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PRICK- AND RADIOALLERGSORBENT TESTS (RAST) TO ALCALASE® IN A POPULATION NOT EXPOSED TO ENZYME DETERGENTS

By

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Alcalase®, a commercial enzyme preparation derived from *B. licheniformis*, has been incorporated in laundry detergents since 1962. Allergy testing has been performed by several investigators mainly due to the possibility of subtilisin provoked respiratory symptoms among workers in the detergent industry. Prick-testing has been the most commonly used procedure (1-11). Other workers have used the radioallerg-sorbent test (RAST), and a good correlation has been found between the results of RAST and prick-test to Alcalase (13, 14).

However, since Alcalase is produced from a common and ubiquitous microorganism the possibility exists that positive reactions to Alcalase may be a result of exposure to that microorganism. It was therefore the aim of this study to determine the frequency of RAST positive and prick-test positive persons living in an environment that had never been exposed to Alcalase via washing powders.

MATERIAL AND METHODS

The material included three groups of individuals in Rumania, where at the time of the investigation (1971), enzyme detergents had not been introduced.

The first group consisted of 100 atopic patients. Sixty-seven suffered from allergic asthma, four from allergic asthma and atopic dermatitis, six from atopic dermatitis alone and 23 from hayfever. The second group consisted of 181 non atopic patients. A total of 176 had asthma, and five had dermatitis of a non atopic type. The third group consisted of 20 healthy volunteers.

Prick-test

The ventral surface of the arm was washed with water and dried. A drop of an Alcalase test solution was placed on a previously marked area. The tip of a no. 20 hypodermic needle was passed through the drop into the skin. The needle was withdrawn and surplus solution wiped off with a paper towel. The test site was observed for 10 min after which the reaction was read and the weal diameter recorded. A control was performed simultaneously with sterile saline. For each test series a freshly prepared enzyme solution was used.

Alcalase test solution:

Crystalline Alcalase 15.2 Anson U/g, dialyzed, freeze dried	1.00 mg
Sodium chloride	9.00 mg
Sodium dihydrogen phosphate	0.19 mg
Di-sodium hydrogen phosphate	1.19 mg
Aqua sterilisata ad	1.00 ml

RAST

Venous blood samples were allowed to clot after which they were centrifuged for 10 min at 2,000 rev/min. The serum was pipetted into polypropylene tubes with polypropylene screw caps and stored in the deep-freezer until approximately 30 samples had been collected. The samples were airfreighted in containers with dry ice to the department of clinical chemistry, University Hospital, Uppsala, Sweden, where the *in vitro* testing was carried out according to the method of Wide et al. (12, 14).

RESULTS

The results of the prick-test examinations of the group of 100 atopic patients are summarized in Table 1. A weal diameter ≥ 5 mm was considered to be a positive reaction while a 3–4 mm weal was registered as a doubtful positive reaction. Control weals are recorded in the Table, and reactions were only considered positive or doubtful positive when the diame-

TABLE 1
Wealing Response to Alcalase® and Saline. First Group of Patients.

Weal diameter (mm)	No. of patients	
	Alcalase	Saline
0	69	81
1-2	18	18
3-4	7	1
≥5	6	0
Total	100	100

ter of the Alcalase weal was at least 2 mm larger than that of the control.

Prick-tests gave six (6 per cent) positive reactions and seven (7 per cent) doubtful positive reactions. There were no positive RASTs. The medical history of the six patients with a positive prick-test is given in Table 2. In five of the six patients the diameter of the weal was at least 5 mm larger than the saline control.

Table 3 shows the results of the second group comprising

TABLE 2
Clinical Data of Six Atopics with Positive Prick-tests to Alcalase®.

No.	Age	Sex	Occupation	Disease	Main allergen	Weal diameter (mm)		Notes
						Alcalase	Saline	
1	44	f	housewife	asthma, atopic dermatitis	house-dust	7	0	fam. atopy
9	28	f	clerk	asthma, atopic dermatitis	pollen	10	0	fam. atopy
12	23	m	foreman	asthma	pollen	5	0	
93	40	m	foreman	hayfever	pollen	5	1	
99	41	f	clerk	asthma, atopic dermatitis	house-dust	10	3	
103	41	m	mechanic	hayfever, atopic dermatitis	pollen	12	2	

TABLE 3
Wealing Response to Alcalase® and Saline. Second Group of Patients.

Weal diameter (mm)	No. of patients	
	Alcalase	Saline
0	107	128
1-2	28	41
3-4	35	10
≥ 5	11	2
Total	181	181

non atopic patients. Only one patient in the second group (no. 34, Table 4) had a positive RAST although there were nine (5 per cent) with positive prick reactions, and five of these had a wealing reaction with a diameter at least 5 mm larger than the saline control. A total of 23 (13 per cent) had doubtful positive reactions when the above mentioned criteria of a 2 mm difference in diameter was observed. The results from examination of the third group of 20 healthy volunteers are presented in Table 5. There were no positive prick-reactions, and only one in the 3-4 mm diameter group had a weal diameter 2 mm larger than the control, and could be considered doubtful positive.

TABLE 4
Clinical Data of Nine Non-atopics with Positive Prick-tests to Alcalase®.

No.	Age	Sex	Occupation	Disease	Weal diameter (mm)	
					Alcalase	Saline
2	59	f	housekeeper	asthma	9	4
4	49	f	" "	"	6	2
22	27	f	caterer	"	6	0
34*	38	m	technician	"	5	2
51	48	f	weaver	"	7	0
65	10	m	schoolboy	"	6	2
76	26	f	laundress	"	14	6
78	61	m	retired	dermatitis	5	3
71	32	m	professor	asthma	5	0

* RAST positive.

TABLE 5
Wealing Response to Alcalase® and Saline. Third Group.

Weal diameter (mm)	No. of persons	
	Alcalase	Saline
0	13	13
1-2	4	5
3-4	3	2
≥ 5	0	0
Total	20	20

DISCUSSION

The results from the first two groups were very similar and showed that positive prick-test reactions occur quite frequently in a non enzyme exposed population. The group of healthy volunteers showed no positive prick-tests, and had a smaller percentage of doubtful positive tests (5 per cent). This group, however, was too small for valid comparison.

In the whole material there was only one positive RAST to Alcalase (0.3 per cent). This patient also had a positive prick-test. It was not possible to obtain information regarding a possible occasional exposure to enzyme detergents (travel abroad, gift parcel, etc.). This finding may be compared with the higher frequencies of positive RASTs to Alcalase found in Sweden by Zetterström & Wide (14).

With 15 positive prick-test reactions and only one RAST positive person no agreement was found between the two tests in this material of non enzyme detergent-exposed individuals. This has a bearing on testing in areas where detergent enzymes are common. Although normally, individuals with a positive prick-test to subtilisin can be assumed to be allergic to these proteolytic enzymes, it can be recommended in doubtful cases to use a RAST as supplement before the final diagnosis of allergy is made.

SUMMARY

A total of 301 persons in a population unexposed to enzyme detergents (Rumania) were prick-tested with Alcalase®, and RASTs were performed on their sera. The material consisted of three groups, one atopic, the second non atopic, and the third healthy. In the first two groups the percentage of positive prick-test reactions amounted to 6 and 5 per cent respectively. Only one positive RAST was found (second group). In the third group no positive prick-tests were found and no positive RASTs.

The majority of the positive prick-tests were considered non specific, and no correlation was found between results of prick-tests and RASTs in this material of non exposed individuals.

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