

Baker's asthma

Clinical and immunological studies

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Summary

Seven bakers with respiratory symptoms were evaluated by skin tests, RAST assay for specific IgE antibodies to rye and wheat, inhalation challenge with methacholine for the determination of non-specific bronchial reactivity, and bronchoprovocation with rye and wheat extracts for the determination of antigen-specific bronchial reactivity. An immediate asthmatic response to antigen challenge was observed in four subjects and all of them had a high level of flour-specific IgE antibodies. The serum RAST values provided a more accurate predictive value than the degree of cutaneous sensitivity determined by skin testing with respect to the bronchial response to antigenic challenge. Among those who reacted positively to antigenic bronchoprovocation, a much lower antigen dose was required to elicit a positive reaction if the subject also had an increased degree of non-specific bronchial reactivity. An elevated RAST value was not found in thirty-eight asymptomatic bakers or in ten asthmatics who had no occupational exposure to flour. Thus, baker's asthma appears to be a form of allergic asthma to cereal flours mediated by specific IgE antibodies. Both the level of serum IgE antibodies and the degree of non-specific bronchial reactivity are important factors which may influence a baker's bronchial response upon inhalation of cereal flours.

Introduction

The occurrence of allergic nasal and asthmatic symptoms in certain workers with occupational exposure to cereal flours has been well known. Most published reports implicated the cereal flours as the responsible allergens (Wilbur & Ward, 1976; Hendrick, Davies & Pepys, 1976; Bush & Cohen, 1977) and specific IgE antibodies have been demonstrated in the affected workers by allergy skin tests and by the radioallergosorbent test (RAST) (Baldo, Krilis & Wrigley, 1980; Blands *et al.*, 1976; Bjorksten *et al.*, 1977). Recently we had an opportunity to study seven symptomatic

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bakers and the results of their clinical, immunological and pulmonary physiological findings are the subject of this report.

Materials and methods

Subject

Fifty bakers from a bakery in Vancouver participated in a health survey, which consisted of filling a medical occupational questionnaire, allergy skin tests by the prick method, spirometric measurements of lung function and taking of 10 ml of venous blood for the measurement of specific IgE antibodies to flours. Thirty-eight bakers did not complain of respiratory symptoms related to flour exposure while the remaining twelve complained of cough, sputum production with or without wheezing. Of these twelve symptomatic bakers, only four consented to participating in more detailed studies including antigenic bronchoprovocation. We have also studied three additional symptomatic bakers who were referred by their family physicians because of their occupationally-related respiratory problems. Thus, a total of seven symptomatic bakers received detailed investigations. Another group of ten asthmatic patients without any occupational exposure to flours also donated a serum sample for the measurement of specific IgE antibodies and served as controls; four of these patients also had bronchoprovocation test, two with rye extract and another two with wheat extract.

The clinical information of the seven symptomatic bakers and the four non-baker asthmatics who had received antigenic bronchoprovocation tests is shown in Table 1. All the bakers were males and had a long history of occupational exposure to flours, mostly rye and wheat flours. The interval between the start of occupational exposure and the onset of respiratory symptoms as well as the duration of the respiratory symptoms varied considerably among the symptomatic bakers.

Methods

Preparation of flour extracts

Extracts of wheat flour (Robin Hood brand which is a mixture of spring wheat, durum wheat and soft wheat) and rye flour were prepared by shaking the flour in phosphate-buffered saline (PBS) at 4°C for 24 hr at a concentration of 10% w/v. The solution was then centrifuged and sterilized through millipore filters before use. The concentrations prepared for skin tests and inhalation tests were 0.001, 0.01, 0.1, 1 and 10% by making ten times serial dilutions of the flour extracts.

Allergy skin tests

Skin tests were done by the prick method using three commercially available allergens: *Dermatophagoides farinae*, mixed Pacific grass pollens, cat epidermal antigens as well as our own preparations of rye and wheat extracts. Phosphate-buffered saline and histamine phosphate (2.5 mg/ml) were used as negative and positive controls respectively.

The weal diameter was measured in two perpendicular directions at 15 min and the mean weal diameter for each dilution was calculated by averaging the two values. Cutaneous sensitivity to the flour extracts was defined as the concentration that produced a mean weal diameter of 2 mm and was obtained from a dose response curve

Table 1. Clinical data of bakers and controls

Subject	Age (years)	Smoking habit	Flour exposure (years)	Symptoms	Medication	Positive skin test reactions	Lung function (% pred.)			
							FVC	FEV ₁	MMF	
<i>Bakers with symptoms</i>										
1	37	Smoker	7	Wheezing, dyspnoea, cough (6 years)	None	Rye, wheat, house dust mite	88	68	32	
2	59	Ex-smoker	45	Dyspnoea, chest tightness (1 year), rhinitis (20 years), dermatitis (2 years)	None	Rye, wheat	142	130	70	
3	63	Ex-smoker	31	Wheezing, dyspnoea, rhinitis (29 years)	Salbutamol, beclomethasone	Rye, wheat, grass, cat	94	71	27	
4	26	Non-smoker	5	Wheezing, cough, rhinitis (2 years)	Theophyllin, cromoglycate	Rye, wheat, house dust mite	89	79	62	
5	50	Smoker	35	Wheezing and cough associated with respiratory infections only (3 years)	None	Rye, wheat, house dust mite	122	132	123	
6	58	Smoker	41	Productive cough (1 year)	None	Rye, wheat, cat	101	105	92	
7	57	Ex-smoker	35	Productive cough (1 year)	None	House dust mite	116	127	114	
<i>Non-baker asthmatic controls</i>										
8	42	Non-smoker	0	Cough, wheezing, (2 years)	None	None	74	83	55	
9	26	Non-smoker	0	Cough, wheezing, (5 years)	Cromoglycate, salbutamol	None	101	114	58	
10	23	Non-smoker	0	Cough, wheezing, (1 year)	None	Grass, house dust mite	67	91	35	
11	28	Non-smoker	0	Cough, wheezing (1½ years)	None	Grass	115	109	110	

as described by Cockcroft *et al.*, (1979). A subject with a high degree of cutaneous sensitivity required a relatively low concentration of flour extract to elicit a positive skin test reaction.

Pulmonary function tests

Spirometric tests were carried out using a 13.5-l Collins spirometer. Forced expiratory volume in 1 sec (FEV₁), forced vital capacity (FVC), and maximal mid-expiratory flow rate (MMF) were measured. Predicted values were obtained from data of Morris, Koski and Johnson (1971). Four of the seven symptomatic bakers had evidence of airflow obstruction on spirometry (Table 1).

Bronchoprovocation tests

The procedures for bronchoprovocation tests with methacholine and antigens have been described previously (Lam, Wong & Chan-Yeung, 1979). The following concentrations of methacholine were used: 0.01, 0.05, 0.1, 1, 5, 10 and 25 mg/ml, and PBS served as control. The solutions were nebulized using a Bennett twin nebulizer with a flow rate of 5 l/min of oxygen.

The subjects inhaled the solutions at tidal breathing for 2 min, starting with the control solution followed by increasing concentrations of methacholine at intervals of 5 min. Measurements of FEV₁ and FVC were made before the test and at 30 sec and every 4 min after each inhalation. The test was terminated when the FEV₁ had fallen by 20% or more from the post-PBS level. The results were expressed as the provocative concentration of methacholine required to induce a 20% fall in the FEV₁ (PC₂₀) which was interpolated from the dose-response curve.

Bronchoprovocation tests with flour extracts were performed on the same day as the methacholine inhalation tests, 2 hr after the FEV₁ and FVC had returned to the baseline level. The initial concentration of the flour extract used was the one which gave a 2-mm weal on prick testing. It was administered in increasingly longer periods (0.5, 1, 1.5 and 2 min) at 10-min intervals. If FEV₁ did not fall by 20% by 30 min after this initial concentration, the procedure was repeated with the next higher concentration until inhalation with a 10% w/v solution was completed. Measurements of FEV₁, FVC and peak expiratory flow rate (PEFR) were measured at various intervals after challenge: immediately, 5-, 10- and 15-min intervals for 1 hr and then at hourly intervals until the patient left the laboratory. All patients recorded peak expiratory flow rate (PEFR) at 2-hr intervals until they went to bed and were instructed to do so if they were awakened at night with dyspnoea. The antigen dose was calculated by multiplying the total volume of extract nebulized by its concentration and the dose of antigen which induced a 20% fall in FEV₁ (antigen PC₂₀) was determined in the same manner as methacholine PC₂₀.

Preparation of allergen discs for RAST

The method of coupling of flour extract to paper discs was modified from that described by Ceska, Ericksson and Varga (1972). Briefly, 6-mm-diameter discs were punched out from Whatman 541 filter papers. One hundred washed discs were put in a beaker with 30 ml distilled water added. After 500 mg of cyanogen bromide was added to the solution, the pH was adjusted to 11.0 for 15 min with 1 N sodium hydroxide. The activated discs were washed in a Buchner funnel with cold distilled water, and then with cold 0.1 M bicarbonate buffer (pH 8.6).

Ten millilitres of the 10% w/v flour extract was added to the activated discs and mixed overnight at 4°C. The discs were washed five times with cold bicarbonate buffer and 20 ml 0.5 M ethanolamine in the same buffer was added to the discs and were mixed at room temperature for 2 hr. The discs were washed once with bicarbonate buffer; once with 0.1 M acetate buffer (pH 4.0), three times with PBS, once with RAST buffer (Pharmacia Inc.). The discs were stored in RAST buffer at 4°C until use.

RAST Test

A 50- μ l serum sample was pipetted into a tube containing an allergen disc. The tubes were shaken on a clinical rotator at room temperature for 3 hr. The tubes were washed four times with RAST buffer; the washing was removed by suction. Then 50 μ l of ^{125} I-labelled rabbit anti-human IgE (Pharmacia Inc.) was added to each tube and the tubes were again shaken on the rotator overnight at room temperature. The tubes were washed four times with RAST buffer and the radioactivity was counted in a gamma counter. The uptake of radioactivity of the sample was expressed as RAST value which was the ratio of the radioactivity of the subject's serum sample to that of the normal control.

Specificity of the serum RAST activity

The specificity of the RAST activity in the bakers' sera was determined by comparing the effects of prior absorption of the serum with either anti-IgE or anti-IgG. The IgG fractions of ϵ chain-specific anti-IgE (Kallstad Laboratories, U.S.A.) and of γ chain-specific anti-IgG (Miles Laboratories, U.S.A.) were prepared by precipitation with ammonium sulphate at a concentrate of 30%. These purified antisera were conjugated with cyanogen-bromide-activated Sepharose-cl-4B (Pharmacia Inc.) to transform into solid phase immunosorbents according to the method described by Wide, Bennich and Johansson (1967). For the absorption study, 0.2 ml of a baker's serum was mixed with an equal volume of either anti-IgE or anti-IgG immunosorbents at room temperature for 3 hr and then the absorbed serum was separated by centrifugation at 3000 rev/min. The serum RAST values were compared before and after such absorption procedures.

Furthermore, the antigen specificity of the serum RAST activity was determined by the degree of inhibition of serum RAST values by prior absorption of the serum specimens with flour extracts. Aliquots of a serum sample in 150 μ l were mixed with different amounts of a 10% rye or wheat flour extract and left at room temperature for 3 hr. The serum RAST activity was assayed before and after the addition of the flour extract and expressed as:

$$\text{Inhibition (\%)} = \frac{(B) - (A)}{(B)} \times 100\%,$$

where (B) represents serum RAST value before incubation with flour extracts and (A) represents the serum RAST value after incubation with flour extracts.

Serum precipitins

The subjects' sera were examined for precipitins against the flour extracts using the agar gel double diffusion method of Ouchterlony (1958).

Results

Skin tests

All but one of the seven symptomatic bakers developed an immediate weal and flare reaction on skin testing with rye and wheat extract, but a late or so called type III skin reaction was not observed in any of them. The non-baker asthmatic controls did not have a positive skin reaction to either flour extract (Table 1). A positive immediate skin reaction to *Dermatophagoides farinae* (house mite) extract was found in four bakers with symptoms and in one control subject.

Antigen inhalation tests

Table 2 shows the results of antigen provocation tests with rye and wheat extracts. Bakers 1-4 showed an immediate bronchospastic reaction to challenge with the rye flour extract, but no late asthmatic reaction was observed in any of these subjects. Subjects 5-7 did not show either an immediate or a late asthmatic reaction to rye extract challenge. Subjects 1 and 2 were also challenged with the wheat extract and again both developed an immediate bronchospastic reaction. Inhalation challenge with wheat extract was not performed on the remaining bakers because they were unable to obtain leave from work for further testing. The four non-baker asthmatic controls had negative bronchial reaction to challenge with wheat or rye extract.

Table 2. Results of inhalation challenge tests with flour extracts

Subject	Concentration of extract (w/v %)	Duration of nebulization (min)	FEV ₁ (l)			FVC (l)		
			Before challenge	After challenge	Change* (%)	Before challenge	After challenge	Change (%)
<i>Rye extract</i>								
Bakers								
1	0.1	0.5	2.09	1.32	-37	3.17	2.67	-50
2	1	5.0	3.63	2.14	-41	5.26	4.65	-12
3	0.01	3.0	2.27	1.82	-20	4.32	3.83	-11
4	1	0.5	3.09	1.70	-45	4.18	2.65	-37
5	1	5.0	3.03	2.98	-2	4.14	4.14	0
6	1	8.0	4.68	4.50	-4	6.27	6.27	0
7	1	8.0	4.01	3.97	-1	4.99	4.99	0
Non-baker asthmatic control								
8	10	5.0	2.67	2.60	-3	3.67	3.66	0
9	10	5.0	3.87	3.79	-2	6.10	6.01	-1
<i>Wheat extract</i>								
Bakers								
1	1	5.0	2.09	1.29	-28	3.30	2.67	-20
2	10	1.5	3.74	2.50	-33	5.08	4.76	-6
Non-baker asthmatic control								
10	10	5.0	2.85	2.54	-11	4.95	4.95	0
11	10	5.0	4.86	4.48	-8	5.79	5.57	-4

* A 20% or more decrease is regarded as a positive reaction to antigenic challenge.

RAST Tests

Figure 1 shows the results of RAST tests on ten non-baker asthmatic controls, thirty-eight bakers with no symptoms and seven symptomatic bakers who had detailed investigations. The mean RAST value pertaining to rye and wheat extract for the ten controls were 1.2 ± 0.4 and 1.34 ± 0.45 respectively; the values for thirty-eight asymptomatic bakers were 1.23 ± 0.18 and 1.10 ± 0.20 respectively. The RAST values pertaining to the rye extract for Bakers 1-4 who developed an immediate asthmatic reaction on challenge with the rye extract were much higher than those of the ten

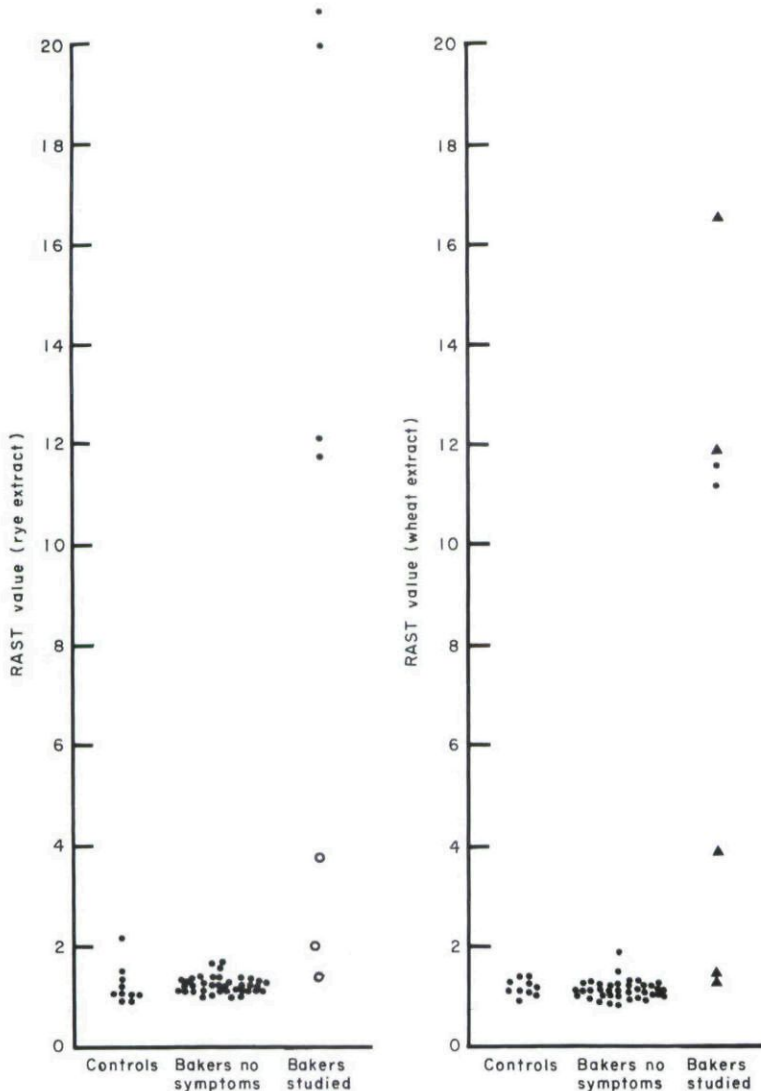


Fig. 1. RAST values against rye and wheat in: (1) ten non-bakers with asthma but without history of occupational exposure to flour, (2) thirty-eight bakers with no respiratory symptoms, (3) seven symptomatic bakers. Subjects with a positive asthmatic reaction on inhalation challenge with rye or wheat extract are represented by solid dots (●), those with negative challenge are indicated by open circle (○), those who had no inhalation challenge are indicated by triangles (▲).

controls, thirty-eight asymptomatic bakers and the three symptomatic bakers with negative inhalation challenge test. The RAST values pertaining to the wheat extract for Bakers 1 and 2 who developed an immediate asthmatic reaction on inhalation challenge with the wheat extract were also much higher than those of the controls and asymptomatic bakers.

Specificity of the RAST activity

Sera from Bakers 1 and 2 with elevated RAST values to flour extracts were absorbed with anti-IgE and anti-IgG immunosorbents, respectively. As can be seen from the results presented in Table 3, absorption of the sera with anti-IgE but not IgG abolished

Table 3. Effects of absorption with anti-IgE and anti-IgG on serum RAST value

Subject	Before absorption	After absorption with anti-IgE	After absorption with anti-IgG
<i>Serum anti-rye RAST value</i>			
1	10.0	1.14	9.50
2	10.6	1.10	9.80
<i>Serum anti-wheat RAST value</i>			
1	3.30	1.24	3.13
2	10.10	1.29	9.46

the RAST activity. This would indicate that the serum RAST value represents the activity of IgE antibodies.

The antigenic specificity of the serum IgE antibodies activity against rye and wheat extract was also determined by the antigen inhibition test. The results displayed in Fig. 2a and b show a dose-dependent inhibition of the serum RAST value when increasing amounts of flour extracts were added to the same two serum samples prior to the RAST procedures. The binding of the specific IgE antibodies by the added flour antigens lead to a suppression of the antibody activity as measured by the RAST.

Correlations between cutaneous sensitivity to flour extracts, RAST values, bronchial reactivity to antigen (antigen PC₂₀) and to methacholine (methacholine PC₂₀)

The results of the examination concerning the possible correlations between these parameters are shown in Table 4. Statistical analysis was not performed because of the small number of subjects involved. In general, the degree of cutaneous sensitivity to flour antigens paralleled the RAST values, i.e. subjects with high RAST values usually had a relatively high degree of cutaneous reactivity (Subject 5 was an exception). The bronchial reactivity to antigen appeared to be dependent on the RAST values and on the degree of non-specific bronchial reactivity (methacholine PC₂₀). Thus Bakers 1, 2, 3 and 4 had high serum RAST values and they reacted positively to antigenic bronchoprovocation while Bakers 5, 6 and 7 had low RAST values and negative reaction to antigenic challenge. Of the four bakers who had positive reactions to inhalation challenge with flour extracts, Subjects 1, 3 and 4 had a significantly higher

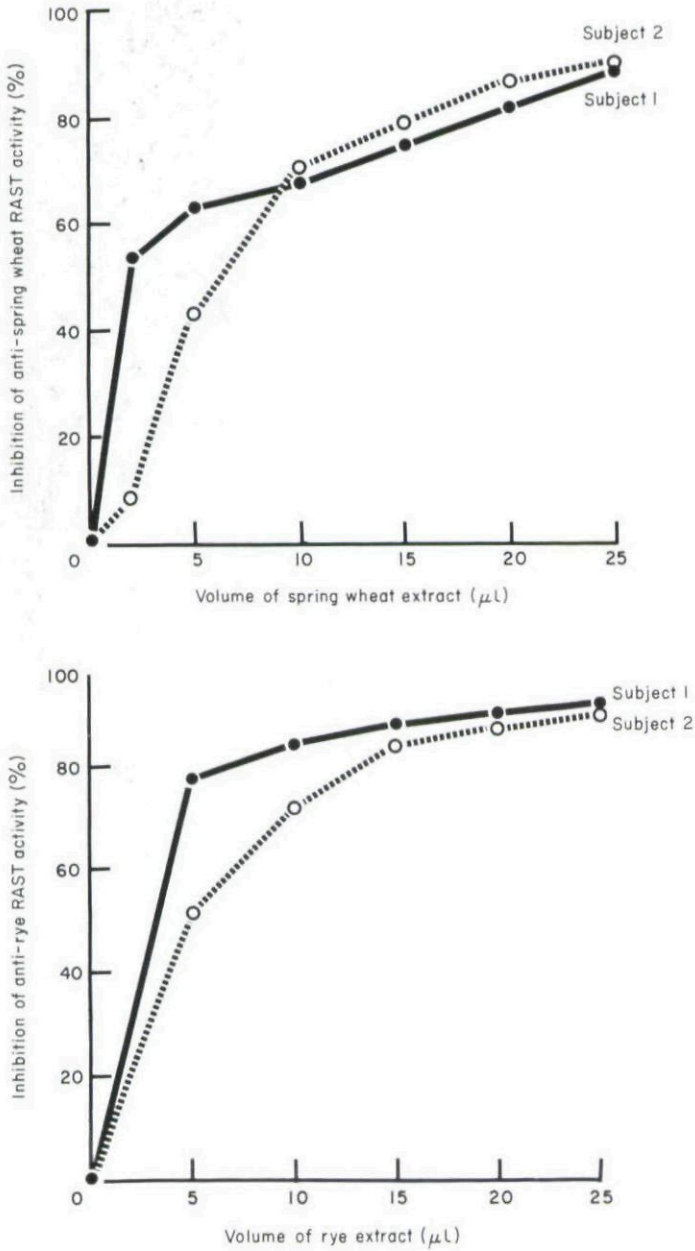


Fig. 2. Antigenic inhibition of serum RAST activity to rye and wheat in Bakers 1 and 2. (a) Percentage inhibition of anti-wheat RAST activity was plotted against the volume of wheat extract pre-incubated with the serum before RAST assay. (b) Percentage inhibition of anti-rye RAST activity was plotted against the volume of rye extract pre-incubated with the serum before RAST assay.

Table 4. Correlation between cutaneous sensitivity, RAST values, antigen-specific and non-specific bronchial reactivity

Subject	Cutaneous sensitivity (mg/ml)	RAST value	Antigen PC ₂₀ (mg/ml)	Methacholine PC ₂₀ (mg/ml)
<i>Rye extract</i>				
Bakers				
1	0.02	11.7	0.28	1.0
2	0.10	12.1	6.90	> 25.0
3	0.003	20.0	0.15	0.2
4	0.1	20.4	0.53	1.0
5	0.006	2.0	NR	> 25.0
6	0.5	3.8	NR	> 25.0
7	NR+	1.4	NR	> 25.0
Non-baker asthmatic control				
8	NR+	1.0	NR+	4.0
9	NR+	1.0	NR+	0.5
<i>Wheat extract</i>				
Bakers				
1	0.5	11.2	5.6	1.0
2	0.5	11.6	30.0	> 25.0
Non-baker asthmatic control				
10	NR+	1.4	NR+	0.7
11	NR+	1.2	NR+	> 25.0

NR+ = no reaction to 10% w/v extract.

NR = no reaction to 1% w/v extract.

degree of non-specific bronchial reactivity (low methacholine PC₂₀ values) and they reacted to a much lower dose of antigen PC₂₀ when compared with Subject 2.

There was no correlation between methacholine PC₂₀ and cigarette smoking in this small study. Of the three smokers (Subjects 1, 5 and 6) two subjects (5 and 6) had methacholine PC₂₀ greater than 25 mg/ml.

Serum precipitins against rye and wheat extracts

No serum-precipitating antibody was detected in any of the subjects in this study.

Discussion

The results of our study, as well as those from other earlier reports, (Bjorksten *et al.*, 1977; Blands *et al.*, 1976; Baldo *et al.*, 1980) indicate that the asthmatic and allergic nasal symptoms in workers with occupational exposure to flours are mediated by IgE antibodies specific for the flour antigens. Elevated levels of specific IgE antibodies as measured by the RAST were found in symptomatic bakers and a significantly elevated RAST value was correlated with quite typical symptoms of asthma in the subject's clinical history and a positive reaction to antigenic bronchoprovocation (Bakers 1-4). The three symptomatic bakers (Subjects 5, 6 and 7) with relatively low serum RAST values and negative response to antigenic inhalation challenge did not seem to have typical symptoms of baker's asthma clinically. They manifested either a chronic cough or bad wheezing only during episodes of upper respiratory infections (Table 1). Thus, the RAST could serve as a useful diagnostic test in the evaluation of workers with occupational asthma resulting from exposure to flours. When the RAST is not readily

available, skin testing with appropriate flour extracts could also provide useful but less accurate information in the diagnostic evaluation of patients with baker's asthma. A good correlation between the results of RAST and prick skin tests has been demonstrated in patients with pollen and animal allergies (Berg & Johansson, 1974). Our present study also showed a fairly good correlation among bakers between the cutaneous sensitivity to flour antigens and their serum RAST values. However, a significantly elevated serum RAST level provided a much more accurate predictive value than the cutaneous sensitivity on the patient's bronchial reactivity to antigenic challenge (Table 4).

In addition to level of flour-specific IgE antibodies in the serum, the degree of non-specific bronchial reactivity (as measured by the bronchial sensitivity to methacholine) was also an important factor which determined a baker's bronchial reaction to antigenic challenge. Among the four bakers with a positive response to inhalation challenge with a rye flour extract, the three subjects with a high degree of non-specific bronchial reactivity responded to a much lower dose of flour antigens (Table 4). This feature of flour-allergic asthma in bakers is similar to that observed in other asthmatics with allergies to pollens, cat or house dust mites as reported by Cockcroft *et al.*, 1979.

It has been shown by Gerrard *et al.* (1980) that bronchial reactivity was increased in smokers with normal lung function compared to non-smokers. In this study involving a small number of subjects we did not find a correlation between smoking and bronchial hyperreactivity. Of the three bakers who were smokers at the time of the study, only one (Subject 1) showed evidence of bronchial hyper-reactivity and this subject also demonstrated allergic skin and bronchial reaction to flour extract; the remaining two had no evidence of bronchial hyper-reactivity. It is difficult from this study to postulate that cigarette smoking was the cause of bronchial hyper-reactivity thus leading to development of asthma in sensitized subjects.

While most published reports implicated cereal flours as the responsible allergens (Wilbur & Ward, 1976; Hendrick *et al.*, 1976; Bush & Cohen, 1977) there has been a case report indicating that the grain mite, *Glycyphagus destructor*, might be an important allergen in a farmer who developed recurrent nocturnal asthma after exposure to grain dust (Davies, Green & Schofield 1976). Subsequent study by doPico *et al.* (1982) on a group of eleven grain-handlers showed that durum wheat was the constituent that was responsible for the bronchial reaction. Extracts of durum wheat airborne dust, grain insects and grain mites did not induce specific bronchial reaction in these subjects. It is however, interesting to note that four out of the seven (57.1%) bakers in this study had positive immediate reaction to extract of *Dermatophagoides farinae*, the house mite. The prevalence of positive immediated skin reaction to *Dermatophagoides farinae* in working populations in British Columbia varies from 10–20% (Chan-Yeung *et al.*, 1980). Although the number is small, it appears that among bakers with symptoms, there is a higher percentage with positive, immediate skin reaction to *Dermatophagoides farinae*. This could be due to cross-reactivity between dermatophagoides and grain storage mite (Cuthbert *et al.*, 1980), which frequently contaminated flour.

The effects of absorption with anti-IgE and with rye or wheat flour extracts (antigenic inhibition) on the serum RAST values indicated that the RAST activity indeed represents flour-antigen-specific IgE antibodies. These antibodies obviously mediated the immediate bronchospastic reaction when the symptomatic bakers were

challenged with rye or wheat flour extracts. A late asthmatic reaction was not observed in any of the bakers in our study and the serum precipitin test was also negative. The fact that an elevated level of flour-specific IgE antibodies was found only in a few symptomatic bakers but not in thirty-eight asymptomatic bakers with similar occupational exposures would suggest either that inhalation of particles of cereal flours through occupational exposure in bakeries induces a relatively low incidence of sensitization or more likely, it represents a survival phenomenon. Many sensitized bakers who have become symptomatic would have already left the industry for other types of work.

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