

IgE—antibodies and skin test reactions to a detergent—enzyme in Swedish consumers

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Summary

The frequency of raised levels of IgE antibodies against a detergent enzyme—subtilisin-A—was investigated in adult Swedish consumers. Of 1132 patient sera which were sent to the laboratory for routine RAST testing against various allergens a positive RAST to subtilisin was found in twenty-one (1.9%). Of 391 blood donors two had a positive RAST and one a doubtfully positive reaction. A positive prick test to subtilisin was obtained in four out of 122 patients with symptoms of allergic airways disease. Sensitization occurred more often among patients with an atopic constitution. About four out of five of the patients in whom IgE antibodies to the enzyme was found were women. Thirty-five patients (seventeen with a positive and eighteen with a negative RAST to subtilisin) were further investigated in order to see if results of RAST and skin test with subtilisin were correlated with clinical symptoms. Eight patients with positive RAST and skin test reported symptoms of an immediate allergic type in connection with exposure to enzyme detergents. Three patients had symptoms which could be attributed to residues of subtilisin in the laundry after washing. In six patients the sensitization did not seem to have any clinical significance. Taking into account the general use of enzyme detergents, sensitization has not been found to be a major clinical problem.

Introduction

Proteolytic enzymes derived from *Bacillus subtilis* have been used as an additive in washing powder in Sweden since 1966. In 1970 detergents containing enzymes held 40% of the market. The dominating enzyme preparation has been Alcalase® (Novo) which contains subtilisin-A.

In the late 1960s it was recognized that such enzymes could have sensitizing properties and that inhalation of the enzyme could cause asthma, rhinitis and type 3 reactions in the lung. Several reports on enzyme-induced allergic disease in workers in the detergent industry have been published (Flindt, 1969; Pepys *et al.*, 1969; Greenberg, Milne & Watt, 1970; Newhouse *et al.*, 1970; Göthe *et al.*, 1972; Witmeur *et al.*, 1973).

Sensitization to the enzymes in consumers was reported in Sweden by Belin *et al.* (1970) and in the USA by Falleroni & Schwartz (1971), Shapiro & *et al.*

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Eisenberg (1971) and Bernstein 1972. Two larger studies on the frequency of sensitization to detergent enzymes among allergic patients have been made. Bernstein (1972) found that a high proportion (about 25% of 353 atopic patients) had positive skin tests to one or two commercial enzyme preparations, while Pepys *et al.* (1973) in the United Kingdom found only two weakly positive skin reactions in 2500 tested patients with a respiratory allergy.

There is evidence that an atopic constitution is important for the development of enzyme sensitization, at least when exposure is low (Pepys *et al.*, 1973). We have studied the frequency of raised levels of IgE antibodies to subtilisin-A in sera from two groups of consumers, one with a high and one with a low proportion of atopics. We have also studied the occurrence of positive skin tests to subtilisin-A in a group of patients attending for allergic investigation for symptoms of asthma and rhinitis.

Patients with suspected allergy to subtilisin were selected as described below and were further investigated and interviewed regarding symptoms, evoked by exposure to enzyme detergents.

Methods

The enzyme preparations were commercial Alcalase® (about 2 Anson units/g) and crystalline subtilisin-A (32 Anson units/g) from Novo A/S Copenhagen. For skin tests both enzyme products were dissolved in Coca's solution to the strength of 10, 1 and 0.1 mg/ml and stored in +4°C. The 10 mg/ml solution was used for 6 months and the weaker solutions for 3 months. Skin testing was made with the prick test method on the volar aspect of the forearm as described by Pepys *et al.*, 1973. The radioallergosorbent technique (RAST) (Wide, Bennich & Johansson, 1967) was used to detect IgE antibodies in serum to the two enzyme preparations. About 3 mg of each enzyme preparation, freshly dissolved in 3 ml of 0.1 M sodium bicarbonate solution, was mixed with 300 mg of CNBr-activated microcrystalline cellulose particles (Wide, 1969) during the coupling procedure. The suspension of the enzyme cellulose conjugates (1 mg/ml) were added in 0.5 ml aliquotes to 0.05 ml serum for each test. To diminish the non specific uptake of radioactivity on the particles during the test, non activated cellulose particles were added to the solution with ¹²⁵I-labelled rabbit anti-IgE antibodies. After centrifugation the supernatant was diluted so that about 80,000 ct/min in 0.1 ml was added to each test tube. About 65% of the immunosorbent purified ¹²⁵I-labelled antibodies were immunoreactive to IgE. For the negative control sera the mean uptake of radioactivity was about 240 ct/min. The result was considered as negative when the activity was less than 500 ct/min which roughly corresponds to mean + 2 s.d. for the negative controls. All reactions exceeding this value were considered as possibly positive and the sera were retested. The results were then regarded as positive only when the reaction was positive in the second assay. The results were considered as doubtfully positive when the activities were between 500 and 800 ct/min in both tests. The clinical significance of a positive or negative RAST reaction against common allergens was recently reviewed by Wide (1973).

Patient material

Three groups of subjects were investigated

(1) At the clinical chemical laboratory of this hospital the RAST method was used in routine investigation of allergic patients. During November 1969–November 1971

sera from 1132 patients were sent to the laboratory for investigation of IgE antibodies to various allergens. These sera were investigated for IgE antibodies to subtilisin-A. Judging from histories and test results about 80% of the patients were atopics. Sex distribution and mean ages ($\pm$ s.d.) are given in Table 1.

(2) During 1971 and 1973 sera from 391 blood donors were RAST tested for IgE antibodies against subtilisin. Before being accepted as blood donors all had signed a statement that they had no allergic symptoms. The frequency of atopics in this group should thus be low. Sex distribution and mean ages ($\pm$ s.d.) are given in Table 1.

(3) During November 1971–February 1972 all patients, totalling 122, coming for their first visit for investigation to the allergy department of the lung clinic were prick

Table 1. Results of RAST and prick tests to subtilisin in various groups of consumers of washing powder in Sweden

				Positive RAST	
				<i>n</i>	%
Sera from patients suspected to have atopic allergy	Total	1132	33.3 ± 12.3	21*	1.9
	male	498	32.7 ± 12.2	4*	0.8
	female	634	33.8 ± 12.4	17	2.7
Sera from blood donors 'non atopics'	Total	39	30.4 ± 9.1	3*	0.77
	male	294	31.8 ± 9.3	1	0.34
	female	97	27.5 ± 7.2	2*	2.0
				Positive skin test	
Patients with symptoms of asthma and/or rhinitis	Total	122	32.2 ± 13.8	4	3.2
	male	49	29.7 ± 13.2	1	2.0
	female	73	33.9 ± 14.0	3	4.1
	Atopics	82	29.8 ± 12.0	4	4.9
	male	38	27.8 ± 11.7	1	2.6
	female	44	31.6 ± 12.1	3	6.8
	Non atopics	40	37.0 ± 16.0	0	0
	male	11	36.4 ± 16.5	0	0
	female	29	37.2 ± 16.0	0	0

* The figure includes one serum with a doubtfully positive reaction.

tested with Alcalase®, 10 mg/ml. The number of atopics in this group was determined from history and result of skin test and RAST. Patients who had either a positive provocation test or a positive RAST or more than two positive skin tests to common allergens were classified as atopics. Four patients had two positive skin tests to common allergens and a negative test to Alcalase®. Three of them were regarded as atopics because of disease and family history. In the non atopic patients the diagnosis in most cases were intrinsic asthma or vasomotor rhinitis. Sex distribution and mean ages ($\pm$ s.d.), and the proportion of atopics and non atopics are given in Table 1.

A clinical evaluation of symptoms, which could be due to Alcalase®, was made in thirty-five patients. This group consisted of patients whose sera gave a possibly positive reaction in the first RAST screening (twenty-four patients) or who had positive or

doubtfully positive results with skin tests (eleven patients). These patients were informed that they might be allergic to enzymes in washing powders and asked to come to the allergy department for further investigation. They were also asked to recall which washing powders they had used and whether they had noted any symptoms from them. Prick tests were in these patients done with Alcalase® and crystalline subtilisin-A in three different concentrations (10, 1, 0.1 mg/ml). To avoid bias in the interpretation of skin tests and histories the investigator had no knowledge of the results of the individual RAST reactions.

Results

The results of RAST and prick tests in the various groups are given in Table 1.

IgE antibodies to subtilisin were found in twenty-one of the 1132 investigated sera from patients with the suspected allergic disease (group 1). Positive tests were 3–4 times more frequent in women than in men. In this group of twenty-one, nineteen were atopics, one was considered non atopic (according to data in the required case record) and in one patient data was not sufficient for classification. Mean age of the patients with a positive RAST test for subtilisin (26.8 ± 8.2 years) did not differ from the mean age of patients in group 1.

Out of the 391 serum samples from blood donors two gave a positive and one a doubtfully positive RAST reaction to subtilisin (Table 1). One of the sera with IgE antibodies to subtilisin was from a 30-year-old man with no allergic symptoms and no atopic diseases in the family. He used enzyme detergent regularly for manual washing without noticing any symptoms. The other subject with a positive RAST to subtilisin was a 31-year-old woman who sometimes had attacks of sneezing, running nose and slight conjunctivitis. Her grandmother and father suffered from asthma and her sister had eczema since childhood and slight allergic rhinitis. She used enzyme detergents regularly but had not noticed symptoms from them. Prick tests were positive to house dust and strongly positive to subtilisin-A and Alcalase®.

The blood donor serum which gave a doubtfully positive RAST reaction to subtilisin was from a 28-year-old woman who had had eczema as a child. In the last 3 years she had had occasional attacks of tightness in the chest especially during the nights. She had a positive prick test to *Dermatophagoides pteronyssinus* and birch pollen and a negative prick test to subtilisin-A and Alcalase®. She used enzyme detergents regularly but had not noticed any symptoms from them.

In the prick test screening of 122 outpatients from the allergy department (group 3) eleven had a positive, weakly positive or doubtfully positive reaction to 10 mg/ml of commercial Alcalase®. These patients were retested with 10, 1 and 0.1 mg/ml of Alcalase® and crystalline subtilisin-A. Three patients got a strong reaction on both extracts, even with the weakest concentrations of 0.1 mg/ml. One patient had a weak reaction obtained with 1 mg/ml of Alcalase® and 10 mg/ml of crystalline subtilisin-A. Three patients had small ($\leq 2 \times 2$ mm) reactions of an atypical appearance with one of the extracts in the highest concentrations. These reactions were considered negative. Four patients had no reactions to the subtilisin when retested.

The agreement between the results of RAST and prick tests to Alcalase® and crystalline subtilisin-A in the thirty-five patients with a suspected allergy to subtilisin and who were subjected to a more thorough investigation is shown in Table 2. A positive RAST test to subtilisin was obtained in sixteen and a doubtfully positive test in one of these patients. Their clinical data are presented in Table 3.

The RAST titres were doubtfully positive to crystalline subtilisin-A and weakly positive to Alcalase® in patient no. 1. In patient no. 2 the titres were considerably higher against Alcalase® than against crystalline subtilisin A. Patient no. 7 had weakly positive RAST titres to both Alcalase® and crystalline subtilisin-A. In the other patients the RAST titres were high to both enzyme preparations.

Prick tests were typically positive even with the most diluted extracts of Alcalase® and crystalline subtilisin-A in fifteen patients—fourteen women and one man. The prick test reactions to the 10 mg/ml extracts were in these patients very strong and in ten patients the reactions were as big as positive intracutaneous tests and in some the testing caused a reddening of the entire forearm.

One patient with a positive RAST test (number 2) had a weakly positive skin test. Another patient (number 1) had a small weal with no flare with the strongest subtilisin extract and this reaction was considered negative.

Table 2. Results of RAST and prick test to subtilisin in thirty-five patients with a suspected allergy to detergent enzyme

	RAST		
	pos.	doubtful	neg.
Prick test			
pos.	15	0	0
weak	1	0	2
neg.	0	1	16

Of the eighteen patients with negative RAST tests to subtilisin two had weakly positive skin reactions. In five patients small ($\leq 2 \times 2$ mm) weals without flare were recorded after testing with one of the strongest extracts. These tests were regarded as negative. The other eleven patients had completely negative skin tests.

A late skin reaction consisting of an oedematous, warm and slightly red swelling of the forearm was noted in five patients (patients number 6, 14, 15, 16 and 17). In three patients the reaction had subsided in 24 hr, in the other two patients it persisted for 2 and 3 days respectively. These patients had strong positive immediate prick tests and their reactions looked like the dual skin tests seen in allergic aspergillosis.

In this group of patients with a suspected allergy to subtilisin all with a positive RAST test to subtilisin were atopics, while five of the eighteen patients with a negative RAST test were non atopics. The sex distribution among the patients with a negative RAST to subtilisin was nine women and nine men and among those with a positive RAST test it was fourteen women and three men. All patients with a positive RAST test to subtilisin reported that they had used enzyme detergents while only thirteen of the eighteen patients with a negative RAST test could recall that they had used them.

Ten of the patients with a positive and seven of the patients with a negative RAST test had noticed sneezing or a feeling of irritation in the airways or in the eyes when exposed to washing powder, e.g. when opening a new pack of detergent or pouring

the powder into the sink or washing machine. In five of these patients, all with a positive RAST to subtilisin (patients number 4, 7, 8, 10 and 17), symptoms of an immediate allergic type (e.g. itch in the eyes) were recorded. Skin symptoms from manual washing was reported by sixteen patients, ten with a positive and six with a negative RAST test. Two patients with a positive RAST test (patients number 4 and 5) had noted itching immediate skin symptoms from hand washing. The other fourteen patients reported itch, eczematous-like reactions or dryness of the skin, which might be a pure detergent effect. Three patients, all with a positive RAST test to subtilisin (numbers 10, 12 and 14) had symptoms which could be attributed to enzyme residues in the laundry. In some other patients with symptoms of house dust allergy (e.g. 4, 9, 15 and 17) it is probable that residues of enzymes in the laundry contributed to their symptoms. Thus, as judged by history, at least eight of the patients were considered to have some allergic symptoms of the immediate type due to enzyme detergents.

Discussion

Raised titres of IgE antibodies in serum against subtilisin were found in about 2% of 1132 patients, investigated for a suspected allergic disease. This frequency must be considered high in a population with only consumer exposure to the detergent enzyme. The frequency corresponds well to the finding of three strong and one weak positive prick test to subtilisin in 122 patients with signs of respiratory allergy.

In spite of using the prick test method the positive immediate reactions were very strong. Prick tests usually are not inconvenient to the patients and do not cause intense late reactions but in some of the tested patients they did. It would probably have been possible to do the tests with equally good results with ten times weaker concentrations, that is 1, 0.1, 0.01 mg/ml. The findings in this investigation support the view that atopics are more readily sensitized than non atopics. In group 1 twenty patients with a positive RAST to subtilisin could be classified regarding their atopic status, nineteen were atopics. Out of the three blood donors with positive RAST reactions two could be considered atopics. In group 3 the four patients with a positive prick test to subtilisin were atopics.

Women in Sweden probably do more washing than men and therefore are more exposed to detergent enzymes. This might be an explanation for the high frequency of females among sensitized patients. This sex distribution and the fact that all patients with a positive RAST had been using enzyme containing detergents makes it probable that they had been sensitized through the use of such washing powders.

The frequency of sensitization to detergent enzymes found in this investigation is very high compared to the findings of Pepys *et al.* (1973) in London. This might be explained by a different exposure to the enzymes. In Sweden from 1966 to 1969 the enzymes were added to the detergents in form of a powder. During 1969 the fabrication procedures were changed and from 1970 almost all enzymes were added in a granulated form. This is in contrast to the situation in Great Britain, where production of enzyme detergents on a large scale took place later. With the exception of small quantities used in a few low volume products in the United Kingdom the enzymes have been added to the washing powders in a low-dust, granulated or capsulated form. The frequency of positive skin tests to detergent enzymes reported by Bernstein (1972) is high compared to the sensitization rate that we have found. The difference may partly be due to different methods of investigation. Some of the positive intracutaneous

Table 3. Clinical data of patients with a positive RAST test to Alcalase*

Patient no.	Sex	Age	Occupation	Disease history	Exposure	Symptoms from washing powder (w.p.), manual washing or laundry	Prick test to subtilisin*
1 LÅ	M	22	Student	Asthma and eczema from childhood. Now only small eczematous manifestations. Pollen rhinitis from 3 years.	Washes sometimes by hand. No regular use of enzyme detergents.	Sneezing from some w.p.	2 × 2 No flare. Regarded as negative.
2 JE	M	24	Student	Severe atopic eczema from childhood. Asthma sometimes in moisty weather and from dust exposure. Rhinitis from animal dander.	Does not wash often because of eczema. Occasional use of enzyme detergents.	No symptoms noted from w.p. Avoids manual washing which always irritates his skin.	2 × 3 Weakly positive
3 BB	M	27	Engineer	Asthma in childhood until age 12. Recurrence of asthma at age 21 after animal contact. Allergic to <i>Dermatophagoides</i> and animal dander.	Mostly machine washing. Sometimes manual washing in enzyme detergent.	Sometimes sneezing from w.p. Not noticed symptoms when washing manually. Sometimes itch around the neck from newly washed clothes.	8 × 6
4 MS	F	19	Student	Rhinitis and conjunctivitis from grass and compositae pollen from age of 7. Slight symptoms from dog and house dust. Spontaneously noticed that w.p. gave the same symptoms.	Manual washing regularly, mostly in enzyme detergent.	Conjunctivitis and slight rhinitis when washing. When using enzyme detergent she is forced to use gloves. If not she gets itching of the hands and then eczema.	21 × 12
5 MC	F	19	Children's nurse	Eczema in childhood. Asthma and slight rhinitis from 12 years of age. Allergic to house dust and animal dander.	Manual washing regularly. Often in enzyme detergent.	Not noticed anything from w.p. Reddening of hands and forearms and sometimes itching, urticaria-like eruptions of the forearms when washing. Sometimes itching from tight fitting clothes.	8 × 9
6 AME	F	20	Housewife	Grew up on a farm. Severe asthma and rhinitis since early childhood. Sensitive to animal dander. Last year living in town and almost symptom free.	Manual washing regularly. Occasional use of enzyme detergents.	Sneezing from w.p. Not noticed symptoms when washing.	5 × 3 Late skin reaction for 24 hr.
7 AS	F	20	Student	Rhinitis and conjunctivitis in the summer from 3 years, occasional slight symptoms in the winter. Allergic to grass and artemisia pollen. Spontaneously noticed that w.p. can give the same symptoms.	Machine and manual washing, mostly in enzyme detergents.	Sneezing and stuffed nose from w.p. Manual washing causes itch on hands and forearms. Almost always rhinitis and conjunctivitis in the college laundromat where enzyme detergents are much used.	9 × 7
8 EE	F	21	Student	Flexural eczema and asthma in childhood. From age 6 only slight asthmatic symptoms until 1970 when she got rhinitis and more intense asthma. Allergic to cat, house dust and grass pollen.	Machine and manual washing. Stopped using enzyme detergents 1971.	Not noticed anything directly from w.p. Stopped using enzyme detergents because they caused itch especially in the elbow flexures. Conjunctivitis with itch and irritation of the bronchi when in the college laundromat.	6 × 7
9 GH	F	23	Housewife and secretary	Asthma from age of 4. Slight rhinitis in the summer. Allergic to house dust and grass pollen. Asthma much better since 1972.	Manual washing often in the bath tub until Nov. 1971. Since then machine washing. Regular use of enzyme detergent.	Not noticed anything from w.p. or from washing. Sometimes urticaria-like eruptions especially after bathing.	16 × 1
10 AL	F	23	Student	Several atopic diseases in close relatives. Attacks of sometimes severe urticaria during the summers 1968, 1971 when living in the summerhouse.	Enzyme detergent for manual washing in cold water during the summers until 1971. Other times machine washing in non-enzyme detergent.	Rhinitis and conjunctivitis from enzyme w.p. Slight itch on her hands when washing by hand. Stopped using enzyme detergent 1971; since then completely free from urticaria.	10 × 9
11 CJ	F	24	Housewife and school-teacher	From age of 4 years sneezing and rhinitis in the mornings. Symptoms much worse in May. Allergic to Birch pollen. Weak RAST and provocation reactions to house dust. Spontaneously noticed that detergents cause eczema of her hands.	Manual washing until 1970, since then machine washing. Regular use of enzyme detergent.	Rhinitis and sneezing from w.p. Gets eczema on her hands after manual washing.	13 × 10
12 FJ	F	25	Secretary	Summertime rhinitis since 1969. Since 1971 perennial symptoms. Wakes up in the night with stuffed nose, sneezing in the mornings. Allergic to grass and artemisia pollen. Only weak skin reaction and negative RAST to house dust and <i>Dermatophagoides pteronyssinus</i> .	Manual washing until 1972, since then machine washing. Regular use of enzyme detergents until March 1973.	Sneezing from w.p. No skin symptoms from manual washing. Night and morning rhinitis completely gone when she changed to a non-enzyme detergent. Observed that clothes washed in enzyme detergents could cause rhinitis.	24 × 14
13 YB	F	29	Housewife	Eczema of hands and elbow flexures since 18 years of age. Asthma especially during winter months since 1968.	Manual washing until 1969, since then machine washing. Regular use of enzyme detergents.	Itch on her hands from w.p. and manual washing.	11 × 8
14 GBJ	F	36	Housewife	Slight asthma from 20 years of age. More intense symptoms especially in the nights from 1971 to spring 1972. Allergic to animal dander and house dust.	Machine washing. Stopped using enzyme detergent April 1972.	Not noticed symptoms from w.p. Severe itch and sometimes small urticaria-like eruptions from newly washed sheets. Symptoms disappeared when she changed to w.p. without enzymes. Asthma much better during 1972 and since 1973 almost quite symptom free.	30 × 25 Intense late reaction for 24 hr.
15 VE	F	37	Nurse in X-ray department	Summertime rhinitis from 10 years of age. Slight asthmatic symptoms during the winter 1970-71. Allergic to grass pollen. Weak i.e. test and neg. RAST to house dust.	Machine washing. Manual washing occasionally. Stopped using enzyme detergents 1971.	Not noticed symptoms from w.p. or from manual washing. She had her asthmatic symptoms during the time she used enzyme detergents.	14 × 6 Weak late reaction for 10 hr.
16 LA	F	39	Housewife	Eczema in childhood. Severe asthma until 15 years of age. Since then only occasional wheezing and rhinitis. Allergic to house dust, <i>Dermatophagoides</i> and animal dander.	Machine washing since 1971. Stopped using enzyme detergents 1971.	Not noticed symptoms from w.p. Manual washing caused itch and eczematous-like reactions on her hands.	17 × 11 Severe late reaction lasting for 2 days.
17 MLP	F	47	Housewife, 1/2 time work in tailor's shop	From 1969 to 1971 eye irritation, sneezing, rhinitis and sometimes slight wheezing. Dog at home since 10 years. Spontaneously noticed that w.p. could give the symptoms. Moderately pos. i.e. test and weakly pos. RAST to dog and house dust.	Machine washing since 1970. Stopped using enzyme detergents autumn 1971.	Sneezing, rhinitis and itch in the eyes from w.p. Eczema under her rings after manual washing. The perennial rhinitis much better since she stopped using enzyme detergents.	15 × 9 Strong, late reaction lasting for 3 days.

* Figures indicate the measured length in mm of two diameters at right angles of the weals after prick test with 10 mg/ml subtilisin-A.

skin reactions in Bernstein's patients might have been caused by factors other than IgE antibodies. The existence of short term skin fixing antibodies of the IgG class have been described (Parish, 1970). The possibility of an irritant effect of the enzymes when injected intracutaneously in highly atopic patients cannot be excluded. Some of our prick tested patients showed small reactions without flare on the strongest extract concentrations. These reactions had an atypical appearance and were considered negative and probably due to irritating properties in the extracts.

Allergy to enzymes in washing powder does not seem to be a great clinical problem in Sweden. In one patient (no. 10) with urticaria the enzyme allergy seemed to be the sole cause for her seeking medical advice. In two patients (nos. 12 and 14) a major part of their symptoms could be attributed to enzyme allergy. In eight patients a minor part of their symptoms might have been due to the enzyme sensitization while in six it seemed to be a coincidental finding.

In three patients there were symptoms which may have been attributable to residues of the enzyme preparations in newly washed laundry. Several patients had a positive RAST and prick test to subtilisin which may have contributed to their respiratory symptoms of house dust allergy. Whilst the risk of sensitization by the enzymes may be very much reduced when detergents with granulated enzymes are used, it seems that their use by atopic subjects is not to be recommended, and it also cannot be ruled out that such subjects may be sensitized by long exposure to small amounts of enzyme residues in the laundry.

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