

Concomitant contact dermatitis due to textile dyes and to colour film developers can be explained by the formation of the same hapten

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p-phenylenediamine derivatives are widely used in industry and in cosmetics, and several of them are well-known sensitizers. One group of allergenic *p*-phenylenediamine derivatives are used as colour film developers. Cross-reactivity between the colour film developers has been reported. In this paper, an occupational facial dermatitis due to colour film developers is described. The patient reacted to colour film developers (CD-1, CD-2, CD-3, and CD-4), but not to other *p*-phenylenediamine derivatives tested. He also showed allergic reactions to Disperse Blue 106 and Disperse Blue 124 and to Disperse Red 17, but not to Disperse Orange 3. The activation of the colour film developers by oxidation at physiological pH was analysed with chemical methods, and the mechanism responsible for the concomitant reactivities to the colour film developers and the disperse dyes at a molecular level is discussed.

Key words: phenylenediamine; PPD; azo dye; Disperse Blue 106; Disperse Blue 124; Disperse Red 17; Disperse Orange 3; benzoquinone; imine; dermatitis; cross-reactivity; skin test; cyclic voltametry; colour film developer. © Munksgaard, 1997.

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A patient occupationally exposed to colour film developers was referred to our clinic because of severely pruritic facial dermatitis. At patch testing, he manifested strong reactions to several colour film developers but also to some textile dyes. Concomitant sensitivity to textile dyes and colour film developers has not previously been described.

Colour film developers are *p*-phenylenediamine derivatives and have been found to be strong sensitizers (1) (Fig. 1), though owing to their limited use, only relatively few people are exposed to these chemicals. However, among staff at film processing laboratories, a high frequency of sensitized subjects has been registered. In an extensive study, Lidén (2, 3) has demonstrated the prevalence of sensitized subjects and their symptoms, and also how modernization of the laboratories is beneficial to staff working with these chemicals. The occurrence both of eczema and lichenoid dermatitis has been described as a result of contact with *p*-phenylenediamine derivatives in colour film laboratories (2, 4-8).

Other *p*-phenylenediamine derivatives are used widely in rubber production and in hair dye products. Some of these compounds are also well-known sensitizers, and the non-substituted compound *p*-phenylenediamine (PPD) constitutes one

of our most common sensitizers, and has long been included in standard series. *p*-phenylenediamine derivatives are supposed to be prohaptens which are activated by oxidation into the true haptens. *p*-benzoquinone mono- and di-imine, oxidation products formed from *p*-phenylenediamine (PPD), are very reactive and can form a number of products depending on the conditions (9). As hydrolysis of the imines proceeds rapidly in water irrespective of pH, special techniques are required to study intermediates and reaction mechanisms in biomimetic systems. In qualitative assessment of the ease with which these prohaptens are activated by oxidation, cyclic voltametry constitutes a simple and efficient method for evaluating oxidation-reduction systems of biological significance (10).

Several textile dyes belong to a group of chemicals referred to as azo compounds. Some of these azo dyes have been shown to cause simultaneous allergic reactions to *p*-phenylenediamine, explained by a cleavage of the azo bond yielding the aromatic amine functionality present in *p*-phenylenediamine (11, 12).

Degradation experiments showing the formation of the colour film developer haptens from some textile dyes are reported in this paper, and

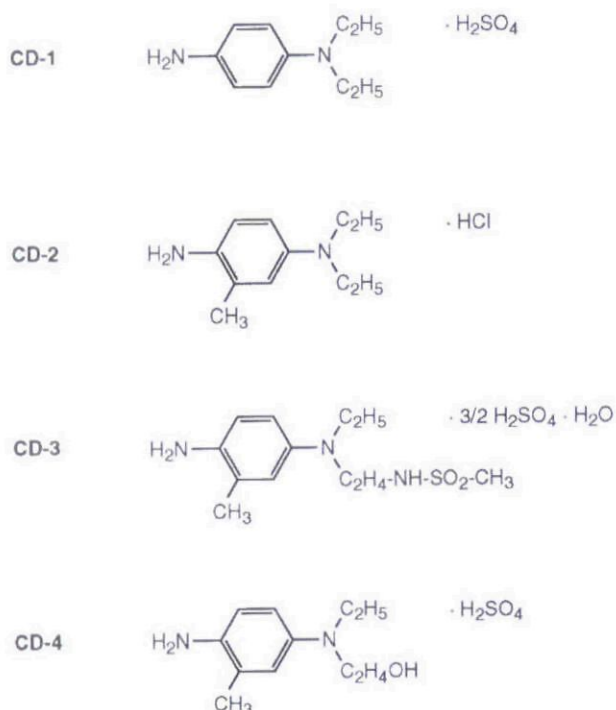


Fig. 1. Molecular structures of CD-1, CD-2, CD-3 and CD-4.

the mechanism underlying the simultaneous allergic reactions is discussed.

Case Report

A 35-year-old man presented with a 2-year history of severe facial dermatitis. At work he had operated an automatic colour film developing machine for the past 5 years. After 3 years of this work, he developed strongly pruritic erythematous dermatitis on his forehead.

Examination showed the presence of erythema, oedema, and scaling on his forehead, a weak perioral erythema, and scaling dermatitis on 2 fingers of his right hand and on the dorsum of the left hand. He reported no history of atopy or any other skin disease. After 2 weeks on sick leave, he was essentially healed and returned to work, but after only 1 day the facial dermatitis relapsed.

He was patch tested with the standard series (TRUE testTM panel 1 and 2, Pharmacia AB), the photographic chemicals series (Chemotechnique Diagnostics AB, Malmö, Sweden), and selected disperse dyes and related chemicals (Table 1). Owing to severe itching, the patches were removed by the patient at as early as 1 day. Readings were performed at 3 and 7 days. In the standard series, he showed a positive reaction to ethylenediamine. He also showed positive reactions to all 4 colour film developers tested, CD-1, CD-2, CD-3 and CD-4, but not to PPD, PPD-mix or *p*-benzoquin-

one. Tests for Disperse Blue 106, Disperse Blue 124 and Disperse Red 17 were all positive, but the Disperse Orange 3 test elicited no reaction.

Materials and Methods

Chemicals

The chemicals used were as follows: sodium dihydrogen phosphate, disodium hydrogen phosphate dihydrate, acetic acid, 100% (Merck), sodium acetate, *p*-phenylenediamine (PPD) (Fluka), *N,N*-diethyl-*p*-phenylenediamine sulphate (CD-1) (Sigma), *N,N*-diethyl-2-methyl-*p*-phenylenediamine hydrochloride (CD-2) (Aldrich), 4-(*N*-ethyl-*N*-2-methanesulfonylaminoethyl)-2-methyl-*p*-phenylenediamine sesquisulfate monohydrate (CD-3), 4-(*N*-ethyl-*N*-2-hydroxyethyl)-2-methyl phenylenediamine sulphate (CD-4) (Acros), Disperse Blue 106, Disperse Blue 124, Disperse Orange 3, Disperse Red 17 (Chemotechnique Diagnostics).

HPLC analysis

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 254 nm were used. All separations were performed on a Lich-

Table 1. Positive patch test reactions in the standard series (TRUE Test, TT) and complementary patch tests

	D3	D7
PPD (TT)	—	—
PPD base (1% pet.)	—	—
PPD mix (TT)	—	—
ethylenediamine (TT)	+++	+++
hydroquinone (1% pet.)	—	—
benzoquinone (0.1% pet.)	—	—
CD-1 (1% pet.)	+	?
CD-2 (1% pet.)	++	+
CD-3 (1% pet.)	++	+
CD-4 (1% pet.)	++	+
2,5-diaminotoluene sulfate (1%pet.)	—	—
4-methylaminophenol sulfate (1% pet.)	—	—
Disperse Blue 106 (1% pet.)	++	+
Disperse Blue 124 (1% pet.)	++	+
Disperse Red 17 (1% pet.)	++	+
Disperse Orange 3 (1% pet.)	—	—

Table 2. Half wave potentials measured by cyclic voltammetry, scan rate 300 mV/s

	pH 7.4 E _{ox1/2} (V)	pH 5.5 E _{ox1/2} (V)
CD-4	0.08	0.20
CD-3	0.09	0.15
CD-2	0.10	0.21
CD-1	0.12	0.23
PPD	0.14	0.22

rospher RP 100 C₁₈ column (5 μ m, 125 $\times$ 4 mm, Merck). All analyses were performed at room temperature at a flow rate of 1.0 ml/min.

Disperse dyes were analysed using acetonitrile: water: tetrahydrofuran (99: 99: 2) as the mobile phase. The retention times were 16.6 min for Disperse Blue 124 and 6.0 min for Disperse Blue 106. CD-4 was analysed using 10% methanol in 0.01 M butylamine (with pH adjusted to 5.2 with acetic acid) as the mobile phase. The retention time for CD-4 was 4.6 min.

Cyclic voltametry

All measurements were performed with a CV-1A Cyclic voltametry instrument. A carbon paste electrode (packed with CP-O carbon paste) was used as the working electrode and a platinum wire as the auxiliary electrode. The reference electrode was an Ag/AgCl electrode. Cyclic voltametry was performed at ambient temperature and a scan rate of 300 mV/s. All components were purchased from Bioanalytical Systems Inc., W Lafayette, IN, USA.

Test chemicals (1.0 mM) in buffer solutions containing 8% ethanol were analysed with cyclic voltametry at pH 7.40 and 5.50. For the preparation of the solutions with pH 7.40 a 0.1 M phosphate buffer was used and the pH of the test solution was adjusted appropriately before analysis. For the preparation of the solutions with pH 5.50, a 1.0 M sodium acetate buffer was used, pH being adjusted appropriately before analysis. Every test solution was analysed several times, the carbon electrode being polished before each analysis.

Results

Analysis data are presented in Table 2.

pH 7.40: This pH was chosen for the first investigation as representing *in vivo* conditions, where the oxidation reactions in question are supposed to take place. As can be seen in Table 2, the 5 compounds examined manifested a distinct sequential oxidation tendency. This sequence demonstrates how easily the 1st step in the proposed sensitization mechanism is passed by the different compounds. According to the $E_{ox1/2}$ values, CD-4 is easiest to oxidize, and PPD is less prone to oxidation.

pH 5.50: This pH was chosen for the 2nd investigation as representing the pH on the skin surface, where the compounds in question might also be oxidized. According to the $E_{ox1/2}$ values, it is easiest for CD-3 to pass the oxidation step, and PPD and CD-1 are most resistant to oxidation.

Discussion

Cross-reactivity between different colour film developers has been reported in a few studies (1, 4, 8). The patient described in this paper reacted to CD-1, CD-2, CD-3 and CD-4. In his work, he was exposed to CD-3 and CD-4, but the possibility of earlier exposure to CD-1 and CD-2 cannot be excluded. In all likelihood the exposure was mainly airborne, since in addition to dermatitis on his hands he also had intensely pruritic dermatitis on his face. Systemic contact dermatitis due to the inhalation of colour film developers has been described (5). Our patient showed no reactions on

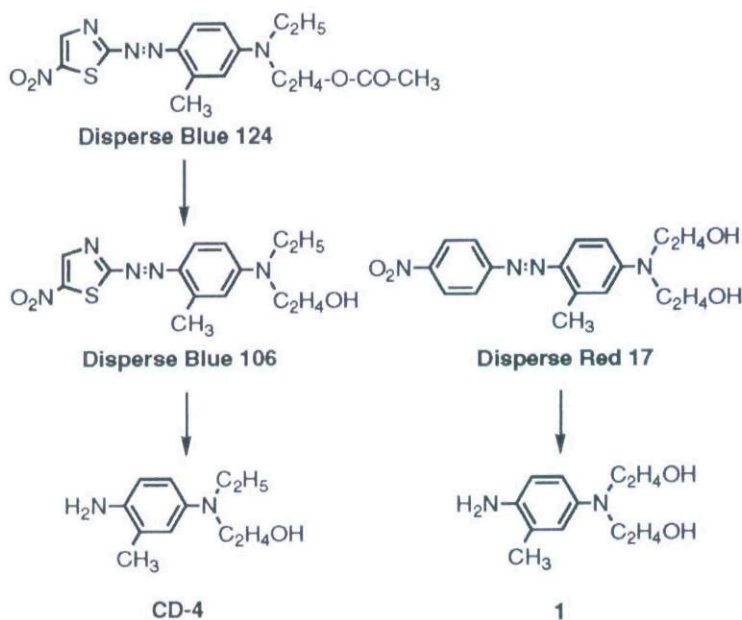


Fig. 2. Molecular structures of some disperse dyes and the products formed from them.

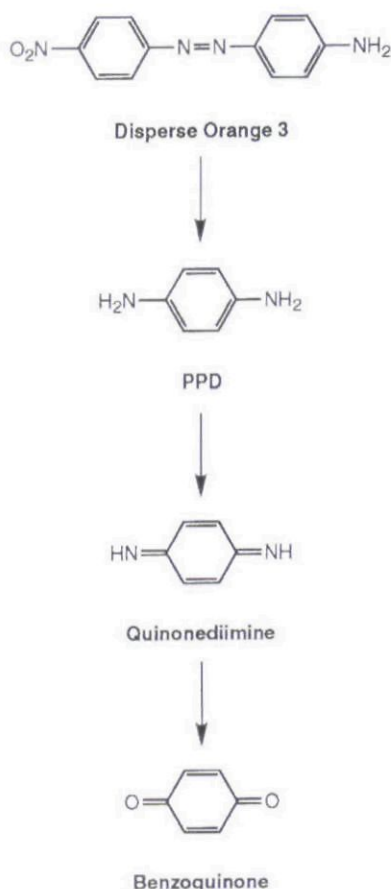


Fig. 3. The formation of benzoquinone from Disperse Orange 3.

skin shielded by clothes, which argues against inhalation as the cause, and emphasises the likelihood of airborne skin contact.

Although our patient had no history of reaction to coloured clothes, we performed patch tests using selected disperse dyes. Positive reactions to several disperse dyes were found, but only to substances with molecular structures which can be converted into one of the colour film developers or to a structure very closely related to them. It has been reported that liver enzymes, for example, can by reductive cleavage of the azo bond split off aromatic amines from azo dyes (13).

Reductive cleavage of Disperse Blue 106 gives CD-4 as 1 of the 2 molecules formed (Fig. 2). To a water solution of Disperse Blue 106 the reducing agent, sodium dithionite, was added until the blue colour disappeared. Analysis of the reaction mixture with HPLC showed that CD-4 was formed during the change of colour. Disperse Blue 124 is identical to Disperse Blue 106 except for the O-acetylation of the 2-hydroxyethyl group on the nitrogen. This acetate ester should be sensitive to hydrolysis by esterase activity yielding Disperse Blue 106 as the product, and concomitant reactions to

Disperse Blue 106 and Disperse Blue 124 has been reported (14). Hydrolysis of the ester was easily achieved by adding a few drops of hydrochloric acid to a water solution of Disperse Blue 124. Disperse Blue 106 was formed immediately after acidification, and the formation was monitored by HPLC. Disperse Red 17 is chemically quite different from Disperse Blue 106, but at reductive cleavage a *p*-phenylenediamine derivative (1) similar to CD-4 is formed. The other substances formed at reductive cleavage of Disperse Blue 106 and Disperse Red 17 are completely different molecules which are probably without relevance concerning sensitivity to colour film developers. Disperse Blue 106 and Disperse Blue 124 are textile dyes which are currently very popular in clothes worn by young women, and several cases of sensitization have recently been reported (15–17).

In a recently published paper (18), the azo dyes are classified according to the non-phenylenediamine part of the molecule. Our findings support an allergic mechanism where, after cleavage from the dye, the *p*-phenylenediamine component is the allergenic part of the molecule. This is borne out by the positive reactions to Disperse Red 17 and Disperse Blue 106, both yielding similar N-substituted 2,5-diaminotoluene derivatives from different types of azo dyes, and the absence of reaction to Disperse Orange 3 which at reductive cleavage yields *p*-phenylenediamine without any substituents.

Disperse Orange 3 was also tested but without reaction, a finding consistent with the absent reaction to PPD since PPD is the *p*-phenylenediamine compound formed at reductive cleavage of Disperse Orange 3 (Fig. 3). Several studies have shown the occurrence of concomitant reactions to PPD and Disperse Orange 3 (12, 18). The formation of benzoquinone as a common activated hapten has been discussed (19, 20). However, the absent reaction to 2,5-diaminotoluene, PPD and benzoquinone are inconsistent with the benzoquinone-hapten concept in the case of CD-4 sensitivity (Fig. 3). In this concept, benzoquinone is formed from azo dyes and PPD-derivatives in several steps. Initial reductive cleavage of the azo bond yields an amino function which in turn is oxidized to imine and finally hydrolysed to benzoquinone. Not even the possible intermediate *p*-aminophenol produced any reaction in this patient.

The negative reaction to the PPD-mix test including IPPD, DPPD and CPPD, each with a mono-alkyl substitution at both nitrogens reflects the complex nature of the cross-reactivity pattern of PPD-derivatives. However, dialkyl substitution at one of the nitrogens is common to all the PPD-derivatives which give rise to positive reactions. The alkyl substituents on the nitrogen may differ

rather widely without affecting the reactivity pattern. It is obvious from the $E_{ox1/2}$ values that at cellular pH all the substances tested were more easily oxidized than PPD. The $E_{ox1/2}$ values also show that a methyl substituent on the aromatic ring lowers the oxidation potential. This is due to electron donation to the aromatic ring. The substituents on the N-alkyl group of CD-3 and CD-4 apparently also lower the oxidation potential of these molecules. These results are in agreement with results achieved at the strongly alkaline pH characteristic of colour film developing processes (21). Although the investigated compounds show small differences in the $E_{ox1/2}$ values, these small differences can be of importance in a complex in vivo activation. The oxidation-reduction behaviour of para-phenylenediamine derivatives and its implication for our understanding of the molecular basis of contact dermatitis are currently being investigated in greater detail.

In most patients, sensitivity to Disperse Orange 3 also produces positive reactions to PPD. Among other derivatives our patient was sensitive to the *p*-phenylenediamine derivative CD-4, and also manifested positive reactions to several disperse dyes. In this case, the primary sensitization was to the *p*-phenylenediamine derivatives, and the positive reactions to the disperse dyes may be regarded as a consequence of the sensitization to the colour film developers. The positive reactions to the disperse dyes can be explained as a metabolic hapten degradation of the azo dyes to the sensitizing CD-4 molecule. Whether CD-4 is the true hapten or only a prohaptent cannot be determined from our present findings. A consequence of these findings is that lichenoid dermatitis, which has been reported to occur in conjunction with exposure to colour film developers, can also be expected to occur on exposure to azo dyes which upon reductive cleavage yield *p*-phenylenediamine derivatives with substituents on the nitrogen as in the colour film developers.

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