_1$ wheat; ◇: lactose exposure; ◆: wheat exposure. b) cumulative dose-response curves for the same subject for lactose (□) and wheat flour (■).

On the first day, a control test with lactose was performed (fig. 1a). If lung function was stable, the flour challenge was performed on another day. Pharmacie Centrale (Assistance Publique, Paris, France) provided the lactose powder and the wheat flour was commercial wheat type 55.

### *Expression and interpretation of the results of the specific challenge*

Changes in lung function were evaluated according to the criterion of the American Thoracic Society for spirometry [9]: if several maximal expiratory manoeuvres are performed, the $FEV_1$ of the subject is the highest value recorded ($FEV_{1,max}$). Therefore, the initial value was not used but instead the highest $FEV_1$ value recorded separately on each day (lactose or flour) was used and the change in $FEV_1$ at the end of each period of exposure was calculated as

$$FEV_1(\%) = 100 - (FEV_1 \text{ observed}/FEV_{1,max})$$

If $FEV_1$ decreased by >15%, the result of the specific bronchial challenge was expressed as the provocative dose of flour (PDf) causing a decrease of 20% ($PDf_{20}$) or 15% ($PDf_{15}$). The reference value was the highest $FEV_1$ value recorded during the test. The provocative dose was determined from the logarithm of the cumulative dose from the beginning of the challenge (fig. 1b). Thus, the PDf was expressed as for the methacholine test [8].

Changes in $FEV_1$ during the lactose test and the wheat flour test were compared using confidence intervals (CI). For each patient, changes in $FEV_1$ during the lactose test were approximately consistent with a normal distribution. The mean and standard deviation were calculated for changes in $FEV_1$ during the lactose test for each patient. By definition, the probability of obtaining a value below the lower limit of the 99.7% CI (mean-3SD) is very low, 0.15%. As the distribution of changes in $FEV_1$ in wheat flour challenge was *a priori* unknown, it was investigated whether the decrease in $FEV_1$ observed in the challenge with flour exceeded the calculated lower 99.7% CI limit for the lactose challenge. In this way, each subject was their own control and no hypothesis was necessary about the distribution of changes in $FEV_1$ during wheat flour challenge. This method did not take into account time, links between values, and the pattern of response and recovery. Therefore, a gradual and moderate decrease below the lower limit of the CI was highly significant.

Finally, three groups were defined according to specific bronchial reactivity: 1) the high reactivity group included subjects with a decrease of >20% in $FEV_1$ from the highest value obtained in the challenge with flour; 2) the negative reactivity group included subjects with no significant difference in results between the challenges with lactose and flour (change in $FEV_1$ in the challenge with flour was <20% and above the lower 99.7% CI limit of the control test); and 3) the intermediate reactivity group included subjects with decreases in $FEV_1$ of <20% but significantly different from the challenge with lactose (maximum decrease in $FEV_1$ in the challenge with flour below the lower 99.7% CI limit of the control test).

*Statistical analysis*

The distribution-free test was used to study the correlation between $PDf_{20}$ (or $PDf_{15}$) and respectively nonspecific bronchial reactivity ($PD_{20}$) and specific wheat IgE. Spearman rank correlation coefficients among pairs of variables were calculated for comparison analysis.

## Results

The demographic characteristics of the population are described in table 1. All subjects were male, except for one female salesperson from a bakery. Their mean age was 29.6±5.7 yrs (mean±SD) and their mean duration of exposure 11.6±6.4 yrs. Seventeen subjects were still exposed to flour at the time of the challenge. All subjects reported work-related asthma. In 20 cases, rhinitis preceded asthma. The mean $FEV_1$ was 97% (range: 71–120%) and the mean total lung capacity (TLC) was 101% (range: 82–137%) of predicted values. The geometric mean of $PD_{20}$ was 245 µg of methacholine (range: 25–>3,100 µg).

The inhalation of lactose did not significantly affect the $FEV_1$ of the 21 patients (table 2). The mean cumulative dose inhaled was 1,945 µg (range: 670–3,495 µg). Slight

Table 1. – Characteristics of the 21 subjects according to specific bronchial reactivity to wheat flour

| No. | Sex | Age yrs | Occupation | Duration of exposure yrs | Interval between last work exposure and challenge months | IgE $kIU \cdot L^{-1}$ | Skin prick test wheat | Initial $FEV_1$ % | $PD_{20}$ methacholine µg |
|---|---|---|---|---|---|---|---|---|---|
| High reactivity group | | | | | | | | | |
| 1 | M | 31 | baker | 12 | 0 | 17 | + | 88 | 25 |
| 2 | M | 43 | pastry maker | 27 | 0 | 18 | + | 71 | ND |
| 3 | M | 27 | pastry maker | 10 | 0 | ND | + | 120 | 133 |
| 4 | M | 38 | pastry maker | 21 | 0 | >100 | + | 87 | 200 |
| 5 | M | 30 | pastry maker | 12 | 0 | 64 | + | 102 | 39 |
| 6 | M | 25 | pastry maker | 10 | 0 | 70 | + | 91 | 100 |
| 7 | F | 22 | saller (bakery) | 6 | 0 | 6 | + | 89 | 100 |
| 8 | M | 30 | pastry maker | 13 | 0 | 12 | ND | 106 | 507 |
| 9 | M | 21 | baker | 7 | 0 | 9 | + | 105 | 80 |
| Intermediate reactivity group | | | | | | | | | |
| 10 | M | 32 | pastry maker | 18 | 0 | 20 | ND | 100 | 245 |
| 11 | M | 27 | pastry maker | 8 | 0 | 8 | - | 99 | 1260 |
| 12 | M | 29 | baker | 14 | 0 | 15 | + | 106 | 1380 |
| 13 | M | 39 | pastry maker | 25 | 0 | ND | + | 118 | 237 |
| 14 | M | 24 | pastry maker | 10 | 0 | 51 | + | 101 | 964 |
| Negative reactivity group | | | | | | | | | |
| 15 | M | 29 | pancake maker | 3 | 6 | 0 | - | 80 | 350 |
| 16 | M | 29 | baker | 12 | 0 | 0 | + | 96 | 279 |
| 17 | M | 35 | pastry maker | 4 | 6 | 4 | - | 100 | 463 |
| 18 | M | 27 | pastry maker | 7 | 1 | 6 | - | 83 | >3100 |
| 19 | M | 21 | pastry maker | 4 | 0 | 0 | - | 105 | 240 |
| 20 | M | 32 | pastry maker | 15 | 0 | 0 | - | 100 | 844 |
| 21 | M | 31 | baker | 15 | 7 | 3 | + | 99 | 245 |

IgE: specific immunoglobulin E to wheat flour; Initial $FEV_1$: percentage of the predicted European value for forced expiratory volume in one second before the methacholine challenge; $PD_{20}$: provocative dose of methacholine causing a 20% decrease in $FEV_1$; M: male; F: female; ND: not done.

Table 2. – Results of the 21 challenges with lactose and wheat flour according to specific bronchial reactivity to wheat flour

| No. | Lactose challenge | | | Wheat flour challenge | | | |
|---|---|---|---|---|---|---|---|
| | Cumulative dose µg | Change in $FEV_1$ % mean | Lower limit of 99.7% CI% | Maximum decrease in $FEV_1$ % | Cumulative dose µg | $PDf_{20}$ µg | $PDf_{15}$ µg |
| High reactivity group | | | | | | | |
| 1 | 889 | 4 | 11 | 25 | 4 | 3 | 2 |
| 2 | 672 | 7 | 17 | 35 | 37 | 21 | 18 |
| 3 | 2719 | 4 | 11 | 31 | 44 | 28 | 21 |
| 4 | 1237 | 5 | 14 | 31 | 94 | 29 | 17 |
| 5 | 1025 | 5 | 13 | 31 | 65 | 40 | 32 |
| 6 | 2344 | 5 | 12 | 26 | 90 | 71 | 52 |
| 7 | 805 | 5 | 10 | 21 | 196 | 178 | 123 |
| 8 | 2353 | 3 | 8 | 27 | 307 | 205 | 146 |
| 9 | 2943 | 4 | 11 | 30 | 629 | 355 | 245 |
| Intermediate reactivity group | | | | | | | |
| 10 | 1499 | 2 | 7 | 19 | 553 | NC | 373 |
| 11 | 3494 | 3 | 8 | 16 | 3318 | NC | 633 |
| 12 | 2147 | 4 | 12 | 17 | 2752 | NC | 2617 |
| 13 | 3252 | 2 | 6 | 18 | 3441 | NC | 3200 |
| 14 | 935 | 3 | 6 | 12 | 2448 | NC | NC |
| Negative reactivity group | | | | | | | |
| 15 | 3124 | 4 | 9 | 9 | 3350 | NC | NC |
| 16 | 3346 | 3 | 7 | 6 | 5938 | NC | NC |
| 17 | 677 | 3 | 9 | 8 | 1449 | NC | NC |
| 18 | 1324 | 2 | 7 | 5 | 2357 | NC | NC |
| 19 | 1854 | 2 | 6 | 6 | 1394 | NC | NC |
| 20 | 2917 | 2 | 7 | 5 | 1870 | NC | NC |
| 21 | 1294 | 9 | 20 | 14 | 1467 | NC | NC |

The decrease during the challenge with flour was below the lower limit of the confidence interval (CI) in the group with high reactivity and in the intermediate group. $FEV_1$: forced expiratory volume in one second; $PDf_{20}$: provocative dose of wheat flour causing a 20% decrease in $FEV_1$; $PDf_{15}$: provocative dose of wheat flour causing a 15% decrease in $FEV_1$; NC: not calculable.

changes in $FEV_1$ were observed, which did not depend on the dose inhaled. These changes were consistent with the typical reproducibility of $FEV_1$ determination.

The total amount of flour inhaled by each subject depended on the level of specific hyperreactivity and, for patients with little or no reactivity it also depended on tidal volume (table 2 and fig. 2).

The patients were assigned to the three groups of specific reactivity: 1) nine subjects were included in the high reactivity group. The challenge with flour was clearly positive with a fall in $FEV_1$ of >20% (table 2). The decrease in $FEV_1$ was significantly different from the distribution of the $FEV_1$ observed during the test with lactose (fig. 2). Bronchoconstriction was immediate with a maximum decrease in airflow ~10 min after the last inhalation period. Reversal of airway obstruction was spontaneous and complete in the nine subjects within 1 h of the end of exposure; 2) seven subjects were included in the negative reactivity group. There was no significant difference in the $FEV_1$ recorded with inhaled doses of flour above 1,390 µg and that recorded with lactose; and 3) five subjects were included in the intermediate reactivity group. There was <20% change in $FEV_1$ but the change recorded was significantly different from the distribution of $FEV_1$ observed during the test with lactose. Time response curves showed a slow decrease induced by exposure, followed by complete reversal.

No late or dual reactions, with fall in peak expiratory flow of >20%, were observed.

Results of the specific bronchial challenge were significantly correlated with those of the methacholine test ($PDf_{15}$ *versus* $PD_{20}$, r=0.67, p<0.02). The correlation between $PDf_{20}$ and $PD_{20}$ was not significant. There was no significant association between $PDf_{20}$, or $PDf_{15}$, and specific wheat IgE. However positive skin prick test and specific wheat IgE were observed more frequently in the high reactivity group (table 1).

## Discussion

In this study, the relationship between the dose of flour inhaled and specific bronchial reactivity was investigated. A wide range of specific reactivity was observed and its relationship to nonspecific bronchial and immunological reactivities was assessed.

The 21 subjects included in this study were selected by occupational physicians and all agreed to undergo bronchial challenge. They were not representative of the population of workers exposed to flour, but they had some common characteristics in terms of their occupational asthma, such as onset as young adults and rhinitis occurring before asthma. More pastry makers than bakers were included in this study. This may be because there are more pastry makers than bakers in the Parisian area, although exposure to wheat allergen is probably lower for the pastry makers than for the bakers. In the Netherlands, HOUBA *et al.* [10] found that the mean exposure to inhalable flour dust was lower for pastry cooks (0.7 $mg \cdot m^{-3}$) than for bread bakers (3.3 $mg \cdot m^{-3}$), with intermediate levels of exposure in mixed bakeries (2.0 $mg \cdot m^{-3}$). The pastry makers included in the current study worked in mixed

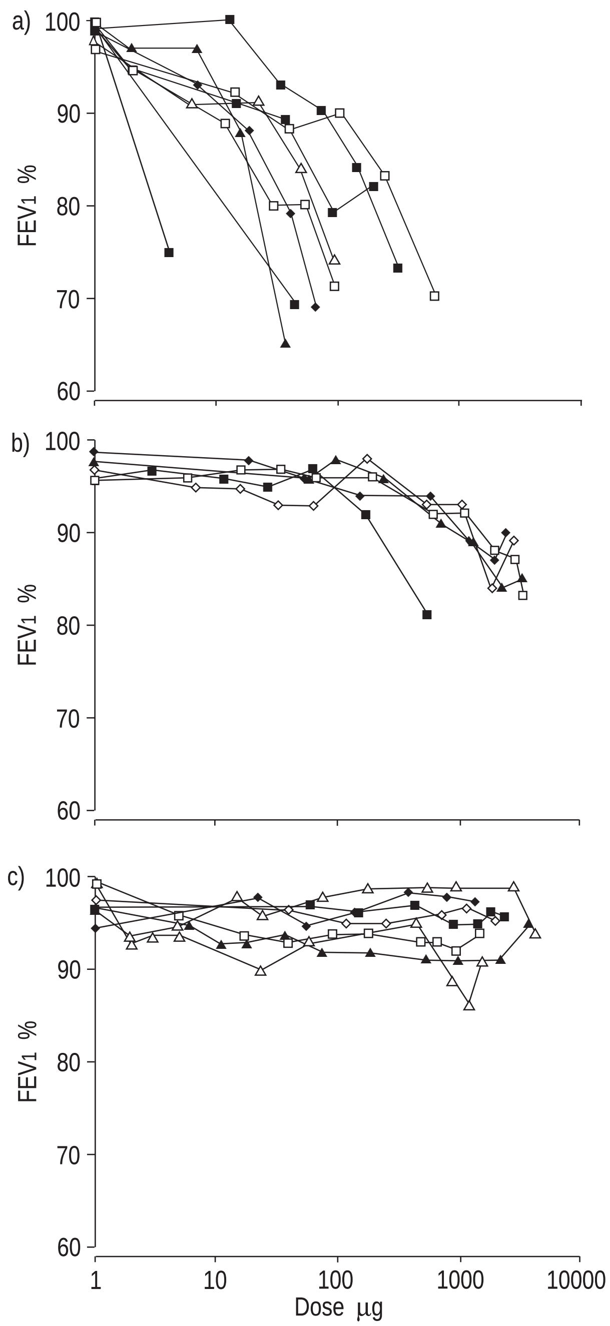


Fig. 2. – Individual patient dose-response curves for wheat flour challenge according to specific bronchial reactivity. a) High reactivity group (n=9). b) Intermediate reactivity group (n=5). c) Negative group (n=7). Each group repesents an individual patient within the reactivity group.

bakeries, and probably had levels of exposure intermediate or similar to those of bread bakers.

TIFFENEAU [11] suggested that there was a relationship between specific bronchial reactivity and nonspecific bronchial and immunological reactivities. The current study has confirmed this relationship since a correlation between the results of specific challenge ($PDf_{15}$) and the methacholine test ($PD_{20}$) was found. Moreover, positive skin-prick test and higher values of specific IgE were observed in the higher reactivity group. The number of subjects was too small to investigate these relationships more precisely. However, CARLETTI *et al.* [12] found that the specific response to flour dust in a group of 37 subjects with suspected occupational asthma due to wheat flour could not be adequately predicted by other clinical, allergological and functional data. They concluded that specific bronchial challenge should always be performed in subjects with suspected occupational asthma.

The authors think that specific challenge is of value not only for the diagnosis of occupational asthma but also for estimating the degree of specific hyperreactivity. The range of specific hyperreactivity was wide in the small group of bakers and pastry makers that were studied. The very high hyperreactivity observed in a few subjects, with PDf <100 µg, emphasizes the need to control exposure during specific bronchial challenge and to begin with very low doses. LIN *et al.* [13] used a rotahaler with capsules containing the allergen, in this case wood dust. Their device was easy to use but the dose of inhaled wood dust (20 mg·capsule$^{-1}$) was very high if the subject had high reactivity. However, the amount of the wood dust inhaled cannot be compared with the results of the current study because the type of allergen, the size of particles and the associated nonallergenic component which may make up most of the weight (starch in wheat flour) are different. MERGET *et al.* [14] carried out bronchial challenges with wheat flour dust administered by Spinhaler (Fisons Inc., Loughborough, UK) and with aqueous wheat flour extract. In 27 bakers, the median dose inhaled per capsule was 229 mg of wheat flour type 55. The patients inhaled 1–7 capsules. The maximum decrease in specific airway conductance was observed 10–20 min after inhalation of the last dose of allergen. CARLETTI *et al.* [12] used another type of device to generate aerosols of wheat flour and observed an immediate decrease in $FEV_1$ in subjects with occupational asthma. The decrease was as much as 76% and 79% in two subjects. However, the concentration of flour generated for the specific challenges varied greatly (geometric mean 12.1 mg·m$^{-3}$; range: 0.8–54.3 mg·m$^{-3}$), and the size of the particles and the doses inhaled were not given.

Specific bronchial challenges are used to confirm diagnosis of occupational asthma [2–4, 12, 14]. However, the expression of the results and their interpretation are not as well defined as for the methacholine test [4, 8]. The establishment of dose response curves involves selecting the most appropriate lung function value to study and determining the dose inhaled and the means of expressing the results.

$FEV_1$ determination was used for the specific test because $FEV_1$ is more reproducible than PEF [8, 15]. This is well known by physiologists and is the reason why nonspecific tests are usually assessed on changes in $FEV_1$ rather than PEF. It has also been observed that $FEV_1$ is more reproducible than PEF in tests with lactose [16].

The expression of the results of the methacholine tests is standardized, with the determination of a provocative dose (or provocative concentration) that causes a 20% decrease in $FEV_1$ relative to the initial $FEV_1$ or to the $FEV_1$ after an aerosol of saline serum [8]. In contrast, results of specific challenges are usually expressed relative to the initial $FEV_1$ only: the test is considered positive if a 20% decrease in $FEV_1$ is observed regardless of the dose inhaled [4, 8]. The latency of the response, (immediate and/or late reaction) has been studied more often than has the real inhaled dose. The relative effects of the duration of exposure during the challenge and of the concentration of the allergen on the reaction of asthmatic workers have been studied only for isocyanates: for a given subject, the reaction depends mainly on the dose inhaled [17]. A first day without exposure and a control lactose test have been used to check the stability of lung function before the specific challenge [2, 4, 8] but not to explain the result of the specific test. The expression of the results of the specific challenge do not in that case take into account the control lactose test or the amount of allergen inhaled. It has been demonstrated herein that $PDf_{20}$ can be used not only to express the result but also the comparison with the lactose control test.

The cumulative dose from the beginning of the challenge was used to determine the provocative dose of flour ($PDf_{15}$ and $PDf_{20}$). This method was suitable for patients with high hyperreactivity because the initial periods of exposure were short and each additional dose caused an additional effect. As for isocyanate, the inhaled dose of wheat was probably the main factor inducing bronchoconstriction, rather than the concentration or the duration of the exposure [17]. However, for patients with low reactivity, the periods of exposure were long and thus the effect of the first doses may have been low or nonexistent. In that case, changes in $FEV_1$ may be due particularly to the last inhaled dose and to the duration of exposure: the rate at which the dose is given may be the main factor in bronchoconstriction. All intermediate cases, between high and low reactivity, may be observed because specific reactivity is a quantitative continuous variable.

Therefore, a negative test with wheat flour cannot exclude the diagnosis of occupational asthma. There may be four reasons for a false negative test: 1) if the subject has not been exposed in the workplace for a long period of time, specific reactivity may be low or absent [4, 18]. In the current study, the 4 subjects no longer exposed at the time of the challenge were in the negative group (table 1). It was found that 9 subjects (43%) had a $PDf_{20}$ <400 µg, and 14 subjects (67%) had dose-response curves to flour significantly different from that of the control test. MERGET *et al.* [14] found a similar prevalence of positive challenge tests with wheat dust in a subgroup of 27 bakers (41%) but nothing is known about the exposure of this subgroup. However, it is difficult to compare the prevalences observed among these small groups and to study the effect of the time interval between the last exposure and the challenge. 2) Sensitization may be directed against rye flour, barley flour or another allergen, with no cross-reaction [18]. In this case, specific bronchial challenge may be negative with wheat flour but strongly positive with another allergen, such as buckwheat in patient No. 15 [19], rye flour in patient No. 11 or α-amylase [18]. 3) bakery workers also report respiratory diseases other than asthma. Chronic exposure to wheat flour may cause nonallergic manifestations such as respiratory symptoms [20–22] and impaired ventilation by direct irritation [23]. 4) another cause of false negative challenge results is low specific reactivity in a patient highly exposed at work. The cumulative dose inhaled during the challenge in the laboratory may then be lower than the amount inhaled at the workplace. HOUBA *et al.* [10] found high concentrations of inhalable flour dust in traditional and modern industrial bakeries. In traditional bread bakeries, the distribution of samples collected during full-shift periods is log-normal with a geometric mean of 3.3 $mg \cdot m^{-3}$. Although this mean is similar to the concentration used for the challenge in the current study, higher exposure during the shift cannot be ruled out for some patients. Moreover, exposure to flour varies over the shift according to the stage in the process and higher concentrations occur for short periods. Such overexposure may induce asthma symptoms in sensitized subjects with intermediate hyperreactivity. In such cases, the duration of the exposure and the dose inhaled should be taken into account. A given inhaled dose may, if given too slowly or too quickly, be inefficient to induce bronchoconstriction. This affects prevention measures because limiting the peak concentration would efficiently prevent the asthma symptoms of these subjects.

In summary, the airborne particle generator made it possible to perform specific bronchial challenge safely and to establish precise dose-response curves. Challenge with flour was standardized so that the results could be interpreted by comparison with the lactose test. A provocative dose of flour that causes a 20% decrease in the forced expiratory volume in one second is useful for evaluating the degree of specific reactivity but is unsuitable in cases of intermediate reactivity, making comparison with the results of the lactose test necessary. Therefore, specific challenge with wheat flour is of value for diagnosing occupational asthma and for demonstrating the wide range of specific hyperreactivity in asthmatic bakers. Specific reactivity is probably a function of immunological and nonspecific reactivities. The level of specific hyperreactivity may also have consequences for the patient's fitness to work.

***Acknowledgements.*** The authors would like to thank the bakery workers for their participation, the occupational physicians C. Alessandrini, T. Schlacther (Centre médico-social de la boulangerie et de la boulangerie-patisserie de Paris et de la Seine) and M.F. Combalot for technical assistance.

## References

1. Meredith SK, Taylor VM, McDonald JC. Occupational respiratory disease in the United Kingdom 1989: A report to the British Thoracic Society and the Society of Occupational Medicine by the SWORD project group. *Br J Ind Med* 1991; 48: 292–298.
2. Cartier A, Malo JL. Occupational challenge tests. *In*: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the Workplace. New York, Marcel Dekker, 1993; pp. 215–247.
3. Pauli G, Soldatov D, Kopferschmitt-Kübler MC. L'épreuve de provocation bronchique est-elle justifiée en pathologie respiratoire professionnelle? *Rev Pneumol Clin* 1996; 52: 97–102.
4. Vandenplas O, Malo JL. Inhalation challenges with agents causing occupational asthma. *Eur Respir J* 1997; 10: 2612–2629.
5. Cloutier Y, Lagier F, Cartier A, Malo JL. New methodology for specific inhalation challenges with occupational agents in powder form. *Eur Respir J* 1989; 2: 769–777.
6. Cloutier Y, Malo JL. Update on an exposure system for particles in the diagnosis of occupational asthma. *Eur Respir J* 1992; 5: 887–890.
7. Cloutier Y, Lagier F, Malo JL. Validation of an exposure system to particles for the diagnosis of occupational asthma. *Chest* 1992; 102: 402–407.

8. Sterk PJ, Fabbri LM, Quanjer PH, *et al*. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. *Eur Respir J* 1993; 16: 53–83.
9. American Thoracic Society. Standardization of spirometry. 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107–1136.
10. Houba R, Heederik D, Kromhout H. Grouping strategies for exposure to inhalable dust, wheat allergens and α-amylase allergens in bakeries. *Ann Occup Hyg* 1997; 41: 287–296.
11. Tiffeneau R. Hypersensibilité cholinergo-histaminique pulmonaire de l'asthmatique. Relation avec l'hypersensibilité allergénique pulmonaire. *Acta allergologica* 1958; suppl V: 187–221.
12. Carletti AM, Talini D, Carrara M, *et al*. II test di provocazione bronchiale specifico con polvere di farina nella diagnosi di asma bronchiale professionale. *Med Lav* 1997; 88: 406–415.
13. Lin FJ, Chen H, Chan-Yeung M. New method for an occupational dust challenge test. *Occup Environ Med* 1995; 52: 54–56.
14. Merget R, Heger M, Globisch A, *et al*. Quantitative bronchial challenge tests with wheat flour dust administered by Spinhaler: comparison with aqueous wheat flour extract inhalation. *J Allergy Clin Immunol* 1997; 100: 199–207.
15. Bérubé D, Cartier A, L'Archeveque J, Ghezzo H, Malo JL. Comparison of peak flow rate and $FEV_1$ in assessing bronchomotor tone after challenges with occupational sensitizers *Chest* 1991; 99: 831–836.
16. Martin JC, Tabka F, Martins J, *et al*. Le volume expiré par seconde est-il plus fiable que le débit de pointe? *Arch Mal Prof* 1998; 59: 418.
17. Vandenplas O, Cartier A, Lesage J, *et al*. Response to isocyanates: effect of concentration, duration of exposure and dose. *Am Rev Respir Dis* 1993; 147: 1287–1290.
18. Alvarez MJ, Tabar AI, Quirce S, Olaguibel JM, Lizaso MT, Echechipia S, Rodriguez A, Garcia BE. Diversity of allergens causing occupational asthma among cereal workers as demonstrated by exposure procedures. *Clin Exp Allerg* 1996; 26: 147–153.
19. Choudat D, Villette C, Dessanges JF, Combalot MF, Fabriès JF, Lockhart A, Dall'Ava J, Conso F. Asthme professionnel á la farine de sarrasin. *Rev Mal Respir* 1997; 14: 319–321.
20. Cullinam P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to flour. *Occup Environ Med* 1994; 51: 579–583.
21. Gimenez C, Fouad K, Choudat D, Laureillard J, Bouscaillou P, Leib E. Chronic and acute respiratory effects among mill workers. *Int Arch Environ Health* 1995; 67: 311–315.
22. Massin N, Bohadana AB, Wild P, Kolopp-Sarda MN, Toamain JP. Airway responsiveness to methacholine, respiratory symptoms, and dust exposure levels in grain and flour mill workers in Eastern France. *Am J Ind Med* 1995; 27: 859–869.
23. Joly N, Martin-Silva B, Choudat D, Vicrey C, Rossignol C, Conso F. Symptômes et fonction respiratoires des artisans boulangers-pâtissiers de la région Poitou-Charentes. *Arch Mal Prof* 1997; 58: 641–647.