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Skin test reactivity in infancy

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Skin tests represent a major tool in the diagnosis of reaginic allergy; however, their interpretation does not appear to be without difficulty in children under the age of 3 yr. Seventy-eight infants from birth to 24 mo were prick tested and compared with 30 nonallergic adult subjects. Skin tests were performed without bleeding by use of two strengths of histamine hydrochloride (1 and 10 mg/ml), a mast cell degranulating agent (codeine phosphate, 50 mg/ml), and allergenic extracts. Negative control solution elicited a small wheal (<1.5 mm) in two infants who were excluded from further results. A clear and significant ($p < 0.001$) hyporeactivity to both histamine and codeine phosphate was observed in infancy, especially before the age of 6 mo. Six infants were allergic and presented positive prick tests to either food or inhalant allergens. These tests were confirmed by serum specific IgE and a suggestive clinical history. The size of the allergen-induced prick test wheal ranged from 2 to 5 mm in diameter, suggesting that prick test wheals may be smaller in infants. This study confirms that prick tests can be performed and interpreted without difficulty in infants, keeping in mind the small wheal size induced by both positive control solutions and allergen-induced prick tests. (J ALLERGY CLIN IMMUNOL 75:646-51, 1985).

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Skin tests represent a major tool in the diagnosis of immediate type hypersensitivity.¹ Different techniques of skin tests have been proposed. The intradermal reaction has been widely used. It has a great sensitivity but is not necessarily reproducible.² The intradermal test is more difficult to perform, especially in children, owing to the texture of the skin and the

pain induced during the test. Moreover, in young children the relatively small area available for skin testing makes it possible to perform only a few intradermal tests. Scratch tests are simple to perform but introduce a variable amount of antigen into the epidermis and therefore are very difficult to standardize. Prick tests,³⁻⁵ when they are properly performed, are sensitive, quick, and simple. It has been estimated that they introduce a volume of 0.001 μ l of extract into the epidermis,⁶ and very few systemic reactions occur when they are carried out. Prick tests are totally harmless, and the reproducibility may be improved by use of the recent standardized devices.⁷ Moreover, the comparison of reactions of the prick tests performed with allergen to that induced by a positive control solution appears to make their interpretation clearer. Allergic symptoms have been recognized to occur very early in infancy,⁸⁻¹¹ and it is therefore desirable to define whether or not the value of skin tests is similar throughout life. Moreover, some investigators have used skin tests to diagnose allergic disorders in infancy,¹²⁻¹⁶ whereas others have stated that skin tests could only be used in childhood.¹⁷⁻¹⁸

The cutaneous reactivity of infants and neonates is reduced,¹⁹⁻²³ but there are no clear data on the infant skin reactivity to prick testing with positive control solutions other than histamine.²⁴ Therefore, the assessment of the reactivity of the neonate to the prick test and infant skin to positive control solutions and allergens in order to determine (1) whether there is a hyporeactivity of the infant skin with this method of skin test, and (2) whether prick tests may be used safely and accurately in infants is important. Two different positive control solutions were used, one to appreciate the whealing capacity of the skin (histamine) and the other to appreciate the cutaneous mast cell releasability (codeine phosphate).²⁵

MATERIAL AND METHODS

Subjects

Seventy-eight infants ranging in age from 4 days to 24 mo (mean $\pm$ SD: 7.75 $\pm$ 6.67 mo) were tested. Twenty-five of the infants were less than 3 mo of age, 16 infants were between 3 to 6 mo, 20 infants were from 6 to 12 mo, and 17 infants were from 12 to 24 mo of age. Of these seventy-eight infants, 35 were girls and 43 were boys. They were compared with 30 nonallergic adult subjects ranging in age from 18 to 32 yr (26.1 $\pm$ 7.2 yr) (mean 16 yr). The study was performed after informed consent was obtained from the parents of the infants and from the adult volunteers.

All infants and adult subjects were carefully selected to exclude all subjects suffering from any skin disease or receiving any medication that would interfere with the skin tests, i.e., no antihistamines, no benzodiazepines, and no theophylline derivatives for 72 hr, no corticosteroids or tet-

racosactide for 1 wk, and no ketotifen or imipramine for the previous 3 wk.

METHODS

Positive control solutions. All subjects were tested with three positive control solutions prepared in 50% glycerol by the Laboratoire des Stallergènes (Fresnes, France), histamine hydrochloride, 1 and 10 mg/mg, and codeine phosphate, 50 mg/ml.

Negative control solution. All subjects were tested with a 50% glycerol solution (Laboratoire des Stallergènes).

Allergen extracts. All infants were tested with three food and two inhalant allergen extracts prepared by the Laboratoire des Stallergènes in 50% glycerol. Inhalant allergens were standardized by means of in vivo and in vitro tests,²⁶ i.e., RAST inhibition and isoelectric focusing.

Foods were fish, whole egg, and milk (10%, w/v), and inhalants were cat and *Dermatophagoides pteronyssinus* (100 index of reactivity (biological unit)). These extracts were lyophilized and reconstituted every 2 wk in 50% glycerol because the shelf life of these extracts was estimated in a previous study.²⁶

Prick tests. All prick tests were performed by the same investigator by use of the modified prick test technique proposed by Pepys³ between 7 and 10 A.M. to avoid possible circadian difference. Prick test was performed by directing the needle (32 by 7/10 mm) through the drop at an angle of 60 to 70 degrees to the skin. The needle point only "catches" the skin and lifts it, thus making a minute canal through which the extract penetrates. The tests were done on the palmar surface of the forearm. The tests were performed a second time if there were bleeding.²⁷ Skin tests were recorded after 10 min for negative and positive control solutions and after 15 min for allergen extracts.

Serum specific IgE. Serum specific IgE to the allergens that were also tested by skin tests was performed by RAST (Pharmacia AB, Uppsala, Sweden).

Statistical evaluation of the results. Nonparametric analysis of the data was performed by the Mann-Whitney U test.

RESULTS

False positive reactions

The negative control solution did not induce any wheal in all but two cases. Two infants 3 and 5 mo of age had a positive reaction with the negative control solution. The wheal size was 1 and 1.5 mm, respectively, in diameter. Because these infants had a false positive reaction, they were not included in the study.

Positive control solutions

Results are illustrated in Figs. 1 and 2 and Tables I and II. Both strengths of histamine and codeine phosphate always elicited in adults a positive wheal-and-flare reaction. The whealing capacity of the skin to histamine was found to be significantly reduced in infants, especially in those who were less than 6 mo

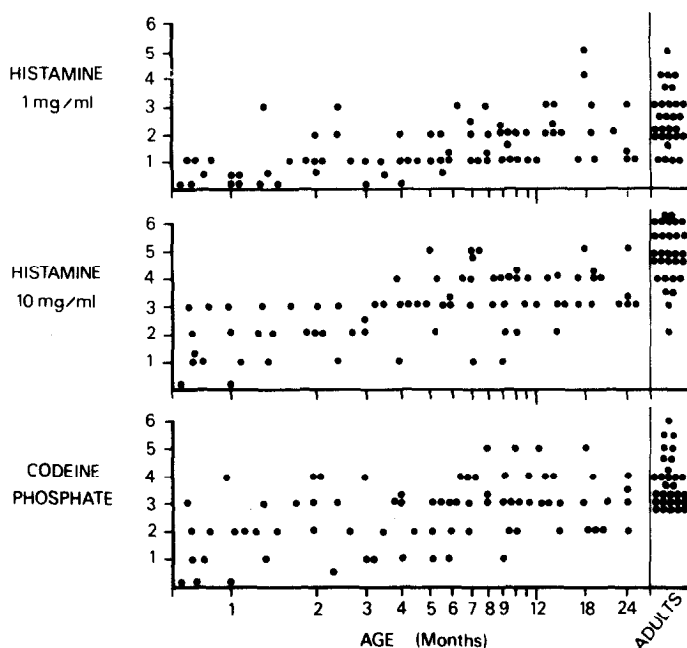


FIG. 1. Wheal size induced by positive control solutions in infants and adult subjects. Results are listed in millimeters.

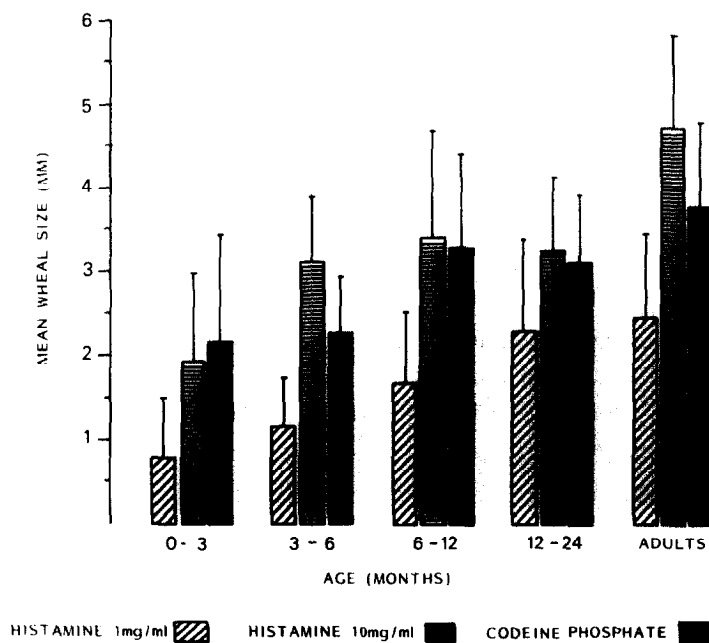


FIG. 2. Mean wheal size of the wheals induced by positive control solutions in infants and adult subjects.

of age. All infants more than 3 mo of age had erythema, whereas a few infants less than 3 mo of age had both a negative wheal and a negative flare reaction. Among infants less than 6 mo of age, 20.5% had a negative prick test to 1 mg/ml of histamine, and 5.1% to 10 mg/ml of histamine; however, for the latter strength of histamine, only very young infants (less than 1 mo of age) had a negative prick test. There is

a significantly reduced mean wheal size in skin tests during the first year that is induced by histamine when wheal size is compared to those wheals induced in older infants. It appears from our study that the whealing capacity of 1 mg/ml of histamine increases in subjects from birth to 24 mo and then reaches a plateau, since the mean value of the prick test wheal is not very different in the second year of life from that

TABLE I. Mean size of the wheal induced by positive controls according to the age of the subjects

	n	Histamine (1 mg/ml)	Histamine (10 mg/ml)	Codeine phosphate
0 to 3 mo	25	0.77 ± 0.75	1.90 ± 0.92	2.06 ± 1.38
3 to 6 mo	14	1.07 ± 0.58	3.07 ± 0.88	2.14 ± 0.87
6 to 12 mo	20	1.67 ± 0.69	3.40 ± 1.18	3.31 ± 1.11
12 to 24 mo	17	2.23 ± 1.14	3.29 ± 0.86	3.08 ± 0.92
Adult subjects	30	2.48 ± 1.00	4.75 ± 1.11	3.70 ± 0.93

observed in adult subjects. On the other hand, the results elicited by the whealing capacity of 10 mg/ml of histamine demonstrate that there is a sharp and significant ($p < 0.01$) rise between the first 3 mo and the next 3 mo. There is a plateau then until the age of 2 yr. Finally, there is a significant ($p < 0.01$) difference between the second year of life (mean wheal size: 3.29 mm) and adulthood (mean wheal size: 4.75 mm).

Three infants less than 1 mo of age had a negative skin test for codeine phosphate. There is a first plateau during the first 6 mo of life and then appears a significant ($p < 0.01$) rise in the mean size of the wheal. A second plateau is observed between 12 and 24 mo of life. Finally, there is a significant difference ($p < 0.04$) between the second year of life and adulthood.

Allergen extracts

Table III illustrates the results of tests of six infants who had a positive skin test to one of the allergen extracts tested. There was a milk allergy in two cases, subsequently assessed by the presence of milk specific IgE in serum at a high titer (RAST class 3 or 4), and gastroenteritis subsided after milk avoidance. One case of allergy to eggs was observed and demonstrated by positive skin tests, RAST, and a suggestive history. The three other allergic infants had positive skin tests and RAST to cat (one case) and mites (two cases). These children suffered from asthma, and there was a significant improvement in their symptoms when cat exposure was avoided or mite preventive measures were applied. All other infants were also tested by RAST with the same allergens, and none of them had any positive serum specific IgE.

DISCUSSION

Prick tests appear to be one of the best methods of investigating skin tests in infants and young children, although a clear histamine hyporeactivity was observed in this study in infants less than the age of 2 yr.

Many techniques of prick testing have been recently proposed. In a former multicentric study in which our

TABLE II. Statistical comparisons between groups

	Histamine (1 mg/ml)	Histamine (10 mg/ml)	Codeine phosphate
0 to 3 mo vs 3 to 6 mo	NS	$p < 0.005$	NS
3 to 6 mo vs 6 to 12 mo	$p < 0.02$	NS	$p < 0.005$
0 to 6 mo vs 6 to 12 mo	$p < 0.005$	$p < 0.001$	$p < 0.001$
6 to 12 mo vs 12 to 24 mo	NS	NS	NS
12 to 24 mo vs adult subjects	NS	$p < 0.001$	$p < 0.04$
Infants vs adult subjects	$p < 0.001$	$p < 0.001$	$p < 0.001$

NS = not significant; statistical analysis performed by Mann-Whitney U test.

TABLE III. Allergen-induced skin tests

Patients	1	2	3	4	5	6
Age (months)	3	6	8	11	17	19
Allergen	milk	egg	mites	milk	cat	mites
Size of test (millimeters)						
Allergen	2	2	3	2.5	4	3
H 1	1	1	2	1	3	1
H 10	2	3	2	4	4	3
CP	2	1	2	2	3	4

H 1 = histamine, 1 mg/ml; H 10 = histamine, 10 mg/ml; CP = codeine phosphate.

group was included,²⁸ three prick test methods were compared (Morrow Brown standardized needle, multitest device, and modified prick test). The reproducibility of the three methods was assessed in the four centers involved in the study, and it was found that the modified prick test was more reproducible in two centers, the Morrow Brown needle in the third center, and the multitest in the fourth center. Thus the use of the modified prick test appears to be the most convenient when there is no bleeding.²⁷

Two different positive control solutions were used. Histamine is the most widely used,²⁹ and codeine phosphate is a mast cell-releasing agent.^{25,30} Anti-IgE was not used in this study because (1) IgE is not found in all neonates,³¹ its synthesis appears to be low in nonallergic infants, and (2) it was previously found that most neonates did not present a positive reaction to anti-IgE.²¹

Results presented herein demonstrate that prick tests can be easily performed in infants and that they could be interpreted without difficulty after the age of 3 mo. It may be difficult to analyse negative results in younger infants. However, it must be said that the wheal size of allergen-induced prick tests is smaller in infants and children,³² (Bousquet J, et al: in preparation) and the criteria of positivity of these tests cannot be the size of the wheal³³ but a ratio between the allergen-induced wheal size and the wheal size induced by positive control solutions. We used a 5% codeine phosphate positive control solution in our clinic and we considered that an allergen test is positive when it induced a wheal size more than 75% of the positive control wheal size.³⁴ Of course the quality of allergen extracts is a prerequisite in this evaluation, and the use of standardized extracts is a great improvement because many of them are based on either the histamine equivalent prick²⁹ or equivalent units.

Histamine hyporeactivity was clearly observed especially in infants less than 6 mo of age, but this condition persists in older infants. Codeine phosphate hyporeactivity was not as great as that elicited by histamine. These data correlate findings in neonates.^{19,21} A similar content of histamine has been found in cord blood basophils as in basophils of adults, but histamine releasability is normal³⁵. Skin mast cells can bind IgE since Prausnitz-Küstner tests have been found to be positive in infancy.²³ Finally, these results agree with recently published data.³⁶

Positive allergy skin tests on the basis of this study on unselected patients are rare in infants less than 2 yr of age and particularly in infants less than 1 yr of age (5%). Positive prick skin tests are especially rare to inhalants in this age group.

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Levels of immunoglobulin G, M, A, and E at various ages in allergic and nonallergic black and white individuals

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Studies were conducted to detect major differences in immunoglobulin levels between allergic and nonallergic individuals. Immunoglobulins G, M, A, and E were quantitated in members of 63 families selected for the presence of children with asthma or allergic rhinitis and compared with a larger group of healthy individuals and families. Mean IgE levels were significantly higher in healthy black than in white individuals. No significant difference was found in IgE levels between healthy parents with and without allergic children. Mean IgE levels were significantly higher in asthmatic children than in healthy children and also much higher in asthmatic children than in their healthy siblings. Asthma occurred more frequently in boys than in girls. IgE levels in healthy children increased rapidly early in childhood, reached a peak before 10 yr of age, and decreased during the teens. This decrease in IgE during the teens may provide the immunologic mechanism by which some children can "outgrow" certain childhood allergies. IgA levels were very low in young children and not significantly different between allergic and healthy individuals. The low IgA level in young children may be of importance in the development of childhood allergies. (J ALLERGY CLIN IMMUNOL 75:651-8, 1985.)

Allergic disorders have long been known to occur more frequently in some families than in others. Mem-

bers of families live in a similar environment and also share more genes in common than unrelated individuals. In complex ailments such as the atopic diseases, presumably both genetic and environmental factors are of importance in the development of the condition, and it may be difficult to establish the relative contribution of genetics and environment. However, one group of factors that may be of importance are the immunoglobulins. IgE is directly involved, and in IgA deficiency the incidence of allergic disorders was demonstrated to be increased.¹ Our previous studies did demonstrate that genetic factors contribute importantly to the variation in immunoglobulin levels.^{2,3}

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