

Activation and cross-reactivity pattern of a new allergen in adhesive plaster

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N,N'-disalicylidene-1,2-diaminopropane is a copper inhibitor present in some adhesive plasters, rubber products and gasoline. Upon contact with water it is hydrolyzed to salicylaldehyde and 1,2-diaminopropane. All patients in this study showed positive patch-test reactions to N,N'-disalicylidene-1,2-diaminopropane, and also to 1,2-diaminopropane and ethylenediamine. None reacted to salicylaldehyde. Patch testing with different N,N'-disalicylidene-derivatives showed localization of the amino groups in positions 1 and 2 to be a prerequisite of cross-reactivity to 1,2-diaminopropane and ethylenediamine. An extraction procedure and a high-performance liquid chromatography (HPLC) method for the analysis of adhesive plasters is described. Studies of the hydrolysis of the copper inhibitor at physiological pH showed rapid formation of 1,2-diaminopropane under biomimetic conditions.

Key words: imine; salicylaldehyde; adhesive plaster; ethylenediamine; diaminopropane; allergic contact dermatitis; high-performance liquid chromatography. © Munksgaard, 2000.

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The adhesive material in adhesive plasters consists of a sticky mass that can be made of acrylic polymers or rubber with tackifiers such as rosin. Allergic contact dermatitis to adhesive plasters may be due to colophony or other resins, rubber compounds, such as dithiocarbamates, or acrylate monomers (1–4). Nowadays, polyacrylate-based adhesives are common. Skin reactions to these adhesives are seldom seen.

We recently reported 2 cases of allergy to a new allergen in adhesive plasters (N,N'-disalicylidene-1,2-diaminopropane) (5). This compound readily forms complexes with different transition metal ions and is therefore used as a copper inhibitor in some adhesive plasters, rubber products and gasoline (6). The presence of transition metal ions in various products increases the rate of oxidation of olefinic constituents in the products. For this reason, metal chelators are often used in cosmetics to enhance the effect of other preservatives added (7). N,N'-disalicylidene-1,2-diaminopropane is an imine (also called Schiff's base) stabilized with an aromatic group attached to the carbon of the imine bond. Imines are condensation products between an amine and an aldehyde or ketone. In this case, the amine is 1,2-diaminopropane, and the carbonyl compound is

salicylaldehyde. Most imines are easily hydrolyzed in an aqueous medium to yield the corresponding carbonyl compound and the free amine. The fast hydrolysis of a similar compound (N,N'-disalicylidene-1,3-diaminopropane) was previously demonstrated when investigating this compound's ability to inhibit the photosynthetic reaction in plants (8).

Here, we present a method for the analysis of N,N'-disalicylidene-1,2-diaminopropane in adhesive plasters. We also show how this compound is hydrolyzed to 1,2-diaminopropane and salicylaldehyde at a pH corresponding to that in the skin. Patch testing was performed to investigate the cross-reactivity pattern of this type of compound, as well as of the corresponding free amines.

Materials and Methods

Chemicals

1,2-ethylenediamine, salicylaldehyde, sodium dihydrogen phosphate monohydrate and di-sodium hydrogen phosphate dihydrate were obtained from Merck, Darmstadt, Germany. 1,2-diaminopropane, 1,3-diaminopropane, 1,4-diaminobutane, and N,N'-disalicylidene-1,3-diaminopropane were

obtained from Lancaster, Lancashire, England. N,N'-disalicylidene-1,2-ethylenediamine, N,N'-disalicylidene-1,2-diaminopropane and N,N'-disalicylidene-1,4-diaminobutane were obtained from TCI, Tokyo, Japan. Salicylic acid was obtained from Acros, Geel, Belgium. Acetonitrile, methanol and methyl tert-butyl ether were obtained from LabScan, Dublin, Ireland. Ammonia and isopropanol were obtained from Farmitalia Carlo Erba, Milano, Italy. The plasters analysed were Hansaplast® Elastic strip, Hansamed® Soft and Hansaplast® Robust. All plasters analysed were manufactured by Beiersdorf AG, Hamburg, Germany.

HPLC

A Waters 600 pump (Waters Chromatography Division, Milford MA, USA) equipped with a Rheodyne Model 7125 loop injector (20 μ l) and a Spectromonitor III (LDC/Milton Roy, Riviera Beach, FL, USA) UV-detector working at 325 nm were used. All separations were performed on a Genesis C₁₈ column (4 μ m, 100 $\times$ 4.6 mm, Jones Chromatography Limited, Mid Glamorgan, UK). All analyses were performed at room temperature at a flow rate of 1.0 ml/min. In the study of the hydrolysis of N,N'-disalicylidene-1,2-diaminopropane, the column was eluted with a mobile phase consisting of 60% solvent A (0.05 M sodium phosphate buffer, pH 7.5) and 40% solvent B (methanol) at 0–1

min, then a linear gradient to 80% B from 1 to 20 min after injection was used. When analysing extracts of adhesive plasters, the column was eluted with 65% methanol in 0.05 M sodium phosphate buffer at pH 7.5.

Because of the rapid hydrolysis, all stock solutions of N,N'-disalicylidene-1,2-diaminopropane must be made in non-aqueous media. Acetonitrile is preferred in this case because of its high solvent capacity. To achieve symmetrical peaks in the chromatogram, it is important to mix the analyte with aqueous buffer, a procedure that starts hydrolysis and must therefore be done immediately before injection on the column.

TLC

In addition to salicylaldehyde, 1,2-diaminopropane was also formed during the hydrolysis. Unfortunately, this small aliphatic diamine could not be monitored with the same chromatographic system. A thin-layer chromatographic method was used instead, using glass plates pre-coated with silica gel 60 F-254 (Merck, Darmstadt, Germany) with a layer thickness of 0.25 mm. For analysis of free amines, the plates were eluted with isopropanol: 25% ammonia (7:3) and the spots were visualized by spraying with ninhydrin solution. The choice of developing system enabled 1,2-diaminopropane (R_f=0.45) to be distinguished from ethylenedi-

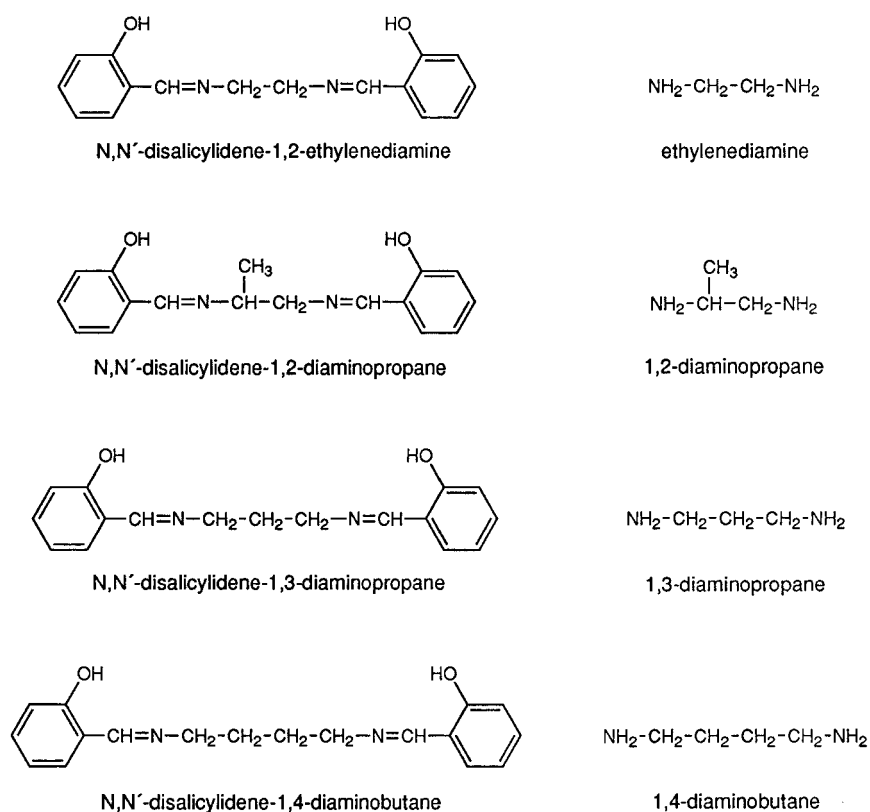
Table 1. Patch test results

Allergen	Concentration (% in pet.)	Patient no.					
		1	2	3	4	5	6
N,N'-disalicylidene-1,2-ethylenediamine	2	+++	+++	++	NT	NT	NT
	0.6	++	+++	+	NT	NT	NT
	0.2	+	++	+	+++	NT	NT
	0.06	?+	+	?+	++	NT	NT
N,N'-disalicylidene-1,2-diaminopropane	2	+++	+++	++	NT	++	+++
	0.6	+++	+++	+	NT	+	++
	0.2	++	++	–	+++	–	+
	0.06	+	+	–	++	–	–
N,N'-disalicylidene-1,3-diaminopropane	2	–	–	–	++	NT	NT
	0.6	–	–	–	–	NT	NT
	0.2	–	–	–	+	NT	NT
	0.06	–	–	–	–	NT	NT
N,N'-disalicylidene-1,4-diaminobutane	2	–	–	–	–	NT	NT
	0.6	–	–	–	–	NT	NT
	0.2	–	–	–	–	NT	NT
	0.06	–	–	–	–	NT	NT
1,2-ethylenediamine	1	++	+++	++	+++ ^{a)}	+	pos ^{b)}
1,2-diaminopropane	1	+++	++	+	+++ ^{a)}	NT	NT
1,3-diaminopropane	1	–	–	–	–	NT	NT
1,4-diaminobutane	1	–	–	–	–	NT	NT
salicylaldehyde	2	–	–	–	–	NT	NT
salicylic acid	2	–	–	–	–	NT	NT

^{a)} 0.2% pet.

^{b)} This patient reacted to ethylenediamine on an earlier occasion.

Fig. 1. Structures of imines and amines used for patch testing.



amine ($R_f=0.26$) as well as from 1,3-diaminopropane ($R_f=0.15$) and 1,4-diaminobutane ($R_f=0.12$).

Extraction of adhesive plasters

To solubilize the adhesive mass from a piece of plaster, the plaster was placed in a test tube with 5 ml of methyl tert-butyl ether and vortexed until the adhesive was loosened from the plaster. 1 ml of the milky extract was diluted with 4 ml of acetonitrile, followed by vigorous shaking of the test tube. This procedure caused the polymer to adhere to the glass surface. 1 ml of the clear solution was mixed with 1 ml of 0.05 M sodium phosphate buffer (pH 7.5) and immediately analysed by HPLC.

Hydrolysis of N,N'-disalicylidene-1,2-diaminopropane

2 ml of a solution of 200 $\mu\text{g/ml}$ N,N'-disalicylidene-1,2-diaminopropane in acetonitrile was mixed with 2 ml phosphate buffer (0.05 M, pH 7.5 or 5.5) in a test tube with a screw cap. After mixing, 1 sample was immediately analysed by HPLC. The test tube was placed on a shaker at room tempera-

ture and samples were taken for analysis at different times.

Patch testing

Patch tests with allergens in pet. were applied to the skin using Finn Chambers[®] (Epitest Ltd Oy, Finland) mounted on Scanpor[®] (Norgesplaster A/S, Norway). The patches were removed after 2 days and read after 3 and 7 days. Gradings were made according to the International Contact Dermatitis Group scale. The concentrations of the different allergens are given in Table 1, and the structures are shown in Fig. 1.

The test panel was selected to show probable cross-reactions between compounds with similar structures with respect to the amine part of the imine and to the free amines. All patients in Table 1 were referred to our clinic for hand eczema except for patient no. 1, who presented with a history of dermatitis after the use of an adhesive plaster, and patient no. 3, who had a facial dermatitis. Some of the patients were initially tested with the standard series and, when a positive patch test reaction to ethylenediamine was noted, the adhesive plaster components were tested. 16 controls tested to ethyl-

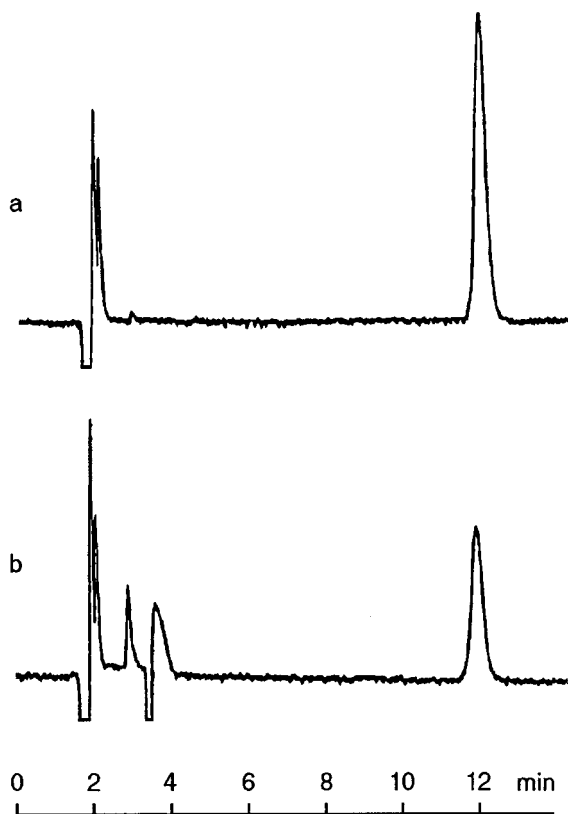


Fig. 2. Analysis of adhesive plaster extracts. (a) Chromatogram of a standard solution of N,N' -disalicylidene-1,2-diaminopropane. (b) Chromatogram of an extract of a single piece of Hansaplast[®] ELASTIC strip.

enediamine and to N,N' -disalicylidene-1,2-diaminopropane were negative.

Results

Analysis of adhesive plasters

About 60 μg of N,N' -disalicylidene-1,2-diaminopropane was found in a piece of Hansaplast[®] ELASTIC strip, weighing 475 mg and with an area of about 18.5 cm^2 (Fig. 2). N,N' -disalicylidene-1,2-diaminopropane could not be detected in Hansamed[®] Soft or Hansaplast[®] Robust.

Hydrolysis of N,N' -disalicylidene-1,2-diaminopropane

The chromatograms in Fig. 3 show how N,N' -disalicylidene-1,2-diaminopropane is hydrolyzed to yield salicylaldehyde during the reaction, and in Figs. 4a, b, the peak heights of N,N' -disalicylidene-1,2-diaminopropane and salicylaldehyde are plotted against time after addition of aqueous buffer in acetonitrile. The half-life of N,N' -disalicylidene-1,2-diaminopropane is about 1–1.5 h under these reaction conditions. It is clear from these

curves that the hydrolysis is a fast reaction at a pH corresponding to that of the skin. Thin-layer chromatography of hydrolysed N,N' -disalicylidene-1,2-diaminopropane showed that 1,2-diaminopropane was formed together with salicylaldehyde.

Patch testing

The patch test results presented in Table 1 show that all 6 patients with positive reactions to N,N' -disalicylidene-1,2-diaminopropane and 1,2-diami-

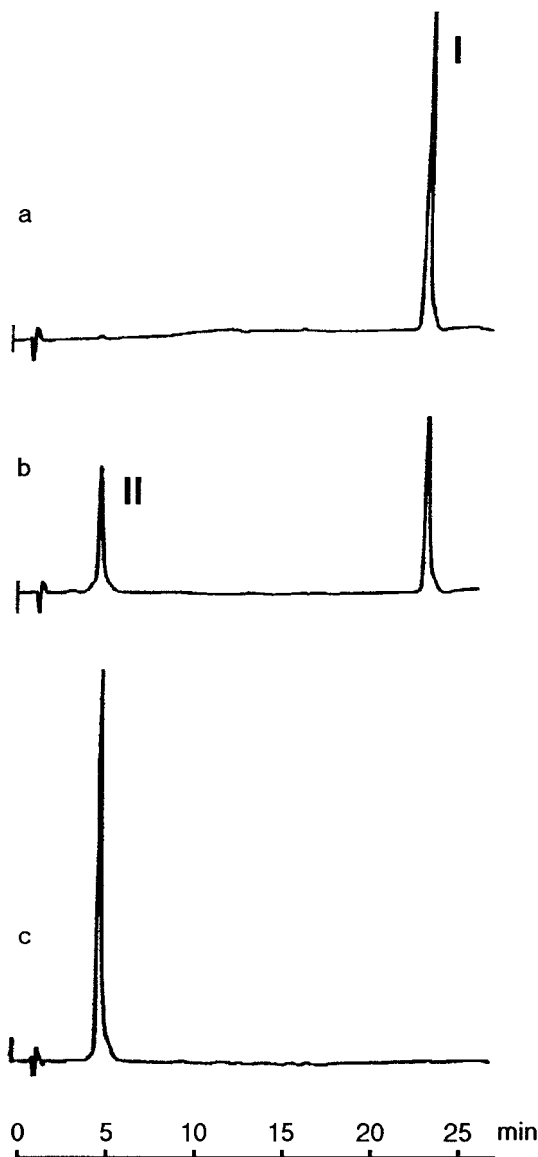


Fig. 3. Chromatograms showing the hydrolysis of N,N' -disalicylidene-1,2-diaminopropane. (a) Injection of N,N' -disalicylidene-1,2-diaminopropane immediately after addition of aqueous buffer (pH 5.5). (b) Injection after 35 min. (c) Injection after 340 min. Peak I represents N,N' -disalicylidene-1,2-diaminopropane and peak II represents salicylaldehyde.

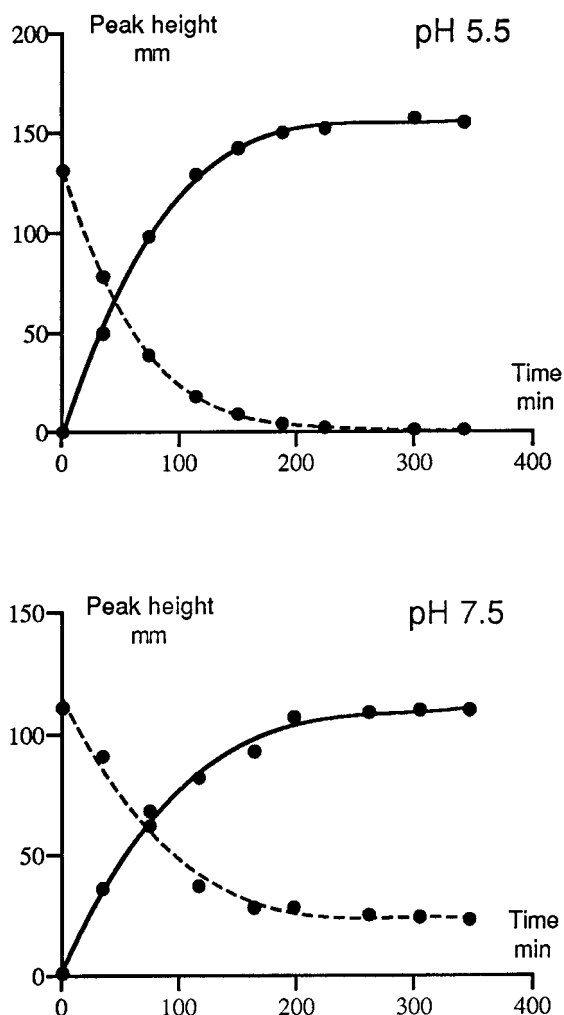


Fig. 4. Hydrolysis of N,N'-disalicylidene-1,2-diaminopropane. Peak heights of N,N'-disalicylidene-1,2-diaminopropane (---) and salicylaldehyde (—) plotted against reaction time at 2 different pH.

nopropane cross-reacted with ethylenediamine but manifested no reaction to salicylaldehyde. 4 patients (nos. 1–4) were also tested with N,N'-disalicylidene-1,2-ethylenediamine, to which they were positive. 1 of these patients (no. 4) manifested

a weak reaction to N,N'-disalicylidene-1,3-diaminopropane.

The results shown in Table 1 demonstrate reaction to an imine to be accompanied by reaction to its corresponding amine. We also found that all patients reacting to ethylenediamine also reacted to both N,N'-disalicylidene-1,2-diaminopropane and 1,2-diaminopropane. Finally, no patient in this study manifested reaction to salicylaldehyde.

Discussion

On several occasions, we have examined patients with a history of dermatitis when using one of the most frequently-used adhesive plasters. The dermatitis proved to be caused by an allergic reaction to a compound never before reported as an allergen and with a structure quite different from those of other known contact allergens (5). This finding was made after we had obtained information and test substances from the manufacturer. In this study, our own analysis of the plaster confirmed the presence of N,N'-disalicylidene-1,2-diaminopropane, and showed a single piece of the plaster to contain about 60 μg of this allergen. Even more noteworthy was the finding that N,N'-disalicylidene-1,2-diaminopropane is rapidly hydrolyzed on contact with water, yielding the free amine 1,2-diaminopropane. The similarities in structure of this amine and of ethylenediamine are clear, and probably account for their cross-reaction on patch testing.

The adhesive in the plaster containing N,N'-disalicylidene-1,2-diaminopropane consisted of a rather thick layer of a white, sticky mass. Extraction of the adhesive proved to be a critical step in the analysis. Firstly, because the extraction solvent had to be able to loosen the adhesive effectively from the plaster and give a homogeneous extract. Secondly, because the polymeric constituents in the extract had to be separated from the analyte before injection on the column. And thirdly, because any contact with aqueous solvents would hydrolyze the analyte.

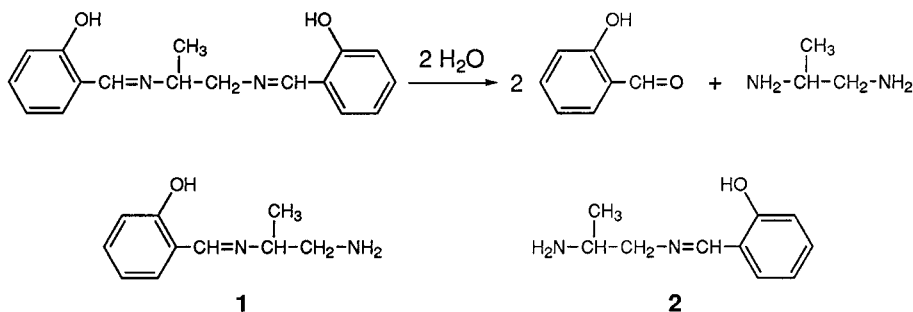


Fig. 5. The hydrolysis reaction. Compounds 1 and 2 are probable intermediates in the reaction.

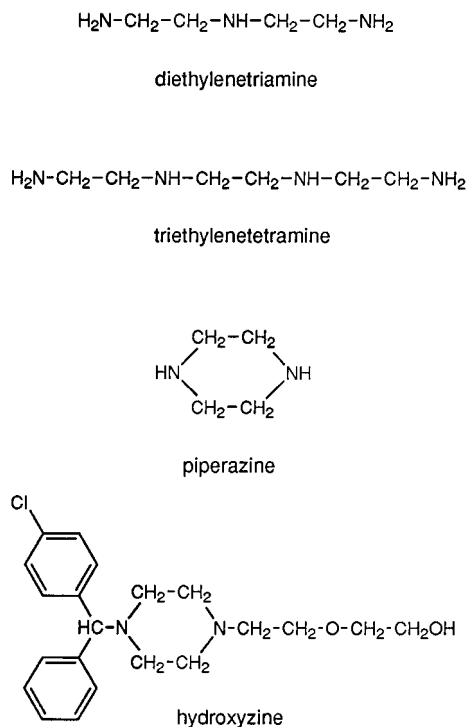


Fig. 6. Some other amines with ethylenediamine moieties in their structure.

Methyl tert-butyl ether (MTBE) was finally chosen for the extraction. We have previously used this solvent to extract mercaptobenzothiazoles in rubber products in high yields (9). MTBE is a suitable solvent for many purposes because it is a somewhat polar and lipophilic solvent with a high solvent capacity. It also has a low boiling point, making it easy to evaporate when necessary. Unlike many other ethers, it forms peroxides very slowly, which is of great advantage for laboratory safety.

That N,N'-disalicylidene-1,2-diaminopropane is hydrolyzed in water is not surprising. This is a feature of most imines, and our experiments showed the reaction to be rapid at physiological pH. The scheme in Fig. 5 outlines the reaction during hydrolysis. Some of the chromatograms manifested a small double peak midway between salicylaldehyde and N,N'-disalicylidene-1,2-diaminopropane, which might have represented compounds **1** and/or **2**. These short-lived intermediates were not isolated.

If we compare equimolar amounts of allergen and assume the imine to be completely hydrolyzed, a patch test with 2% N,N'-disalicylidene-1,2-diaminopropane should correspond to a patch containing about 0.5% 1,2-diaminopropane. A patch test with 2% imine would probably give a stronger reaction than 0.5% amine, because of the more

lipophilic character of the imine. N,N'-disalicylidene-1,2-diaminopropane is a lipophilic and non-ionic compound that presumably has a manifest tendency to penetrate into the skin, especially as it is a constituent of the adhesive of a plaster attached directly to the skin. When N,N'-disalicylidene-1,2-diaminopropane comes into contact with water in the skin, it is hydrolyzed and salicylaldehyde and 1,2-diaminopropane are formed.

In all likelihood, the allergic reaction to N,N'-disalicylidene-1,2-diaminopropane is dependent on the formation of 1,2-diaminopropane during hydrolysis of the imine. We also believe the cross-reaction between 1,2-diaminopropane and ethylenediamine to be due to the structural similarities between these free amines. The ethylenediamine moiety in other structures (Fig. 6) has been discussed earlier in connection with contact dermatitis. Diethylenetriamine and triethylenetetramine, found for example in epoxy hardeners, have been shown to cross-react with ethylenediamine (10). These 2 amines contain the ethylenediamine substructure, which explains such cross-reactions. Piperazine is another amine in this category, often found as part of a larger structure, for example in the antihistamine drug hydroxyzine. Piperazine has also been reported to cross-react with ethylenediamine (11). However, 2 reports on hydroxyzine allergy have recently been published. In 1 of the reports, 3 patients with positive patch test reactions to hydroxyzine were negative to ethylenediamine and piperazine (12). In the other report, a patient with contact allergy to ethylenediamine developed systemic contact dermatitis when taking hydroxyzine (13).

Ethylenediamine can also be an ingredient in medical preparations and in that connection act as an allergen. The asthma drug aminophylline is a complex of ethylenediamine and theophylline. Systemic reactions have been reported after use of this preparation, and have been explained as hypersensitivity reactions to ethylenediamine (14–16). Ethylenediamine has also been used as a stabilizer in antibiotic and corticosteroid creams, and has been a common cause of allergic reactions (17). In this paper, we report that a new allergen in a frequently used adhesive plaster, after hydrolysis in the skin, cross-reacts with ethylenediamine. This finding provides a new explanation of the common observations of positive reactions to ethylenediamine on patch testing, where no other obvious explanation can be found.

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References

1. Gäfvert E, Färm G. Rosin (Colophony) and zinc oxide in adhesive bandages. An appropriate combination for rosin-sensitive patients. *Contact Dermatitis* 1995; 33: 396–400.
2. O'Donnell B F, Hodgson C. Allergic contact dermatitis due to lanolin in an adhesive plaster. *Contact Dermatitis* 1993; 28: 191–192.
3. Taylor J S. Adhesives, gums and resins. In: Fischer A A (ed): *Contact dermatitis*, 3rd edition. Philadelphia, Lea and Febiger, 1986: 644–674.
4. Fischer A A. Dermatitis due to medical mechanical devices. In: Fischer A A (ed): *Contact dermatitis*, 3rd edition. Philadelphia, Lea and Febiger, 1986: 362–365.
5. Hansson C, Bergendorff O. Allergy to adhesive plaster. *Allergy* 1998; 53: 324–325.
6. Hochhauser A. Gasoline and other motor fuels. In: Kroschwitz J, Howe-Grant M (eds): *Kirk-Othmer encyclopedia of chemical technology*, 4th edition. New York: Wiley Interscience, 1994: 12: 362.
7. Hart R. Chelating agents as preservative potentiators. In: Kabara J (ed): *Cosmetic and drug preservation: principles and practice*. New York: Marcel Dekker, Inc, 1984: 323–337.
8. Ireland C, Goldwin G. Are the in vitro effects of disalicylideneopropanediamine mediated by salicylaldehyde? *Plant Physiology* 1979; 63: 1210–1211.
9. Hansson C, Bergendorff O, Ezzelarab M, Sterner O. Extraction of mercaptobenzothiazole compounds from rubber products. *Contact Dermatitis* 1997; 36: 195–200.
10. Rudzki E, Krajewska D. Cross-reactions between ethylenediamine, diethylenetriamine, and triethylenetetramine. *Contact dermatitis* 1976; 2: 311–313.
11. Price M L, Hall-Smith S. Allergy to piperazine in a patient sensitive to ethylenediamine. *Contact Dermatitis* 1984; 10: 120.
12. Michel M, Domp Martin A, Louvet S, Szczurko C, Castel B, Leroy D. Skin reactions to hydroxyzine. *Contact Dermatitis* 1997; 36: 147–149.
13. Ash S, Scheman A. Systemic contact dermatitis to hydroxyzine. *American Journal of Contact Dermatitis* 1997; 8: 2–5.
14. Terzian C G, Simon P A. Aminophylline hypersensitivity apparently due to ethylenediamine. *Ann Emerg Med* 1992; 21: 312–314.
15. Allergy to aminophylline (editorial). *Lancet* 1984; 2: 1192–1193.
16. Guin J D, Fields P, Thomas K L. Baboon syndrome from i.v. aminophylline in a patient allergic to ethylenediamine. *Contact Dermatitis* 1999; 40: 170–171.
17. Fisher A A. Unnecessary addition of ethylenediamine hydrochloride to “generic” nystatin creams. *J Amer Acad Derm* 1989; 20: 129–130.

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