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Anomalous cutaneous absorption of allergens as cause of skin prick testing adverse reactions in adult patients. Clinical and experimental evidence

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skin prick-test; skin tests adverse reactions; skin absorption; skin permeability barrier; prick-test inoculum volume variability

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Summary
Background. *Paediatric age, active eczema and high number of allergens tested in poly-sensitized patients have been pinpointed as possible risk factors of systemic reactions by skin prick testing. As far as atopic eczema concerns, the higher penetration of the allergens into the skin because of the scraping or micro-injuries is an intuitive rationalization. Purpose of the present study is to provide documentary evidence that adverse reactions elicited by anomalous absorption of allergens can occur also in adult patients with apparently normal skin.* ***Methods.*** *Report of some exemplifying clinical and experimental observations. Measuring the inoculum volume into impaired skin and its variability in relation to the variation of the chemical-physical characteristic of the solutions used for the tests by means of a method of direct assay based on the use of a gamma-camera.* ***Results.*** *Localized impairments of the skin permeability can cause a significant increase in inoculum volume by prick-test. Critical amounts of allergens can be introduced into the skin because of the possibility of direct absorption, also without pricking, of allergy diagnostic solutions. The greater water content of the solutions used for prick-testing can significantly increase the inoculum volume.* ***Conclusions.*** *This study adds clinical and experimental evidences that localized impairments of permeability can occur in adult patients with apparently normal skin. Special precautions should be taken when a change of the drops' normal shape and cohesion is seen, because allergy prick-testing in such areas is potentially associated with increased risk of large local or systemic reactions.*

Introduction

Skin prick test is currently the technique more widely used to diagnose allergic sensitization to common allergens. The fast and painless execution and the high number of allergens tested in the same session are some of the unquestionable advantages of the method. Considering the smallest amounts of allergen injected into the skin (1), the prick test must be considered on the whole a safe diagnostic procedure. If not altogether absent, the risk of systemic adverse reaction is very low.

Some large clinical-epidemiological studies have suggested that the overall risk of inducing anaphylactic reactions by skin prick testing with common allergens is about 0.02% (2,3). The progress on extracts standardization and diagnostic methods has further reduced the rate of reactions with commercial extracts to less than 0.002% (4), being latex or fresh foods more likely to cause adverse events (5-8). Paediatric age, active eczema and high number of allergens tested in poly-sensitized patients have been pinpointed as possible risk factors (9,10).

Systemic reactions are usually mild to moderate in severity and can be easily controlled by recommended therapy (11). No fatalities have been reported in the last decades.

Unusual conditions of hyperactivity, an overload of allergens by non-standardized or much more concentrated extracts, or a lot of positive reactions can be seen as a possible explication of some cases of systemic reactions.

As far as eczema concerns, the higher penetration of the allergens into the skin because of the scraping or micro-injuries is an intuitive rationalization. However, a clear demonstration of this probable mechanism does not exist.
Aim of the present work is to report clinical and experimental evidences that an anomalous absorption of a critical amount of allergens, potential cause of systemic reaction, can occur also in adult patients with apparently normal skin. What's more, we studied the effect on inoculum volume of the variation of the chemical-physical characteristic of the solutions used for the prick-test.

Methods

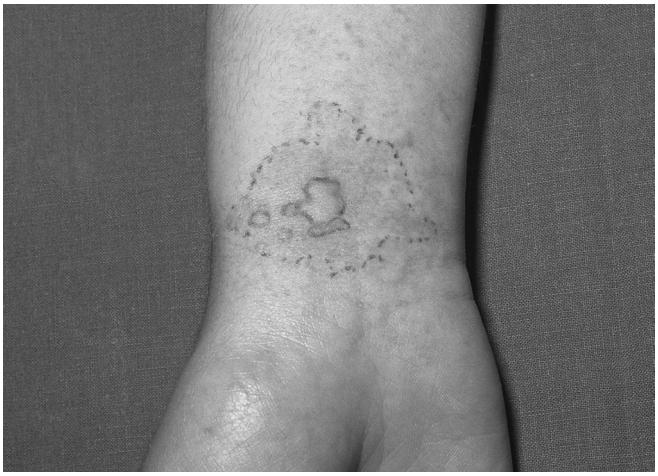
We report some explanatory clinical observations taken out from our files to prove with documentary evidence that, in some adult patients with impairments of skin permeability, there is the possibility of significant increase of allergens load by absorption and penetration through the skin of diagnostic solutions also without doing prick/puncture tests.
The amount of allergen extract which could penetrate into the skin by a prick test altered by simultaneous absorption of the solution used for testing, has been assessed by means of a method of direct assay based on the use of a gamma-camera. In short, a 50% glycerol-saline solution routinely used as diluent in allergy work was labelled with 99m Tc-pertechnetate (Tc99m). The inoculum volume was calculated with precision measuring the activity of the solution penetrated into the skin by means of a gamma-camera. This assay method and its overall reliability in terms of sensitivity, precision and accuracy, and the results of the assay of the inoculum volume by prick testing have been extensively reported elsewhere in literature (1,12).
The possible variations of the size of inoculum volume in relation to the variations of the chemical-physical characteristic of the solution used for the tests have been studied with the same technique. For this aim, some series of prick test were carried out in 15 health subjects (average age 43 ± 8; 13 female) by means of two glycerol-saline solutions respectively at the concentration of 10 and 50%. Four rows of prick test were carried out on the volar side of the forearms of each subject (i.e. two rows of 4 prick test for each forearm, the one with 50% and the other with 10% solution, alternating the radial and ulnar side) for a total of 16 tests per person. The data series have been compared by Wilcoxon matched-pairs test.
All patients gave their written informed consent and the study with radioisotopes was then approved by Local Ethical Committee (Del. N. 665, 16.04.96).

Results

Case report 1

This clinical observation concerns a 23-year-old female patient, who had referred to our service because of the onset, for about two years, of perennial rhino-conjunctivitis and asthma. She reported a personal history of atopic dermatitis recovered at school-age and a vague story of food allergy. At the same time, together with the respiratory symptoms, frequent occurrence of widespread pruritus and of recurrent, fleeting episodes of dermatitis of flexural surfaces of the joints, mainly in winter, were started. No active skin lesions were present at the time.
Performing skin tests we noted a fast spread out of the allergen drops put down, and their near complete disappearance, adsorbed by the skin. Actually, the forearm skin was very dry, lackluster with a fine scaling by gentle rubbing and accentuated skin markings in the areas of elbow and wrist folders. Skin test procedure was stopped. To verify the effective penetration into the skin, a drop of the positive control (histamine 10 mg/mL in 50% glycerol-saline solution) was put down on the wrist without pricking. The drop spread out and was adsorbed by the skin, with provocation of a large flare and a number of wheals of different size (**figure 1**).

Figure 1 *- Positive cutaneous response to a drop of histamine control put down on the wrist without pricking. Wheals of different size are the result of percutaneous absorption of the solution which was spread out on the skin.*

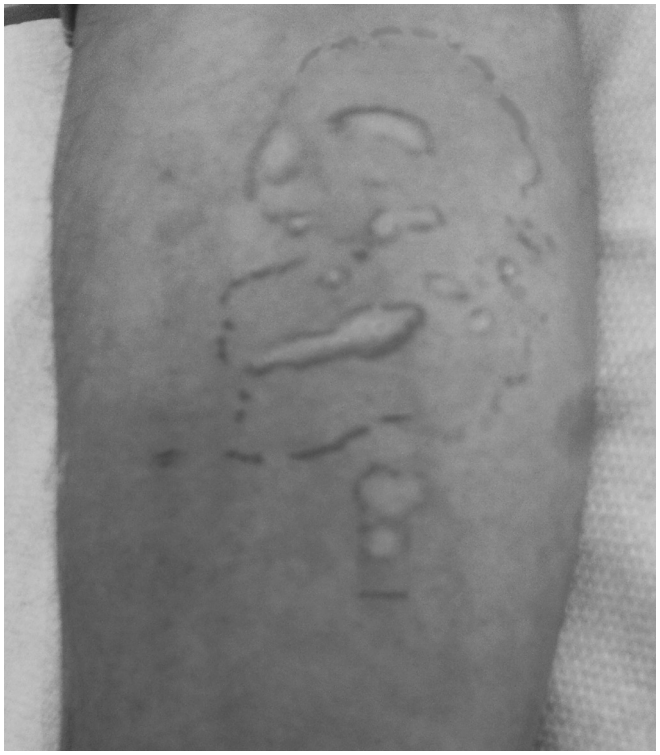


Case report 2

Similar case concerning a 32-year old bricklayer with perennial rhinitis and mild asthma. The patient had never suffered from atopic dermatitis or other cutaneous diseases and skin appeared to be normal. Prick-tests were normally carried out. However, soon after skin pricking a slow spreading and adsorption of some allergen drops near to elbow, including house-dust mites extract, was noted. Drops were immediately wiped by blotting paper. Nevertheless a strong reaction, with a very large flare and a lot of wheals of different size involving skin areas of other

prick tests, was triggered, making a reliable interpretation of skin test results impossible (**figure 2**). At a later stage, specific sIgE dosing resulted positive only to mites.

Figure 2 *- Spreading and absorption of an allergen soon after skin pricking. A very large flare and a number of wheals of different size involving skin areas of several other prick tests make a reliable interpretation of results impossible.*



Case report 3

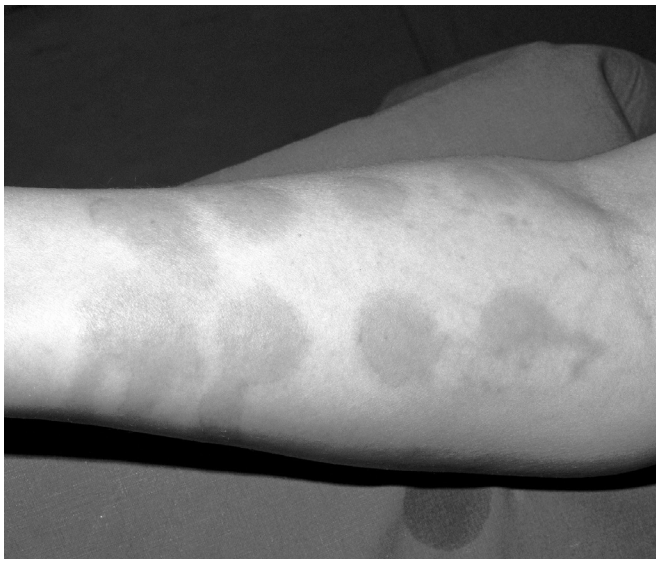
A 43-year old woman, housewife, addressed to our allergy unit for an episode occurred about two months before, of a severe, delayed generalized skin reaction, presumably a maculopapular rash, resulted from the intake of some capsules of amoxicillin. She was not suffering from atopic diseases, but reported intolerance to the costume jewellery and an episode of mild hand eczema in the past.

The patient was skin tested according to ENDA/EAACI guidelines (13). In particular, skin prick tests with ampicillin and amoxicillin were performed at the concentrations of 0.2, 2, 10 and 20 mg/mL.

Also, in this case we noted that the drops put down did not maintain their spherical shape but spread and formed rivulets on the forearm surface. The skin looked apparently normal. Since an IgE-mediated reaction was really improbable, prick tests were quickly performed and the test solution summarily dried by blotting paper. No immediate-type positive cutaneous responses were seen. At the end, as usual, the skin was wiped with a cotton wad wetted of disinfectant solution.

The next morning the patient came to our service because of the occurrence of delayed skin reaction which involved not only the points where the prick-tests were performed, but the entire area of contact where the liquid had been spread and dripped, and clearly absorbed by the skin (**figure 3**).

Figure 3 *- Delayed skin reactions to prick-tests with ampicillin and amoxicillin (see text). The shape and the extensive size of the patches reflect the skin areas where the solution drops were put down, spread out and formed rivulets, and where the antibiotics had been absorbed.*

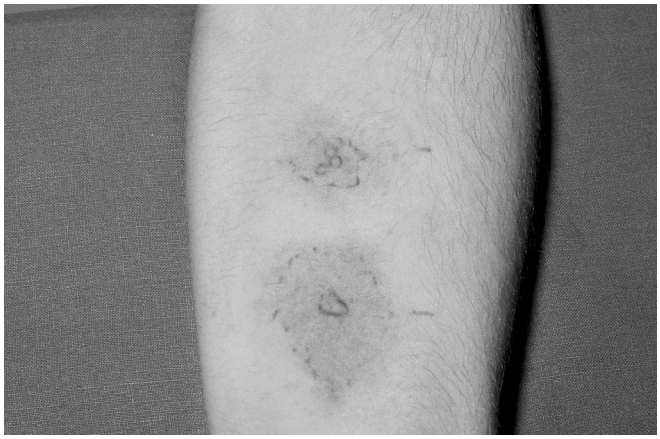


Case report 4

A 19-year-old boy had been sent to our service for persistent rhinitis on progressive worsening with secondary asthma. The respiratory symptoms started at age of 12 about and during the babyhood he suffered from a mild, short lasting form of atopic dermatitis. Also in this case we noted that the drop of extract went slowly losing its spherical form, spread and partially penetrated into the skin. Skin testing was stopped. The forearm skin was dry but no other alterations were seen.

Two drops of the histamine control were put down. Skin prick test was carried out through one of them. Both gave a positive skin response, but the one pricked provoked a strong reaction with a flare of unusual breadth (**figure 4**).

Figure 4 - *Positive cutaneous responses to two drops of histamine control. The one above is the result of percutaneous absorption without pricking. The one below is the response to a prick-test, which provoked a strong reaction with a flare of unusual breadth.*



Case report 5

To assess the size of inoculum volume, series of prick tests had been performed on the forearm volar aspects of a number of healthy volunteers with a 50% glycerol-saline solution, routinely used as diluent in allergy work, labelled with Tc99m (1,12). As for the clinical cases previously reported, in one subject out of them (a healthy 64-year-old woman, with apparently normal forearm skin), we observed the spreading and the partial adsorption of one drop of the solution. In this way we had the opportunity to calculate the size of the volume which could penetrate into the skin in an example of prick test modified by the simultaneous absorption of the solution into the skin area surrounding the pricked point, and to match it with the average volume of inoculum of the prick test carried out on normal skin areas of the same subject.
In this example, the volume of solution penetrated into the skin (0.232 µL) was about 19 folds greater than the average volume of inoculum (0.012 µL) by prick testing in healthy skin (**figure 5**).

Variability by solutions' water content

The use of a solution at higher water content (glycerol-saline solution 10%) produced a significant increase in the size of inoculum volume as compared to one with lower water content (glycerol-saline solution 50% routinely used as diluent in allergy work) in more than seventy percent of the cases (11/15 = 73%). For the remaining cases not significant variations have been observed (**table 1**). The data show great differences between the different subjects. The average increase of the inoculum size has been of about two hundred percent (median = 184), but within a wide range of variability, for one case over seven hundred percent (range 85-765%).

Figure 5 - *Monitor image of a series of prick tests performed on the forearm with glycerol-saline solution labelled with Tc99m and acquired by gamma-camera. The dimensions of one inoculum, compared to other ones, appear very outsized. The enlarged image shows the spread of solution and at least three different sources of penetration into the skin.*

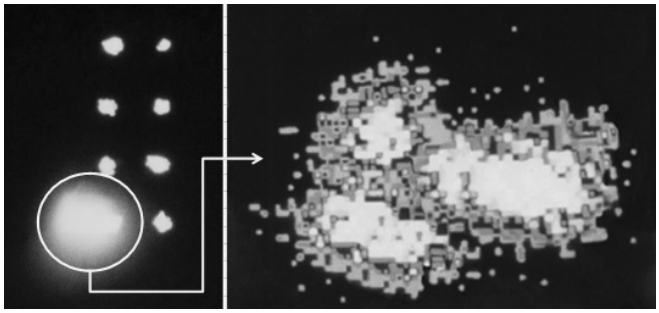


Table 1 - *Average inoculum volume (πL) by prick test carried out with 50% and 10% glycerol saline solution in 15 healthy subjects. In 11 of them, the inoculum size results significantly greater with solution at higher water content (10% solution) compared to more concentrated one (50% solution).*

Patients	Count	50% Solution	Count	10% Solution	P
N	N	(πL)	N	(πL)	
1	8	20626	8	32281	NS
2	8	27660	8	76370	< 0.001
3	8	2704	8	6976	NS
4	8	1447	8	6835	< 0.01
5	8	11298	8	17672	NS
6	8	10193	8	13275	< 0.01
7	8	2115	8	3916	< 0.05
8	8	4950	8	14370	< 0.001
9	8	8345	8	7054	NS
10	8	37355	8	72447	< 0.001
11	8	30510	8	102850	< 0.05
12	8	4290	8	72300	< 0.0000
13	8	3064	8	7200	< 0.05
14	8	49861	8	141770	< 0.0000
15	8	26140	8	226310	< 0.0000

Discussion

We hypothesize that an impairment, more or less localized and maybe transient of skin barrier-function could provide a reasonable and exhaustive explication of observed phenomena.
The most obvious function of the skin is to protect the body against the environmental *noxae*.
The epidermal permeability barrier, which controls the transcutaneous movement of water and electrolytes, is probably the most important protective function of the skin. Most part of this barrier function resides in the stratum corneum, composed by many layers of anucleate corneocytes embedded in an intercellular lipid matrix. A second level of defense is formed by the tight junctions of the keratinocytes, and by the lamellar bodies of the stratum granulosum resulting in the formation of an impermeable, lipid-containing membrane. The permeability barrier is largely represented by the epidermis. When the epidermis is disrupted, the underlying dermis is almost completely permeable. It is important to remark that even minimal injuries predispose to more penetration of fluids or other materials applied topically on the skin surface (14).
The surface of the skin is sheltered by a lipid film, composed of both sebum and the lipids of the epidermal cells (15). This film acts as a hydrophobic, low wettability surface. For this reason a fluid put on the skin will tend to minimize contact with the surface and will form a compact liquid droplet. On healthy skin water drops maintain their spherical shape, will not roll off and not fall even if the forearm is tilted. Because in normal conditions (at least for not lengthened applications), there is not significant absorption of the aqueous liquid or other substances put on the skin, pricking through the drop is necessary to produce a micro-lesion by which a tiny amount of solution is introduced into the skin.
The homeostasis of the epidermal permeability barrier is finely and actively regulated. Impairment or loss of barrier-function are primary pathophysiologic factors in a number of skin diseases, including atopic dermatitis, ichthyosis and many other xerotic skin conditions (14).
Abnormalities in lipid processing metabolism and genomic defects concur to the skin barrier abnormalities in atopic dermatitis (14,16). Filaggrin gene mutations and ineffective keratinocyte differentiation, decreased levels of ceramides and pyrrolidone carboxylic acid result in abnormal keratinization of skin, abnormal lipid organization and deficiency of the natural moisturizing factors. Alterations in sebum secretion and chemical composition of skin surface lipid are a common feature in atopic dermatitis and several inflammatory chronic skin diseases (15). Because of these structural and functional changes, permeability barrier function is impaired displaying both increased trans-epidermal water loss and lowered water-binding capacity.
Atopic skin proves very dry and more vulnerable to the penetration of exogenous substances.
As a consequence of alterations and reduction of its lipid film, in atopic dermatitis and other xerotic skin condition, the normal hydrophobicity of the skin surface is frequently lost. In the case, water and fluid drops put down on the skin cannot maintain their form, but spread out. As in clinical cases reported, this occurrence should be considered a warning of significant damage of the skin with impaired permeability barrier function, allowing for a fast substances penetration (15).
Occasionally, spreading and dripping of extract drops put on the skin can be seen also in some patients with no structural change or impaired function of epidermis. Soaps, synthetic detergent or bath foam, but also some cleansing and moisturizing cream used for cleanliness and body care can deplete or damage the lipid film. In this cases, water drops can spread out and form rivulet. If the damage is only limited to the superficial external lipid film, there is no significant water adsorption.
That is because skin barrier-barrier function is mainly (although not exclusively) fulfilled by underlying corneus stratus (the so called "brick and mortar" structure), and the damage of the corneus stratum is a necessary condition for the impairment or loss of skin barrier-function. Barrier creams (topical formulations used to place a physical barrier between the skin and external *noxae*) could provide a protective film, replacing the function of the natural outside hydrolipidic film which covers the skin. However, prick-puncture tests produce a micro-lesion by which the liquid is introduced into the skin diffusing through the epidermis. For this reason, just restoring the function of the external lipid film is not enough to prevent an abnormal penetration and spreading of the allergen solutions.
However, housekeeping products, soap with high content of free alkali and/or harsh chemicals in cosmetics can go deep into the skin dissolving the lipids of underlying epidermal layers, impairing skin barrier and increasing permeability (17). This is probably the case of the housewife we reported (case 3). Here we must stress the point that, if the patient had had an IgE-mediated sensitization, in all probability, skin prick testing would challenge a severe anaphylactic reaction.
The normal skin acts as a two-way barrier to prevent the inward or outward passage of water and electrolytes. Studies on drugs delivery by transdermal patch demonstrate that the penetration of substances through the skin surface depends upon different factors, which include skin conditions (e.g. injured or abraded skin surfaces, hydration, etc.), age, physical and chemical characteristics of considered substances and time of application. The absorption through the skin acts by a slow process of passive diffusion through the corneum layer. Defects in epidermal permeability barrier, by skin diseases or injuries enhance and accelerate the diffusion processes (18).
Clinical cases reported demonstrate that the absorption of the glycerol-saline solution normally used for skin prick tests can be

really fast. Moreover, puncture-prick tests carried out on dry, injured skin seems to enhance considerably the fluid penetration and diffusion. As a matter of fact, the prick-test in the reported cases provoked skin reactions of unusual breadth (cases 2 and 4). In a similar situation (case 5), we have demonstrated that the volume of solution penetrated into the skin was by far higher than the mean size of inoculum in normal skin. The monitor image visually explains the spread of the solution into the skin and the scale of the phenomenon (**figure 5**).

Water is an effective penetration enhancer. Results of our study show that an aqueous solution produced a significant increase in the size of inoculum volume, as compared to one with low water content. In clinical practice, it means that when prick tests were carried out using extemporary, aqueous extracts or food with high water content (like milk or some fruits), also on healthy skin an amount of allergens much higher than expected can be introduced into the skin. In conditions of pathologic skin with altered permeability, critical amount of allergens, sufficient to induce systemic reactions in a sensitized patient, could be reached with a single prick test.

In conclusion, we have added clinical and experimental evidence that prick-testing in patients with atopic dermatitis and other skin diseases or conditions with impaired permeability of the skin is a risk procedure. Areas of normal skin should be carefully chosen to prevent large, scattered local reactions for which test results could be very difficult to interpret, and suitable precautions should be taken to avoid risk of systemic allergic reactions.

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