

ASSESSMENT OF CHROMIUM ALLERGY: FEATURES OF PATCH TEST REACTIONS AT ELECTRON MICROSCOPIC RESOLUTION

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Abstract. The possible correlation of clinically recorded test reactions and morphological changes at electron microscopic resolution in human subjects allergic to chromate (CrVI) was investigated. The effect of simple occlusion and of vehicles, e.g. distilled water, an alkaline buffer, and petrolatum was also studied 72 hours after application. The results indicate that even simple occlusion causes reactive changes in living epidermal cells. These changes become conspicuous in occlusion with distilled water, more pronounced with petrolatum, and were destructive in the case of the alkaline buffer. Chromate solutions produced varying degrees of morphological change but were less pronounced in a vehicle of distilled water, compared with the changes seen when the chromate was combined with the alkaline buffer. Destructive cellular changes were observed in the electron microscope even in sections from test areas where no macroscopical reactions were recorded. When the clinical evaluation of hypersensitivity was done 72 hours after the application of the patch test the morphological changes were too advanced to provide specific pathognomonic information.

Key words: Chromium allergy; Clinical experimental study; Electron microscopy; Human subjects; Patch test reactions

The assessment of an allergic reaction at patch testing is at present based on macroscopic evaluation. Light microscopic study of biopsies from skin areas of "positive" reaction does not generally allow a differentiation between an allergic reaction and one of primary irritancy. Fine structure data correlating to the clinical findings in patch testing are exceedingly rare (14, 20) both with regard to the test substance and the vehicles and to the occlusion as such.

Accounts of experimentally induced skin reactions as seen in the electron microscope have recently been published (1, 4, 9, 10, 17, 22, 23). The

present report is part of a study confined to the epidermal effects of (CrVI) chromium.

The morphology of patch test reactions in persons allergic to chromium is described in relation to various factors such as simple occlusion, the type of vehicle and the type and concentration of the chromium compounds. The methods at present available for electron microscopic routine preparation of pathologic skin material are discussed in this context.

MATERIALS AND METHODS

Subjects. Five hypersensitive males, 40-60 years of age. Their chromium allergy was diagnosed by prior testing.

Chromium compounds. Sodium chromate ($\text{Na}_2\text{CrO}_4 \cdot 4 \text{H}_2\text{O}$), potassium bichromate ($\text{K}_2\text{Cr}_2\text{O}_7$).

Vehicles. Distilled water, petrolatum, and an alkaline buffer, pH 12 (19).

Concentrations. Serial dilutions (19): 0.25 %, 0.125 %, 0.0625 %, ... 0.0039 %

Test patches. Al-test (Imeco, Sweden).

Tape. Leukoflex (Beiersdorf, Germany), Blenderm (3M, USA).

Patch test method. The patches were applied in vertical rows on the skin of the back. They were removed after 48 hours' exposure, and the gross skin changes were read and biopsied 24 hours later. Apart from the testing with the chromium compounds in various vehicles, the effects of simple occlusion with a dry Al-test patch, the tapes and the three vehicles (without chromium) were studied.

Assessment of test reactions

No reaction	—
Erythema and infiltration	+
Papules	++
Vesicles	+++

Biopsies were taken from areas in the range of — to +++.

Biopsies were taken without local anesthesia with a

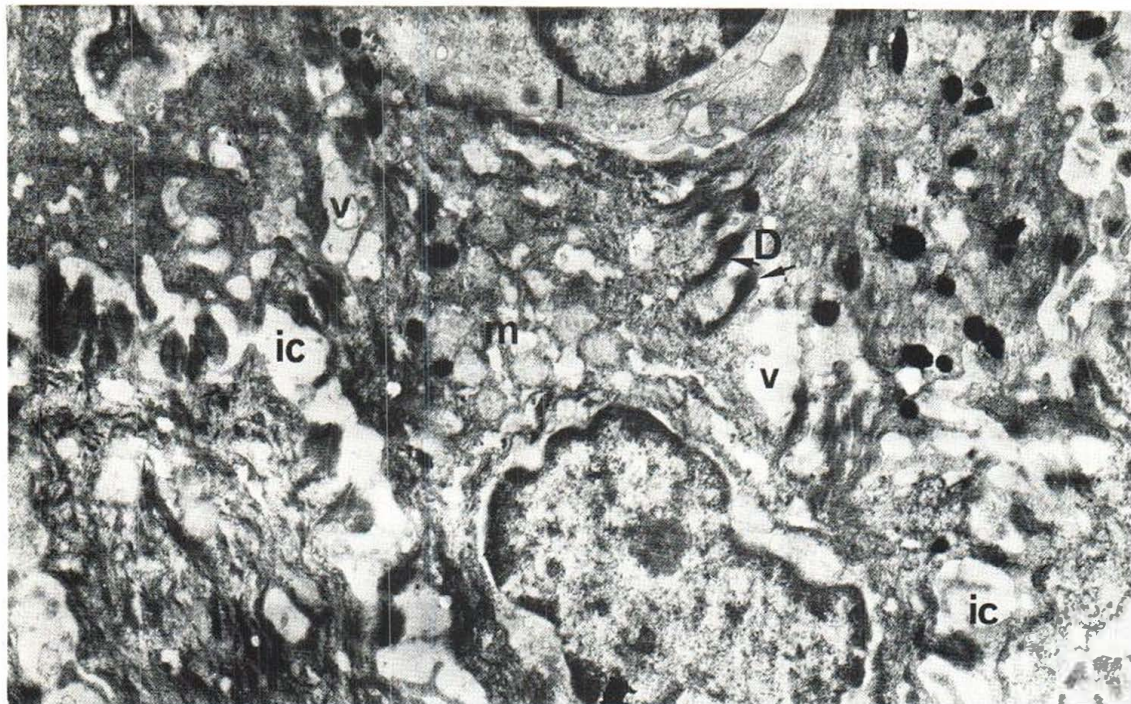


Fig. 1. Stratum spinosum of epidermis exposed to distilled water under occlusion. A number of vacuoles are seen in

the cytoplasm. The perinuclear cytoplasm is rarified. Top: an invading cell (a Langerhans cell). Male 58 ys. $\times 14\,400$.

2 mm punch. Unexposed skin samples for controls were taken well outside the test area on the contralateral side of the back. More than 60 biopsies were taken for this study.

Fixation, preparation. The biopsies were fixed in 3–5% glutaraldehyde in phosphate buffer at 4°C for 3 hours. During the fixation the specimens were split and one part was subsequently post-osmicated in 2% OsO_4 in phosphate buffer at 4°C for 1 hour. The fixed specimens were processed for Epon embedding according to Luft (8). Sectioning was performed on an LKB Ultratome set for 500 Å sections. The sections were contrasted with uranyl acetate and lead.

Electron microscope. Philips EM 301 G operated at 40 and 60 kV.

RESULTS

In conformity with the practice in our clinic the assessment of test reactions was made 24 hours after removal of the test patches. This facilitates differentiation between allergic reactions and those of primary irritancy. At this time no macroscopic reactions were recorded in areas exposed to tape, dry test patch or test patch with a vehicle (i.e. distilled water; alkaline buffer pH 12; petrolatum). Areas exposed to chromate solutions whether in

distilled water, petrolatum or in alkaline buffer displayed varying degrees of macroscopic reactions, depending on the concentration of the allergen.

At electron microscopic resolution (here 15–24 000 $\times$) cellular changes were observed in all biopsies from test areas. The changes were most conspicuous in the stratum spinosum but were also found in the basal layer and the stratum granulosum to a lesser extent. These changes could be specifically related neither to the occlusion as such nor to the vehicles or to the chromate solutions (CrVI). When moderate, the changes consisted of perinuclear vacuoles which frequently distorted the contour of the nucleus (Fig. 1). The intercellular space was widened (Fig. 2); sometimes the intercellular contacts appeared broken close to desmosomes but never through these structures. The mitochondrial fine structure was frequently disturbed in simple occlusion and occlusion with distilled water and

Figs. 1–4 are all from cases of chromium allergy. **Abbreviations:** D=desmosome, I=invading cell, K=keratinocyte, k=keratin filaments, M=melanin granules, ic=intercellular space, m=mitochondrion, v=vacuole.

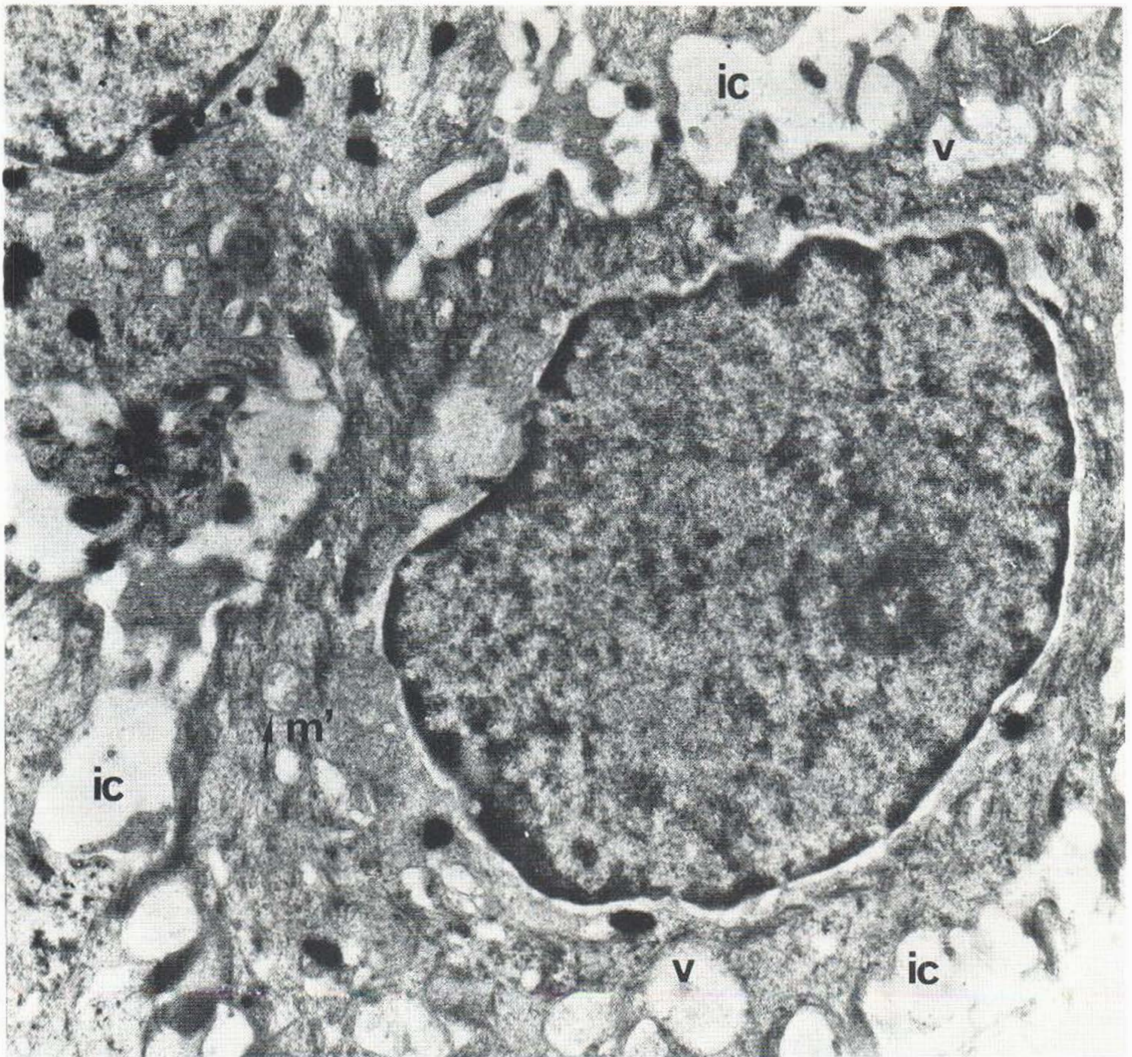


Fig. 2. Stratum spinosum of epidermis exposed to an alkaline buffer, pH 12. In the perinuclear zone the cytoplasm is rarified (top left). A number of vacuoles are

seen in the cytoplasm. A disintegrating mitochondrion is seen (*m'*). The intercellular space appears wider than normal. Male 52 ys. $\times 22750$.

petrolatum. When exposed to the alkaline vehicle, only mitochondrial fragments could be detected in the sections. There were no changes in other subcellular structures which could be related to positive clinical findings of a hypersensitivity reaction to chromium in the present study (Fig. 3).

The possibility of specific fixation artefacts was avoided by identical preparation of test biopsies and control skin biopsies obtained simultaneously. At electron microscopic resolution the epidermis of the control sites conformed to previous descriptions of the normal human epidermis (2, 3) (Fig. 4).

As regards the chromate (CrVI) solutions, no specific difference was observed between the action of $K_2Cr_2O_7$ and that of Na_2CrO_4 . No qualitative difference could be detected in the reaction patterns due to the vehicle used. The cellular damage caused by a certain concentration of allergen varied with the individual under investigation.

DISCUSSION

At electron microscopic resolution, no previous descriptions of the human skin in chromium allergy

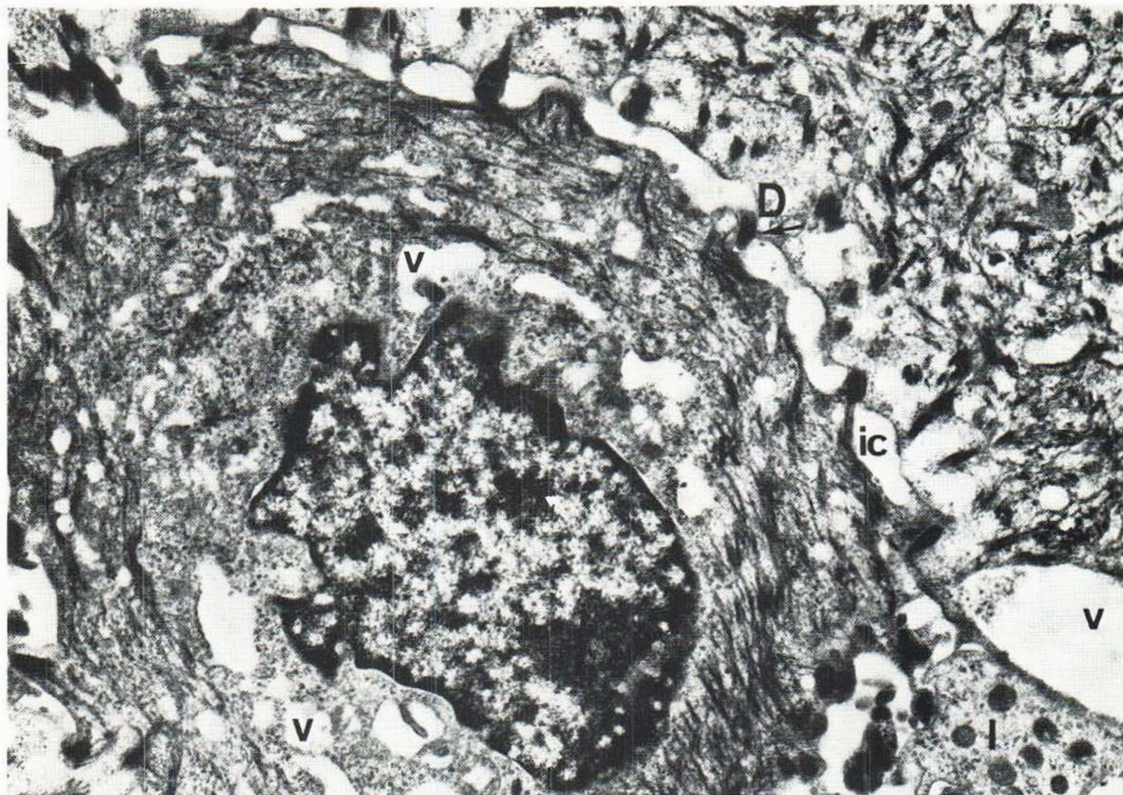


Fig. 3. Stratum spinosum of epidermis exposed to 0.25% solution of chromate in distilled water. The cytoplasm of

the keratinocytes contains vacuoles of varying sizes. Male 52 ys. $\times 14\ 850$.

have been published. The patterns of reaction are discussed in relation to findings in other experimental skin reactions and disorders.

The oedema

Oedemas in contact allergy are claimed to be extracellular. A defective membrane function is the most likely cause for the oedematic tissue in experimental contact eczema. Thus, in a tissue exposed to a foreign substance expected to act on membrane permeability, fixation and dehydration are likely to produce a redistribution of non-structural matter, i.e. physiological salts, amino acids, peptides and small protein molecules. In the present case this is likely to occur to a much higher degree than can be expected in the case of normal tissue. A papule recorded at the examination of the test area might therefore correspond to an oedema which is intra- as well as intercellular *in vivo*. The analysis of oedema localization in diseased skin is greatly hampered by the fact that no data are available on

the absolute or relative volumes of the intra- and extracellular compartments of normal and oedematous skin. This problem of oedema localization is preferably solved by non-conventional preparation techniques, i.e. cryo-ultratomy.

Vacuoles

After exposure to a vehicle with or without chromate, keratinocytic vacuoles appeared below the level of the transitional cells (3). Nucleus-deforming vacuoles appeared partly membrane-bound, partly membrane-free (figs. 1–3). Some were observed to contain granular material while others were translucent. Some might well be destroyed mitochondria.

Vacuole formation appears to be a non-specific type of reaction in keratinocytes. Hence the finding of vacuoles has been reported in several studies on skin reactions to solutes such as acetones and kerosene (9), to alkali and acids (12), to ultraviolet light exposure (13), and X-ray exposure (15), as well as



Fig. 4. Stratum spinosum of macroscopically normal epidermis from a case of chromium allergy. Mitochondria and melanin granules are seen in the perinuclear zone.

Bundles of keratin filaments are seen in the cytoplasm. Male 52 ys. $\times 34\,300$.

stripping of the stratum corneum (6, 11), and dermatographism (5). Cytoplasmic vacuoles in stratum spinosum and granulosum are sometimes reported to be membrane bound. They may deform the nucleus. No clearcut evidence has been presented for an autophagic role of the vacuoles. Thus the vacuoles may be formed as a result of the destruc-

tive effects of the agent or due to a functional reaction of the living cell to this agent.

The effect of occlusion and distilled water

Little is known about the effect of occlusion (7). Even simple occlusion without any fluid in the patch was shown to give rise to obvious cellular

changes such as vacuole formation in the perinuclear zone of the keratinocytes. Such changes are also seen with distilled water in the test patch. The cause of the changes is not apparent. They may be due to an increased hydration of the tissue, resulting in the blockage of normal water transport through the stratum corneum (6, 16). The possible effect of substances dissolved from the tapes or from the dry test Al-patch due to the increased hydration of the epidermis under occlusion could not be evaluated. Further experiments need to be performed in order to elucidate this aspect of the patch test situation.

The effect of occlusion plus vehicle

In patch testing, an alkaline vehicle is sometimes used to mimic the conditions of occupational exposure (i.e. that of cement workers). At patch testing, this vehicle is more effective in detecting chromium allergy (20) than is distilled water and petrolatum. It gives no macroscopical reactions. After a 72 hour patch test it is difficult at electron microscopic resolution to differentiate between the effect of the alkaline vehicle as such and the more specific effect of the allergen, i.e. the chromate solution. Hence, correlation between the structural and the clinical findings in a 72 hour patch test is not feasible. The morphological changes recorded in the present study as being due to an alkaline solution were, however, not as excessive as those found by other authors on application of 1 N NaOH (i.e. pH 13.0) (12). The less pronounced changes recorded in our investigation are probably related to the exposure to a smaller volume of alkali and/or a less concentrated solution as well as to the buffering capacity of untreated epidermis, not exhausted by our small volumes.

The morphological changes seen at occlusion with petrolatum further stress the need for information about the effect of vehicles on the living cells in the epidermis. Since petrolatum is a complex mixture, the cell damage may be related to one or several of its ingredients. Knowledge of the possible differences in reaction in normal, non-allergic individuals and in persons with any kind of allergic disorder may provide a rationale for the future composition of vehicles of this kind.

The Langerhans cells and mononuclear cells

Langerhans' cells, identified by their characteristic granules, were seen both in control skin and in

tested skin. The integrity of the cells appeared undisturbed also in skin samples exposed to chromate solutions, irrespective of vehicle. No association with monocytes could be recorded (18). Such a cellular reaction is, however, most likely to occur at a much earlier stage of the test period. No signs of phagocytic activity in the Langerhans cells were observed (18, 21).

As the present investigation was limited to a small number of individuals who volunteered to participate in the tests, no attempt has been made to assess the number of mononuclear cells in a quantitative manner.

The problem of occlusion:

Ways of experimental approach

The reactions elicited by the occlusion as such should be separated from reactions evoked by vehicles separately and in combination with chromates. To avoid concentration effects due to evaporation, the test solutions may be introduced into the epidermis by iontophoresis. Biopsies should then be taken at different time intervals, starting very soon after the application (i.e. 1–2 hours) up to at least 24 hours. Ethical viewpoints on the exposure of healthy human individuals to known allergens such as chromium limit the use of such a technique in our country.

CONCLUSIONS

With the electron microscope, structural changes can be detected earlier than has been possible by light microscopy. However, macroscopic reactions suitable for clinical recordings and quantification of hypersensitivity cannot be obtained after short intervals of test solution exposure, i.e. <24 hours. The present investigation shows that the composite pattern of morphological changes in 72 hours patch testing cannot be interpreted in simple straightforward terms. Great care must therefore be exercised in interpreting the epidermal fine structural changes at patch testing due to the coinciding effects of occlusion, vehicle and allergen.

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