

Allergen-coated lancets (Phazet) for skin prick testing in children

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With a new allergen-coated lancet (Phazet), skin prick tests can be made directly on the skin without separate use of liquid extract (the Phazet dry method), and reactions are recorded as for the ordinary method with liquid allergen extract (Pharmalgen) (the Pharmalgen wet method). 100 children were tested in parallel with the two methods. As a double control each Phazet was used twice (Phazet 1 and 2) in each child, who was tested with nine standard allergens. Good agreement was found between the results obtained with the two methods and also between Phazet 1 and Phazet 2. No general reactions were shown in relation to the numerous skin prick tests with allergen extract of a strength up to 100,000 BU/ml. 66% of the children preferred the Phazet while 22% had no preference.

Key words: allergy; children; skin prick test.

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CLINICAL ASPECTS

The Phazet dry method for skin prick testing has further simplified the diagnosis of childhood allergy. Since individual Phazets can be used at least twice without a weakening of the allergic reaction, routine double controls are now greatly facilitated. The results indicate that in future skin prick tests a cut-off limit of 7 mm² (diameter 3 mm) will be acceptable for a positive reaction with either the Pharmalgen wet method using 100,000 BU/ml or the Phazet dry method, and the histamine reference will only be used to confirm skin reactivity towards histamine.

The skin prick test (SPT) is still the cornerstone of allergy diagnostics, and the introduction 10 years ago (4) of a new single-use 1 mm lancet simplified the diagnosis of allergy since its use required less experience than existing methods involving, e.g. hypodermic needles or longer lancets. The method was simple in that the 1 mm lancet was applied at an angle of 90° to the skin, directly through liquid allergen extracts. Reproducibility was better with the new technique and could be further improved by using stronger allergen extracts of concentrations up to 100,000 BU/ml, together with a histamine reference concentration 10 mg/ml (2).

The 1 mm tip of the lancet has now been coated with freeze-dried standardized allergen

extracts of a strength corresponding to 100,000 BU/ml. With this allergen lancet (Phazet) tests can be made directly on the skin (the Phazet dry method), and reactions are recorded in the same way as with the Pharmalgen wet method (1), which involves the use of a separate liquid extract.

Agreement between the two methods has recently been demonstrated in adults (3). The aim of the present study was to compare the two methods in children.

MATERIAL AND METHODS

The subjects were children attending the Allergy Clinic of the Pediatric Department at Vi-

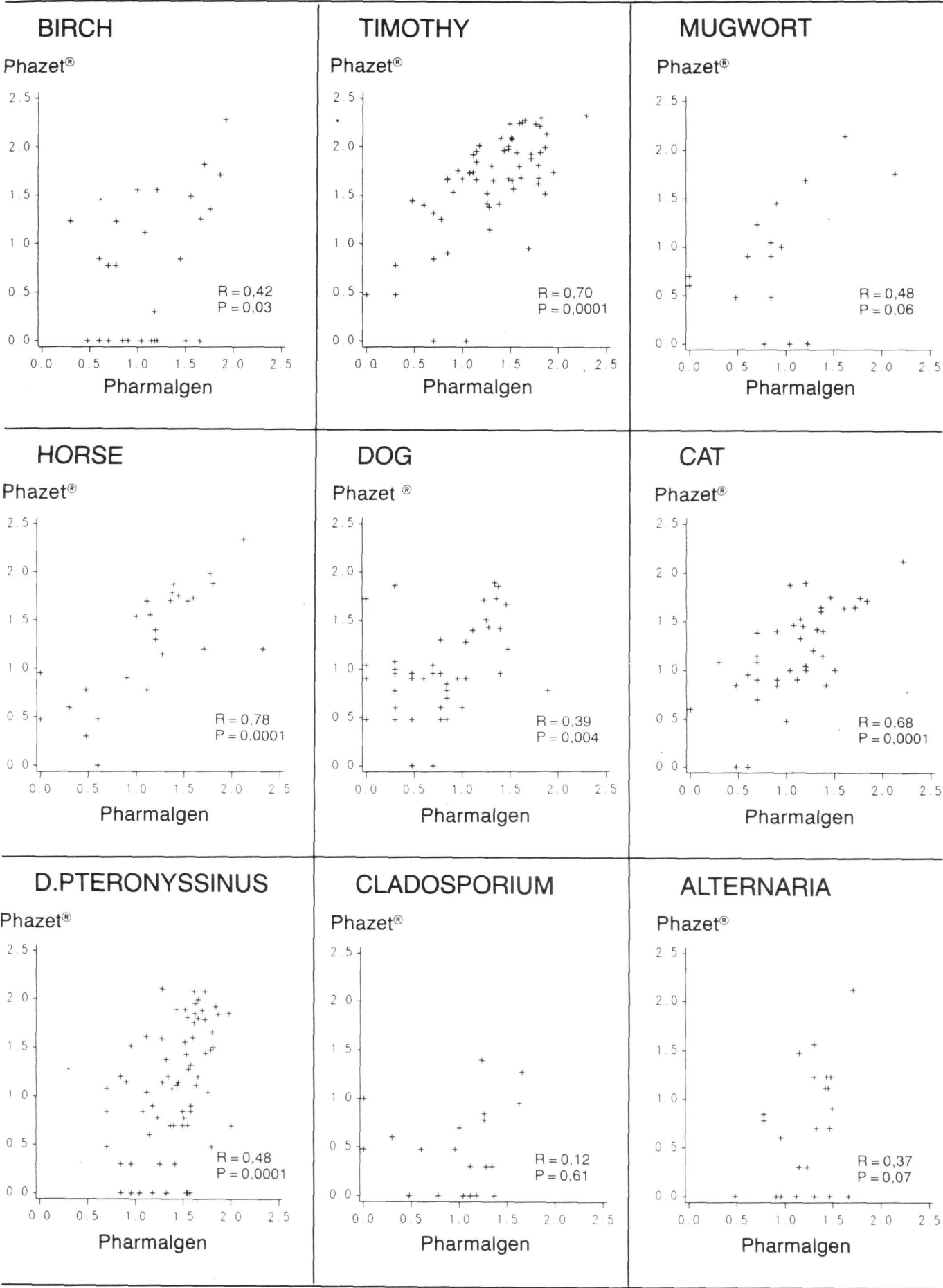


Fig. 1. Log wheal areas in mm² for the individual allergens using 100,000 BU/ml Pharmalgen liquid extracts vs. Phazet dry method. Pearson's correlations coefficient (R) and the P-values are indicated on each graph.

Table 1

Number of reactions for the individual allergens using Pharmalgen 100,000 BU/ml versus Phazet. 4 × 4 tables with cut-off values of 7 mm², and relative to histamine HCl 10 mg/ml

Pharmalgen 100,000 BU							
		Birch		Timothy		Mugwort	
		-	+	-	+	-	+
Phazet							
≥ 7	+	3	10	5	51	2	7
mm ²	-	77	10	43	1	88	3
≥ His	+	7	11	7	50	3	2
10 mg	-	82	6	43	0	91	4

<i>D. Pter.</i> Cladosp. Altern.							
		-	+	-	+	-	+
Phazet							
≥ 7	+	2	45	1	4	1	9
mm ²	-	27	26	85	10	78	12
≥ His	+	3	53	2	3	0	9
10 mg	-	31	13	86	9	81	10

Horse Dog Cat							
		-	+	-	+	-	+
Phazet							
≥ 7	+	1	18	17	15	7	28
mm ²	-	80	1	62	6	64	1
≥ His	+	2	18	11	14	6	23
10 mg	-	80	0	73	2	70	1

borg County Hospital, Denmark. 92 atopic children with one or more allergic reactions were consecutively included in the study together with 8 non-atopics. The children were aged from 6 to 17 years (28 girls and 72 boys). 62 had asthma with or without rhinitis or atopic dermatitis, 29 had allergic rhinitis with or without atopic dermatitis, one had atopic dermatitis and 8 were non-atopics. During the SPT the children were symptom-free without medication. Conventional antihistamines were not allowed for 72 h, and local steroids or depot bronchodilators during 24 h prior to skin testing.

Pharmalgen wet method

Nine freeze-dried and standardized Pharmalgen allergen extracts (Pharmacia AB, Uppsala,

Sweden) of birch, timothy and mugwort pollens, horse, dog and cat danders, *Dermatophagoides pteronyssinus*, cladosporium and alternaria strength 10,000 BU/ml and 100,000 BU/ml were used throughout the study. The freeze-dried material was reconstituted at least once a week (for cladosporium and alternaria every 3rd day) with albumin diluent. Histamine HCl solutions at concentrations 1 mg/ml and 10 mg/ml (5.43 and 54.3 mmol/l), respectively, served as positive controls, and the albumin diluent as negative control solutions. The uncoated Phazet lancets were used in the wet method for SPT.

Phazet dry method

Phazet is a 1 mm lancet coated with standardized Pharmalgen allergens or histamine (Phar-

Table 2

Total number of histamine reactions with histamine HCl 1 mg/ml and 10 mg/ml and with histamine Phazet. 4 × 4 tables with cut-off values of 3 mm² and 7 mm²

			Histamine 10 mg	
			-	+
Histamine	≥ 3	+	0	92
1 mg	mm ²	-	0	8
-	≥ 7	+	1	59
	mm ²	-	4	36
Histamine	≥ 3	+	0	59
Phazet (1)	mm ²	-	0	41
-	≥ 7	+	2	34
	mm ²	-	3	61

macia AB, Uppsala, Sweden). The Phazet lancets were delivered in separate envelopes and had previously been shown by calibration in adults to have an allergen extract strength of 100,000 BU/ml (1). Uncoated Phazet lancets were used as negative controls. The Phazets and allergen extracts were stored at temperatures between +4 to +8°C before use.

Skin prick tests

Each of the 100 children was tested in parallel by the Pharmalgen wet method (allergen concentrations 10,000 BU/ml and 100,000 BU/ml) on one arm and the Phazet dry method on the other. As a double control each Phazet was used twice (Phazet 1 and 2), and each child was tested with all 9 standard allergens. Including the positive and negative references each child was tested with 44 SPT. i.e. a total of 4400 skin prick tests including 3600 with allergens. All tests were performed by the same two experienced nurses. Skin prick tests with liquid extract as well as with Phazet were performed as recommended, i.e. the tip of the lancet/Phazet was pressed against the skin for approx. 1 s at an angle of 90° guided by the index finger on the top of the lancet.

The wheal reactions were assessed after 15 min. The perimeters of the swellings were

Table 3

Total number of histamine reactions following double control with the same histamine Phazet (1) and (2). 4 × 4 tables with cut-off values of 3 mm² and 7 mm²

			Histamine - Phazet (2)	
			-	+
Histamine	≥ 3	+	8	51
Phazet (1)	mm ²	-	24	17
-	≥ 7	+	13	23
	mm ²	-	49	15

traced with a black marker pen and transferred to the registration scheme using transparant adhesive tape. Results were assessed partly on the basis of area measurement with a digitizer and partly on the basis of the wheal size relative to a histamine reference of 1 mg/ml or 10 mg/ml. Wheal areas of at least 3 mm² (diameter approx. 2 mm) and 7 mm² (diameter approx. 3 mm) were also used as cut-off values.

Statistics

Pearson's test was used to compare the correlation coefficient (R) between the absolute wheal areas obtained by the two methods. Wheal areas have been 10-log transformed. The inclusion criterion is Phazet or Pharmalgen wheal areas of at least 3 mm². If the wheal areas happen to be zero the value is replaced by 1 before log-transformation. A 5% significance level was used. The protocol was approved by the Human Research Ethics Committee and informed consent was obtained from all participants.

RESULTS

Fig. 1. shows log wheal area in mm² for the individual allergens using the Phazet dry method and Pharmalgen wet method. All reactions with an area of at least 3 mm² (diameter approx. 2 mm) are included, although only reactions with an area of at least 7 mm² are normally regarded as being clinically relevant.

Table 4

Total number of allergen reactions with 100,000 BU/ml Pharmalgen versus 10,000 BU/ml Pharmalgen and Phazet. 4 × 4 tables with cut-off values of 3 mm² and 7 mm², and histamine HCl 1 mg/ml and 10 mg/ml

			Pharmalgen 100,000 BU/ ≥ His 10 mg	
			-	+
Pharmalgen 10,000 BU	≥ 3	+	5	228
	mm ²	-	581	86
-	≥ 7	+	1	151
	mm ²	-	642	106
-	≥ His	+	29	182
	1 mg	-	643	46
-	≥ His	+	3	148
	10 mg	-	669	80
Phazet (1)	≥ 3	+	33	254
	mm ²	-	553	60
-	≥ 7	+	39	187
	mm ²	-	604	70
-	≥ His	+	35	183
	10 mg	-	637	45

Excluding for cladosporium, alternaria and mugwort (few analyses) the figures show a statistically significant, good correlation between the results obtained by the two methods for the individual allergens. Fig. 1 shows a greater number of negative reactions (below 3 mm²) to birch, house dust mite, cladosporium and alternaria allergens using the Phazet method, whereas for dog dander allergen a greater number of negative reactions are seen using the Pharmalgen method.

When Phazet double control was used there was no difference between the Pharmalgen wet method and the Phazet dry method with respect to the negative allergen reactions with the exception of dog dander allergen. The present study did not include double control with the Pharmalgen wet method, but using the Pharmalgen extracts of strengths 10,000 and 100,000 BU/ml as parallel controls, with histamine references of 1 mg/ml and 10 mg/ml, respectively, there was still a greater number of

reactions to dog dander allergens with the Phazet than with the Pharmalgen method, in agreement with results obtained from adults (3). This aspect should be further investigated by organ provocation.

The results obtained with cut-off values of 7 mm² in relation to the results for a histamine reference of 10 mg/ml are given in Table 1. If the reproducible positive values are taken as indicative of allergy (cutaneous allergy), with a cut-off limit of at least 7 mm² the following frequencies of reaction are obtained for the patient group studied: birch 10 %, timothy-grass pollen 51 %, mugwort 7 %, horse dander 18 %, dog dander 15 %, cat dander 28 %, house dust mite 45 %, cladosporium 4 % and alternaria 9 %. Table 1 shows that the frequencies of reaction with a cut-off limit of 7 mm² correspond to positive reactions related to a histamine reference of 10 mg/ml.

Table 2 shows the total number of histamine reactions, obtained with histamine in concentrations of 1 mg/ml and 10 mg/ml, and with histamine Phazet with cut-off limits of 3 mm² and 7 mm². With a histamine concentration of 1 mg/ml, 92 % of the patients showed a reaction of at least 3 mm², while only 59 % showed a reaction of at least 7 mm². In contrast, almost all reactions to histamine at 10 mg/ml concentration had relevant size, i.e. at least 7 mm². The reactions to the histamine Phazet corresponded roughly to those to 1 mg/ml histamine, and neither the results for the histamine Phazet nor those for 1 mg/ml histamine can therefore be regarded as suitable for use as positive reference values in the present investigation.

Table 3 shows that the same histamine Phazet (Phazet 1 and Phazet 2) could be used twice for the same patient, there being equally many positive and negative reactions with Phazet 1 and Phazet 2 when employing the relevant cut-off value of 7 mm². Only 23 % of the patient group showed reproducible positive reactions of 7 mm² with the histamine Phazet.

The overall results for the allergen reactions with the different cut-off values are shown in Table 4. Thus with a cut-off value of 7 mm², 100,000 BU/ml Pharmalgen gave 41 % more allergen reactions than 10,000 BU/ml Pharmal-

Table 5

Total number of allergen reactions following double control with the same Phazet (1) and (2). 4 × 4 tables with cut-off values of 3 mm² and 7 mm², and relative to histamine HCl 10 mg/ml.

			Phazet (2)	
			-	+
Phazet (1)	≥ 3 mm ²	+	26	261
		-	586	27
-	≥ 7 mm ²	+	34	192
		-	639	35
-	≥ His 10 mg	+	27	191
		-	651	31

gen. With the latter cut-off value, the number of positive reactions for 10,000 BU/ml Pharmalgen corresponded to the number of positive reactions related to a 10 mg/ml histamine reference, precisely as for the Phazet reactions. When related to the 1 mg/ml histamine reference there were roughly equally many positive reactions for 10,000 BU/ml and 100,000 BU/ml Pharmalgen, but about 1/4 of the reactions were weak, i.e. between 2 mm (3 mm²) and 3 mm (7 mm²) in diameter.

As seen from Table 5, the allergen Phazet was well suited for double control, since one Phazet could be used twice without a weakening of the reaction. The table summarizes the results for the two cut-off values. There was a correspondingly good correlation between the Phazet 1 and Phazet 2 reaction areas for the individual allergens (results not shown). It is apparent from Table 5 that there were only 10-15% false positive reactions with Phazet 1 and Phazet 2. The good agreement between Phazet 1 and Phazet 2 for allergen Phazets and histamine Phazets shows that the prick test technique was satisfactory.

The results indicate that in the case of a positive skin reaction it is not necessary to use a histamine reference, regardless of whether the Pharmalgen wet method with 100,000 BU/ml or the Phazet dry method is used, provided the cut-off limit chosen is sufficiently high, i.e. at least 7 mm² (at least 3 mm in diameter).

No general reactions were seen in relation to the 3600 SPT with allergen extract of a strength corresponding to 10,000 BU/ml or 100,000 BU/ml, and 66% of the children preferred the Phazet while 22% had no preference. Delayed local reactions were not recorded.

DISCUSSION

The present results for children confirm that allergen Phazet gives rise to reactions corresponding to 100,000 BU/ml Pharmalgen, in agreement with previous results for adults (3). We have not correlated the SPT results with the results of organ provocation tests or with case history, but have merely compared the two skin prick test methods.

Double control with a single Phazet for one patient must be regarded as a significant advance. The 10-15% so-called false positive allergen reactions with double control should primarily be judged by carrying out a new skin prick test, and only the reproducible positive test results should be evaluated with respect to their clinical significance for non-skin allergy, e.g. allergic asthma.

Judging by the good reproducibility of the histamine Phazet reactions, the problems with Phazet histamine do not appear to be related primarily to wrong technique, a conclusion which is also confirmed by the good agreement of the allergen reactions with the Phazet. At the same time, practically all the patients give a relevant reaction with 10 mg/ml histamine, thus establishing the skin reactivity of histamine.

In future clinical tests it will be acceptable to use a cut-off limit of 7 mm² (diameter 3 mm) for positive SPT, both for the Pharmalgen wet method and the Phazet method, and to use the histamine reference only to confirm skin reactivity towards histamine. In the case of repeated (two) failures of the histamine Phazet test, 10 mg/ml histamine HCl liquid extract should be used to confirm the skin reactivity.

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