

From the Institute of Dermatology, London, W.C. 2.

ALLERGIC SENSITIVITY TO EOSIN

By

C. D. CALNAN

Over the past five years, at St. John's Hospital for Diseases of the Skin in London, we have been making an extensive clinical study of allergic contact eczematous dermatitis, as a prelude to more specific problems. Like most workers in this field, we have encountered an appreciable number of patients allergic to cosmetics (Calnan 1957), and the commonest responsible agent was found to be lipstick. Although there are a large number of potential allergens in the base, perfume, and colour content—and most of them have caused dermatitis on occasion—for all practical purposes, fluorescein derivatives are the only important sensitizers in modern lipsticks and must account for more than 95 per cent of lipstick cheilitis (Calnan & Sarkany 1957). This was recognised twenty years ago by Hecht, Schwarzschild & Sulzberger (1939) as well as by many others investigators; its frequency was soon appreciated by the cosmetic industry, and many manufacturers now market non-indelible, non-eosin—containing lipsticks under proprietary names.

During the investigation of nearly a hundred lipstick sensitive patients, we have come up against a number of unusual findings which merited further study. The main clinical features have been described elsewhere (Calnan & Sarkany 1957). The observations which especially attracted our attention were:

- 1) Ten per cent of patients who reacted to lipstick on the lips gave a negative patch test on the skin of the back or arm.
- 2) Patients sensitive to eosin who reacted to one manufacturer's eosin-containing lipstick sometimes found that they could use another stick from a different manufacturer with impunity, even though it also contained eosin.
- 3) Purified laboratory eosin gave weaker patch test reactions than commercial products.

These observations are not original, for they were previously noted by Sulzberger and his colleagues, although we were not aware of their work at the time.

They suggested three possible explanations:

- a) The allergen is not pure eosin but an impurity.
- b) The combined action of the pure eosin and certain impurities elicits the allergic response.
- c) The pure eosin is rendered non-allergenic by the purification method.

To elucidate this they tested patients to the purified eosin and to the residues obtained in the process. Nearly all their patients reacted strongly to the residues and weakly, or not at all, to the purified dye. Hence they concluded that the allergen was an impurity in the commercial product. But, although they appreciated the importance of this work to allergy, immunology, biology, commerce and industry, they did not pursue it further (Sulzberger personal communication 1957).

We first studied the negative patch test. About 10 per cent of our genuinely lipstick sensitive patients had a negative test on the skin of the back. The eosin content of most lipsticks varies from 1 per cent to 5 per cent. A patch test to 5 per cent eosin in oil or petrolatum was negative, and yet these patients were only able to wear a non-eosin-containing lipstick without discomfort. Patch tests at other sites such as the inner arm and the side of the neck were also negative. Sulzberger (1939) believed that the allergic sensitivity was localised to the lips in such cases, and not present over the whole skin surface as

is usual. Exposure to ultraviolet light did not make the test site positive, nor did such measures as skin scratching, or mixing the allergen with 1 per cent sodium lauryl sulphate. However when we applied the eosin in a concentration of 50 per cent in wax every such case showed a positive reaction. The explanation of the negative lipstick patch test, therefore, is that the stratum corneum on the vermilion of the lips is so thin that it is readily penetrated by the allergen. On the skin of the body, however, the stratum corneum may be sufficiently thick to absorb all the eosin, for which it has a strong affinity, when applied in low concentration (1-5 per cent). When applied in high concentration (50 per cent) the stratum corneum at the test site is saturated and sufficient of the dye penetrates to the prickle cell layer so as to elicit the allergic reaction. This is, of course, only likely to be necessary in cases of a low degree of sensitivity to the dye. In a few biopsy specimens from test sites, we have been able to confirm these views on the penetration of the dye. Magnus (1958) has shown that eosin will bind with keratin at an acid pH only.

As far as the practical problem is concerned, one should not regard a patient as being non-allergic to eosin until they have failed to react to a 50 per cent concentration or to the pure powder.

Although it can be shown that the vast majority of lipstick sensitive patients are allergic to eosin, clinical experience presents perplexing facts. Quite a number of these patients can use a lipstick from certain cosmetic firms but not others, even though they all contain eosin. Sometimes the concentration of eosin varies but often these variations are not responsible for the presence or absence of reactions. I patch tested a number of known sensitive patients to samples of eosin from different sources and compared the results. It is not easy to do quantitative patch tests accurately with eosin, because of the staining by the dyestuff, and the nature of the reaction, apart from the method of application. Sulzberger and his co-workers always used the pure powder in their tests, but I used an ordinary lipstick base in which to dilute the eosin. However I found that

the eosin supplied by one manufacturer gave consistently stronger reactions than the others; and a cosmetic firm told me that when they used this eosin in their lipsticks they received more complaints of dermatitis from customers. It was also found that laboratory prepared eosin gave weaker reactions. Commercial preparations of eosin, although they may satisfy the requirements of the U.S. Certified Colours List of the Food and Drug Administration, can be "purified" to a greater degree by repeated sodium salt recrystallisation. Sulzberger & Hecht (1941) showed that such a purification process reduced and often abolished the capacity of the dye to produce a reaction in a sensitive patient. They also found that the residues from the recrystallisation process still contained the active allergen. I have been able to confirm these findings. Patients were tested with eosin after each recrystallisation, and the skin reaction was found to diminish and finally disappear after the sixth recrystallisation, except in one patient. These patients reacted to the residues. The six times recrystallised eosin was then incorporated in lipsticks at a concentration of 5 per cent. The patients who were patch test negative on their backs to this eosin at 50 per cent were asked to use the lipsticks. Out of 26 patients, 14 said their lips reacted adversely to it, and only 12 were able to continue using it without ill effects.

The most obvious explanation of these results is that purification of the eosin by recrystallisation six times has failed to remove all of the allergen, and enough is present in a 5 per cent concentration of the purified dye to elicit a reaction on the lips, which, as has been shown, are so much more sensitive than the skin of the back, and the patients who were able to tolerate this special lipstick did not react, simply because they had a lower degree of allergic sensitivity. However, one cannot disregard the possibility that there may be more than one allergen in commercial eosin amongst the impurities, and not all patients become sensitized to the same substance. Some patients may be allergic to absolutely pure tetrabromofluorescein; and again, if there is more than one allergen, these may be so closely related chemically that cross-sensitization takes place.

The identification of the precise chemical nature of the allergen or allergens is likely to be a most difficult problem. Strong evidence has been given that lipstick dermatitis patients are not often primarily allergic to the molecule of tetrabromofluorescein itself. The majority of patients who react to commercial preparations of tetrabromofluorescein also react to diioda and tetraiodo fluorescein, although the degree of sensitivity is generally much lower, but some show no reaction at all when tested at the same concentration as that of the tetrabromo-compound. This might indicate either that the halogen groups are not important in the constitution of the allergen, or that the antibody does not distinguish between the specific halogens. The latter explanation seems more likely since eosin sensitive patients do not react to fluorescein itself.

Fluorescein is made commercially by the condensation of two molecules of resorcinol with one molecule of phthalic anhydride, using anhydrous zinc chloride as a catalyst. The temperature used is usually in the region of 160°C for about three hours. The degree of completion of the reaction will naturally vary; some residual free resorcinol and phenolic by-products are probably present in the final preparation marketed as fluorescein. Commercial eosin, which is also known as D. and C. Red 21, tetrabromofluorescein, or 2,4,5,7-tetrabromo-3,6-fluorandiol, is made by brominating fluorescein. The product from this reaction is also variable. The U.S. regulations for certified colours require the following specifications: free bromine—not more than 0.02 per cent; range of organically combined bromine in pure dye—47.5 to 51.5 per cent; pure dye as determined gravimetrically—not less than 93 per cent.

In the course of bromination of fluorescein any free resorcinol and other contaminants will also be brominated. Various brom-resorcinals and bromphenols will be produced. It is possible that the allergens in commercial eosin may be found amongst the brominated by-products, as much as in the brominated fluorescein.

Paper chromatography of four samples of eosin from different sources showed that there was more than one fluores-

cent component in each. One sample showed an additional fluorescent substance which moved at an entirely different rate from the main materials. The solvent used was a mixture of 50 per cent ethanol and 5 per cent ammonium hydroxide. Paper chromatography was then carried out with the same solvent system on the following specimens:

- 1) a commercial brand of eosin,
- 2) eosin refined once by sodium salt recrystallisation.
- 3) eosin refined six times by sodium salt recrystallisation,
- 4) the residues from the first recrystallisation.

The chromatograms were examined in ultraviolet light. All of them showed a broad band of tetra-bromofluorescein. The original eosin (specimen 1) showed a small quantity of free fluorescein and one other orange coloured contaminant. The once-recrystallised refined eosin (specimen 2) revealed no free fluorescein, and a small amount of the orange coloured material seen in the original eosin. However, a trace of an additional light purple substance was seen, which was not present in specimen 1. The eosin which has been refined by six sodium salt recrystallisations did not show any visible or fluorescing impurity. The residues from the refining process contained at least three fluorescent contaminants, including the orange and purple materials mentioned above.

These substances can also be separated by column chromatography. Eosin sensitive patients have not yet been tested to the individual fractions. Because of the problems of penetration and the affinity of eosin for keratin, necessitating the use of a relatively high concentration of the test substance for patch tests, it may be difficult to obtain the fractions in large enough amounts to elicit a response, although, if the allergen has been purified, it should be active in low dilution. It must also be remembered that there is no evidence that the active allergen is fluorescent. If the allergen is a brom-resorcinol or a halogenated phenolic compound, it may prove very difficult to detect, unless the biological test for its presence can be made more sensitive.

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