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REACTIONS OF EPIDERMAL KERATINOCYTES IN SENSITIZED AND NON-SENSITIZED GUINEA PIGS AFTER DICHROMATE EXPOSURE: AN ELECTRON MICROSCOPIC STUDY

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Abstract. The reaction in epidermal keratinocytes in sensitized and non-sensitized, normal guinea pigs after dichromate exposure has been investigated by electron microscopy. The results of the investigation show that the morphological alterations in keratinocytes in irritant and contact allergic reactions to dichromate are of a non-specific nature. This underlines the fact that morphological reactions in the keratinocyte are uniform. The reactions were dose- and time-dependent. The morphology of the contact allergic reaction to dichromate in the guinea pig is consistent with that described for DNCB.

Key words: Dichromate; Irritant reactions; Contact allergic reactions; Guinea pig; Keratinocyte; Electron microscopy

In occupational dermatology, allergic reactions to heavy metal compounds are frequently observed. Among men, chromate is the most common cause of allergic contact dermatitis (7).

During the past decades much work has been carried out to reveal the mechanisms of contact allergic reactions in skin (1, 4, 8, 23, 26) with particular regard to chromate (19). The morphology of epidermis in irritant and contact allergic reactions (2, 3, 9, 10, 11, 14, 15, 25) as well as the cellular response to a great variety of chemical and physical agents (12, 13, 16, 17, 22, 27, 28) have been described in both guinea pigs and man. However, the morphological alterations in chromate reactions at electron microscopic resolution has previously been recorded only in relation to patch test reactions in sensitized human subjects (5) and in a preliminary study in guinea pigs (6).

When reviewing the work done up to the present, the keratinocyte appears to have a uniform cellular response to a great number of stimuli. In most instances cell damage and cell necrosis conform to the pattern seen in autolysis (29) and comprise cytoplasmic vacuolization, destruction of mitochondria, disintegration of cytoplasm, and aggregation of chromatin. Finally there is lysis of the cell. Another, distinct type of cell death, apoptosis, has been described (30, 31). The phenomenon is proposed to represent a sort of a pre-programmed cell death and to play a central role in maintaining a steady state in proliferating tissues. Morphologically it is seen as a condensation of the chromatin and the cytosol. The condensed cell is fragmented and subsequently phagocytosed. Apoptosis does not lead to cytolysis. In epidermal cells apoptosis has been reported in various conditions, i.e. after exposure to ultraviolet light, in lichen planus, in graft-versus-host reactions (review by Weedon et al. (31)). Dyskeratotic keratinocytes with the resemblance of apoptotic cells have been reported after exposure to croton oil (27).

In experimental work on chromium allergy, guinea pigs have been used extensively . The aim of the present study was to describe the morphological changes in keratinocytes of normal and sensitized guinea pigs exposed to dichromate at different concentrations.

MATERIALS AND METHODS

Animals. Male guinea pigs weighing 300–700 g.

Sensitization was performed according to Polak & Turk (18). The outcome of the sensitization procedure was checked with an intradermal test (24). Animals with the strongest reaction, i.e. test reaction larger than 10 mm after a 0.1 ml intradermal injection of a 0.024% dichromate solution, were selected for further studies.

Dichromate solutions. In the investigation, 0.006% and 0.024% solutions of potassium dichromate ($K_2Cr_2O_7$) (Merck) in saline were used. Each animal was injected intradermally (0.1 ml) with both solutions and with a ve-

Table I. *Morphological findings in both sensitized and non-sensitized guinea pigs after injection of 0.006 % and 0.024 % dichromate solutions*

In both groups of animals the most advanced reactions were seen after injection of the 0.024% solution. Non-sens. = non-sensitized guinea pigs, Sens. = sensitized guinea pigs. *Grading of the morphological changes:* 0 = normal, + = only a few, occasional findings, ++ = intermediate frequency of findings, +++ = findings of general occurrence

Morphological changes	Time after injection of the dichromate solutions		
	3–6 h	24–48 h	96 h
Cytoplasmic vacuolization in keratinocytes			
Non-sens.	+++	+++	0
Sens.	+++	+++	++
Destruction of mitochondria in keratinocytes			
Non-sens.	+++	+++	0
Sens.	+++	+++	+
Keratinocytes with electron-translucent cytoplasm and diffuse dispersion of filaments			
Non-sens.	++	++	0
Sens.	++	++	+
Keratinocytes with a more electron-dense cytoplasm than normal			
Non-sens.	++	++	0
Sens.	++	++	0
Keratinocytes showing cytolysis			
Non-sens.	0	++	0
Sens.	0	+++	0
Keratinocytes with the feature of apoptosis			
Non-sens.	+	+	0
Sens.	+	+	0
Loss of desmosomal attachments			
Non-sens.	0	++	0
Sens.	0	+++	0
Widening of intercellular space			
Non-sens.	+	+	0
Sens.	++	+++	0
Inflammatory cell infiltrate			
Non-sens.	0	++	0
Sens.	+	+++	0
Breaks in the basal lamina			
Non-sens.	0	+	0
Sens.	0	++	0

hicle control (saline). The same concentrations were used for the sensitized and the non-sensitized animals.

Electron microscopy. Sensitized and non-sensitized guinea pigs were injected 3, 6, 24, 48, and 96 hours prior to killing with an overdose of barbiturate. In each group 3–5 animals were used at each time interval. Biopsies were taken from initially marked and clipped areas whether or not any visual skin reaction was evidenced. Non-exposed skin taken well outside the exposed areas served as controls. Specimens were divided and fixed for 4 hours in 2.5% glutaraldehyde or in 2% osmium tetroxide in a 300 mOsmol phosphate buffer, pH 7.4. Post-fixation for 1–2 hours in 2% osmium tetroxide in the same buffer. After dehydration in ethanol the specimens were embedded in Epon and approx. 60-nm thick sections were cut on an LKB Ultratome. Uranyl acetate in water or uranyl acetate in 50% methanol was used for contrast enhancement. Electron microscopy was performed with a Philips EM 301G at primary magnifications of ×600–40 000.

RESULTS

In both sensitized and non-sensitized guinea pigs the morphological changes developed as a function of time. In both groups the reactions were most pronounced after injection of the 0.024% dichromate solution, and the morphology was most affected in the sensitized animals. Variations in the

Abbreviations used: D = dermis, G = granular substance, I = infiltrating cell, K = keratinocyte, L = lymphocyte, M = mononuclear cell, N = nucleus, S = intercellular space, SC = stratum corneum, –▷ = desmosome, ▷–◁ = basal lamina. Figs. 1–5 and Fig. 10 are from normal, non-sensitized skin. Figs. 6–9 and Fig. 11 are from sensitized skin.

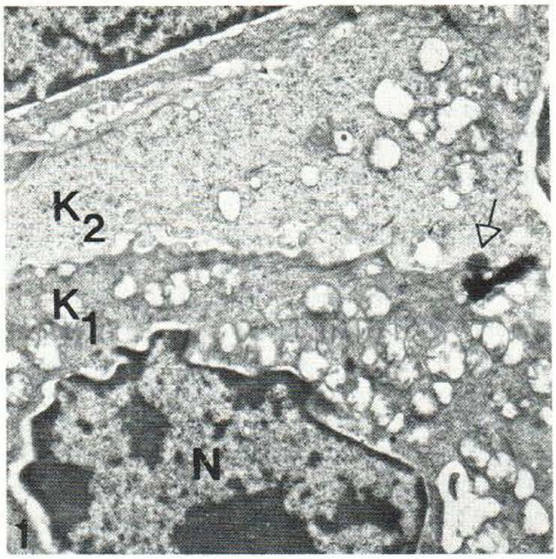
Fig. 1. The cytoplasm of two keratinocytes in normal skin 3 h after exposure to the 0.006% solution. Both cells contain vacuoles and destroyed mitochondria. K_1 has a more electron-dense and K_2 a more translucent cytoplasm than is normally seen. ×9 800.

Fig. 2. A basal keratinocyte in normal skin 3 h after exposure to the 0.024% solution. The cell has a condensed cytosol and a condensed chromatin. ×7 700.

Fig. 3. (A) A basal keratinocyte in normal skin 24 h after exposure to the 0.006% solution. The cell shows signs of cytolysis. ×7 700. (B) Part of the cell in A at higher magnification (K_1). Loss of desmosomal attachment, i.e. the desmosomes no longer join neighbouring cells. Widened intercellular space with granular substance, loss of cytoplasmic structures, cytoplasmic vacuoles and break in the cell membrane (filled arrows) are seen. ×17 800.

Fig. 4. Separation of a basal keratinocyte from the basal lamina in normal skin 24 h after exposure to the 0.006% solution. Hemidesmosome structures (arrows) are seen in the cell membrane. ×27000.

Fig. 5. A basal keratinocyte with electron-dense cytoplasm, cytoplasmic and perinuclear vacuoles found in non-sensitized skin 48 h after exposure to the 0.024% solution. ×7 700.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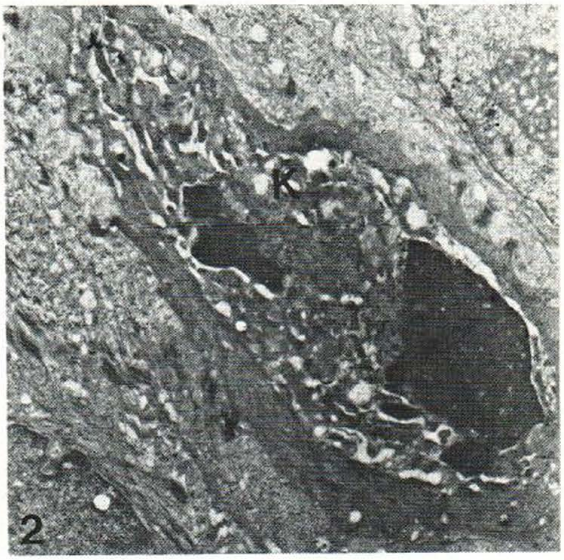
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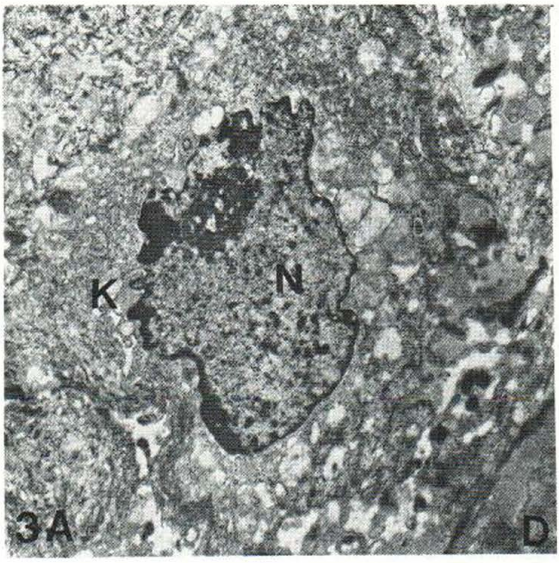
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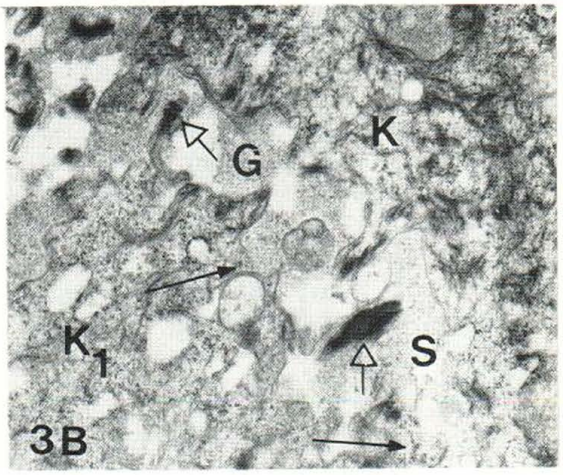
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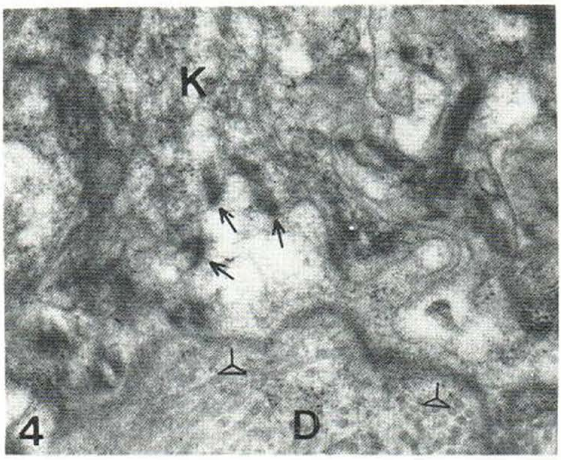
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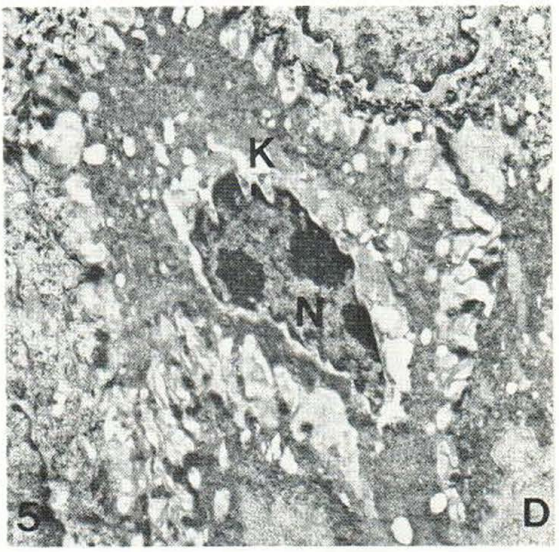
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degree of change were observed between specimens but also within one and the same specimen (biopsy). Thus, necrotic keratinocytes could be found adjacent to cells with an apparently normal morphology. Injection of the vehicle (saline) did not affect the morphology at any time interval. The findings are summarized in Table I.

Reactions in non-sensitized guinea pigs

At 3 and 6 hours after injection of the dichromate solutions, distinct changes were observed in keratinocytes in stratum basale and to a lesser extent in stratum spinosum. Cytoplasmic vacuoles, both with and without membrane, perinuclear dilatation, perinuclear vacuoles and mitochondria with loss of the internal structure appeared in keratinocytes (Fig. 1). Some cells displayed an electron-translucent cytoplasm with loss of cytoplasmic structure and dispersed filaments, while other cells had a more electron-dense cytoplasm than normally seen (Fig. 1). In skin exposed to the 0.024% solution a few cells with homogenously condensed chromatin and a compact cytoplasm with condensation of cytoplasmic structures were found (Fig. 2).

At 24 and 48 hours the cellular changes in the keratinocytes were more pronounced than at 3 and 6 hours. Thus we observed cytolytic keratinocytes with peripherally aggregated chromatin, cytoplasmic vacuoles, loss of subcellular structures and dissolution of the cell membrane (Fig. 3A, B). Other keratinocytes had aggregated chromatin, cytoplasmic vacuoles, but an electron-dense cytoplasm (Fig. 5) Loss of cellular cohesion due to disappearance of desmosomal attachment was seen in basal parts of the epidermis (Figs. 3B and 10). Occasionally desmosomes were seen cleaved through the middle (inset, Fig. 10). A focal loss of contact between basal keratinocytes and the basal lamina appeared (Fig. 4). The intercellular spaced showed no obvious widening and contained granular masses, sometimes membrane bound (Fig. 3B). In some specimens a complete separation of epidermis–dermis at the basal lamina occurred at 24 hours after injection of the 0.024% solution (Fig. 10) Only a few infiltrating cells were seen in the epidermis.

At 96 hours the epidermis appeared normal.

Reactions in sensitized guinea pigs

At 3 and 6 hours the morphological changes in the keratinocytes were consistent with those seen in non-sensitized guinea pigs at the same time interval. An increase in mononuclear cells not seen in normal skin was present in the dermis and at 3 hours a contact between lymphocyte-like cells and mononuclear cells was observed in the stratum basale (Fig. 6). The intercellular space was wider than that in non-sensitized skin.

At 24 and 48 hours the most prominent features were the occurrence of a large epidermal intercellular oedema, with loss of cell contacts and a dermal and epidermal cell infiltrate (Fig. 11). Compared with 3 and 6 hours, more advanced morphological changes were seen in the keratinocytes. Necrotic cells with aggregated chromatin, cytoplasmic vacuoles and a structureless cytoplasm (Figs. 7 and 11) occurred and in some cells, rupture of the cell membrane. A few, rare cells had condensed chromatin and cytosol. Dissociation of basal keratinocytes from the basal lamina occurred, while in places the remains of disrupted keratinocytes could still be seen attached to the basal lamina (Fig. 7). Granular masses, often membrane bound, appeared in the intercellular space. Loss of cell cohesion was due to disappearance of desmosomal attachment. Intact desmosomes were seen just inside and adjacent to the cell membranes, in addition to residual desmosomal structures which were found in the cell membranes (Fig. 8A, B) Focal disappearance of the basal lamina was observed. Non-keratinocytes were seen penetrating the dermal–epidermal junction and the inflammatory cell infiltrate consisted in granulocytes and lymphocytes. Many of the granulocytes contained granules having a crystalline structure and were identified as basophil leukocytes.

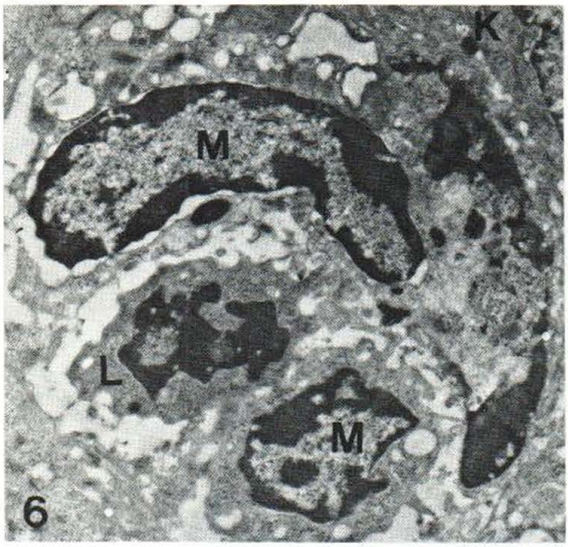
At 96 hours a restoration of the epidermal structure had taken place. The epidermis was thicker than normal and keratohyalin granules were seen in

Fig. 6. Contact between a lymphocyte and mononuclear cells in basal epidermis in sensitized skin 3 h after exposure to the 0.006% solution. ×7700.

Fig. 7. Detail of a cytolytic keratinocyte still partly attached to the basal lamina in sensitized skin 48 h after exposure to the 0.024% solution. ×27000.

Fig. 8. Loss of desmosomal attachment in sensitized skin 24 h after exposure to the 0.024% solution. (A) Complete desmosome just inside the cell membrane (arrow). ×45000. (B) Residual desmosomal structures in the cell membrane of cytolytic keratinocytes. ×27000.

Fig. 9. The upper part of epidermis in sensitized skin 96 h after exposure to the 0.024% solution. More cell layers than normal contain keratohyalin granules (++). Cytoplasmic vacuoles (+) are seen. ×3700.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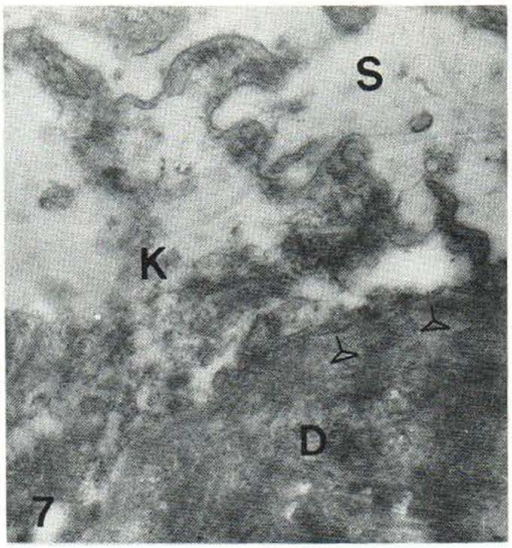
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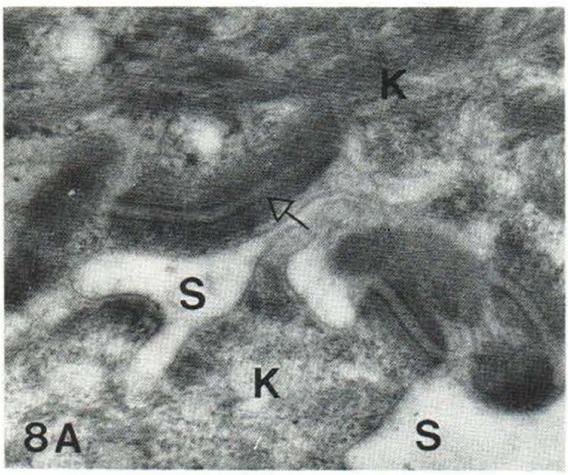
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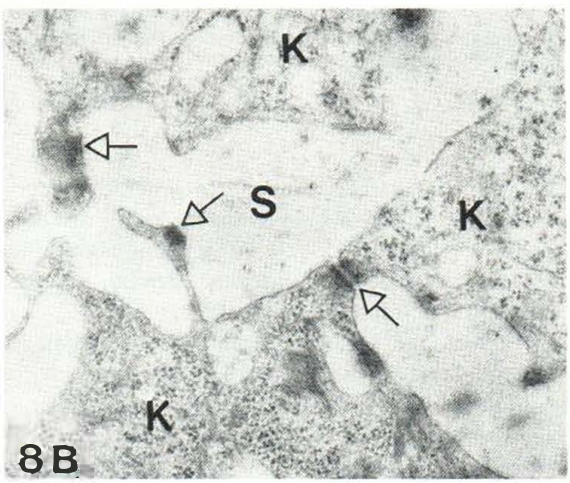
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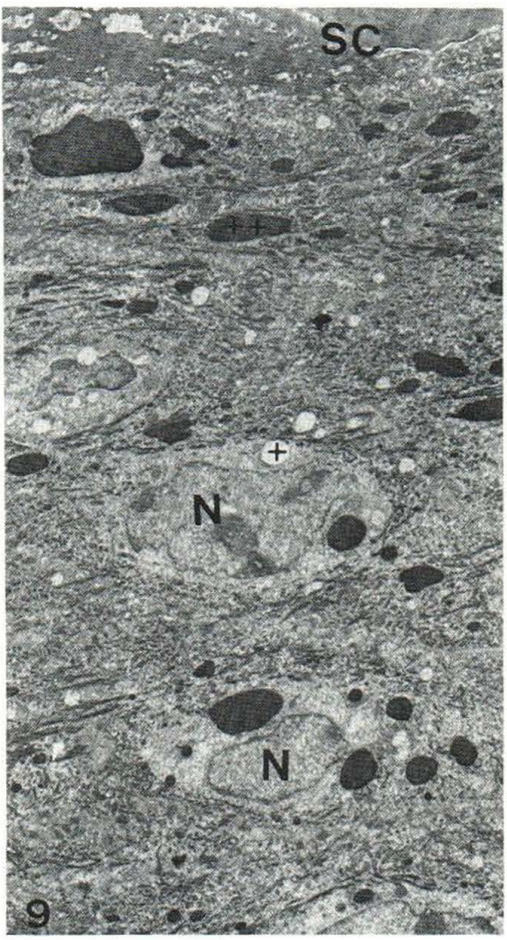
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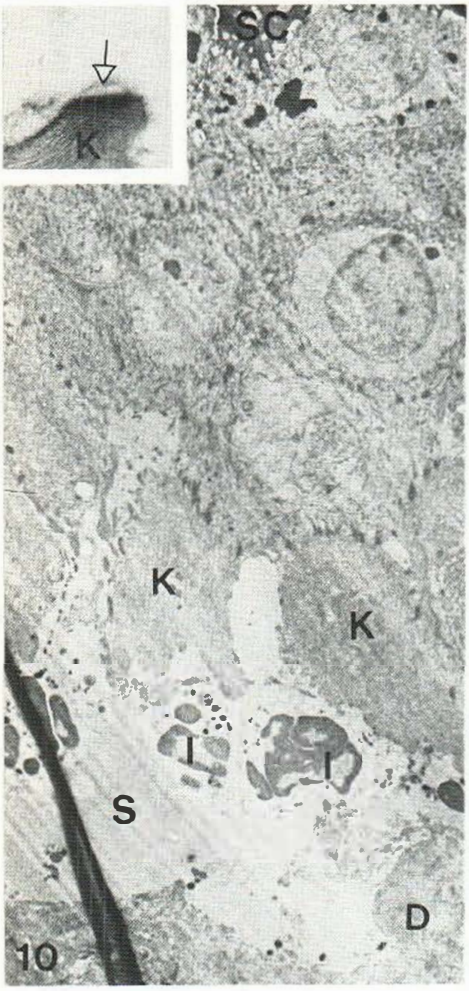

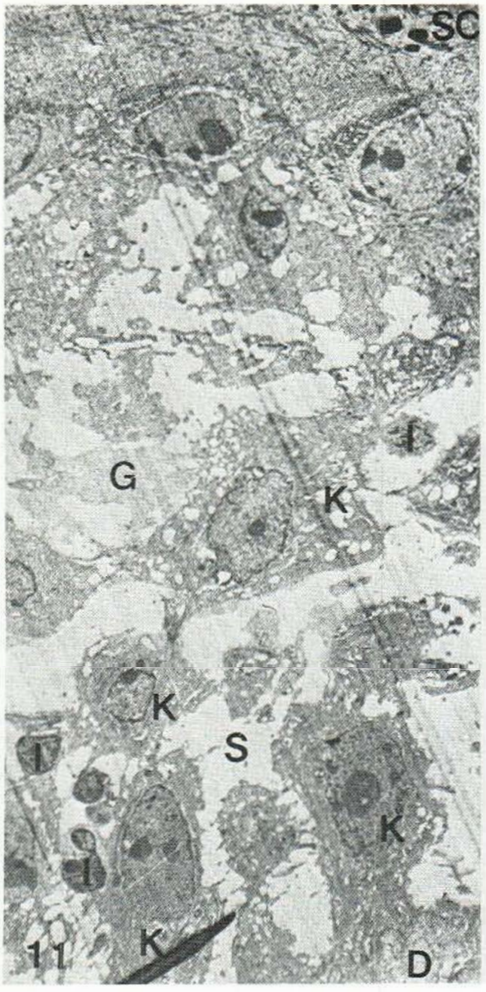

Fig. 10. Separation at the dermal–epidermal junction seen in normal skin 24 h after exposure to the 0.024% solution. A few infiltrating cells are seen. ×2 430. *Inset:* Part of a desmosome cleaved through the middle, found in this biopsy. ×53 000.

Fig. 11. Intercellular oedema and infiltrating cells seen in sensitized skin 24 h after exposure to the 0.024% solution. A granular substance is observed in the intercellular space. ×2 400.

more cell layers than normal (Fig. 9). Cytoplasmic vacuoles and phagosomes were found in keratinocytes of the differentiating epidermis.

DISCUSSION

One of the primary functions of the epidermis is to act as a barrier to noxious substances in the environment (21). Upon contact with such substances, two main types of reaction may arise, either solitary or superimposed on each other in sequence (25). These are the irritant reaction and the contact allergic reaction. In order to understand the mechanisms governing these two reaction patterns we must obtain information about the reactions between the noxious agent(s) and the tissue in which they take place.

A direct comparison between observations made in normal and sensitized guinea pigs on exposure to chromate vis-à-vis the corresponding reactions recently described in man, is not directly feasible. However, the guinea pig experimental system does allow systematic investigation, the data of which may shed light on phenomena observed in man.

The aim of the present investigation was to follow the development of morphological changes in the epidermis during a period of 96 hours after intradermal injection of dichromate solutions (0.006% and 0.024%). A physiological saline solution was used as vehicle in order to avoid the side effects of plain water on the morphology (5). The use of Freund's adjuvant when sensitizing the guinea pigs did not seem to affect the results as compared with normal animals. Nor did the intradermal injection of the vehicle (saline) affect the morphology, as revealed by electron microscopy.

The observed morphological changes in the keratinocytes were of a non-specific nature in both sensitized and non-sensitized animals and corresponded closely to changes described in the literature. The degree of changes was dependent on the concentration of the dichromate solution applied. Cytolytic changes ranging from cytoplasmic vacuolization and destroyed mitochondria, to complete lysis of keratinocytes, were observed. In the non-sensitized guinea pigs, reactions seemed to reach a peak at 24–48 hours after the injection of dichromate, with a subsequent restoration of epidermis at 96 hours. Sensitized animals displayed a fully developed allergic reaction with a massive inflammatory cell infiltrate at 24 and 48 hours. The morphology was consistent with that described for DNCB reactions in guinea pigs (4, 9, 25). Thus, in sensitized guinea pigs an allergic reaction was superimposed on an initial irritant reaction. In this group too a restoration of the epidermis took place at 96 hours.

One interesting finding in our specimens was the occurrence of keratinocytes with the features of apoptosis, i.e. condensed chromatin *and* cytosol. It must be pointed out that in our specimens only a few, occasional cells were found displaying such morphological changes and the significance of this is not clear. These cells may represent a normal, physiological event in the epidermal cell turnover coinciding with the changes induced by dichromate. But, to our knowledge, the phenomenon of apoptosis has not been described in normal epidermis yet. It is therefore tempting to speculate on the possibility that the changes observed are due to the application of dichromate, and that the fate of the individual keratinocyte is dependent on the phase of the cell cycle in which the stimuli (noxae) is applied.

Our electron microscopic results are consistent with the suggestion that the keratinocyte has a stereotyped reaction pattern to different stimuli, with quantitative rather than qualitative variations.

In the contact allergic reaction the reduction of hexavalent chromium (CrVI) to trivalent chromium (CrIII) appears to be crucial (20). This reduction (of CrVI) is likely to occur during the passage of chromate from the environment into the organism, but the precise site of this reaction is not known. Use of an intradermal injection as the mode of introduction for the chromate (CrVI) also allows a great variety of reduction paths. The administration route, consequently, might affect the cellular response in different ways.

When comparing the results of different electron microscopic investigations it must be remembered that the choice of buffer system(s) for the fixative(s) and the preparation techniques will influence on the results. As long as no quantitative evaluation of the electron microscopic information is at hand, e.g. by the use of morphometry, the evaluation of data, such as size of intercellular space, tonofilament density, etc., will be a matter of subjective speculation.

The morphological changes in the keratinocytes in irritant and contact allergic reactions appear to be the same in both human and guinea pig epidermis. This fact must not be confused with the fundamental differences appearing in the overall immunological reaction patterns that occur in the contact allergic reaction.

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REFERENCES

1. Chase, M. W.: Developments in delayed-type hypersensitivities 1950–1975. J Invest Dermatol *67*: 136, 1976.
2. Dvorak, H. F., Dvorak, A. M., Simpson, B. A., Rickerson, H. B., Leskowitz, S. & Karnovsky, M. J. Cutaneous basophil hypersensitivity. II. A light and electron microscopic description. J Exp Med *132*: 558, 1970.
3. Dvorak, H. D., Mihm, M. C. & Dvorak, A. M.: Morphology of delayed-type hypersensitivity reactions in man. J Invest Dermatol *67*: 391, 1976.
4. Dvorak, H. F., Galli, S. J. & Dvorak, A. M.: Expres-

sion of cell-mediated hypersensitivity in vivo—recent advances. Int Rev Exp Pathol *21:* 119, 1980.
5. Forslind, B. & Wahlberg, J.-E.: Assessment of chromium allergy: Features of patch test reactions at electron microscopic resolution. Acta Dermatovener (Stockholm) *57:* 29, 1977.
6. — The morphology of chromium allergic skin reactions at electron microscopic resolution: Studies in man and guinea-pig. Acta Dermatovener (Stockholm) Suppl. 79: 43, 1978.
7. Fregert, S., Hjorth, N., Magnusson, B., Bandmann, H.-J., Calnan, C. D., Cronin, E., Malten, K., Meneghini, C. L., Pirilä, V. & Wilkinson, D. S.: Epidemiology of contact dermatitis. Trans St Johns Hosp Dermatol Soc *55:* 17, 1969.
8. Godfrey, H. P. & Gell, P. G. H.: Cellular and molecular events in the delayed-onset hypersensitivities. Rev Physiol Biochem Pharmacol *84:* 1, 1978.
9. Hunziker, W. & Winkelmann, R. K.: Cell populations in experimental contact dermatitis. Arch Dermatol *113:* 1543, 1977.
10. Komura, J. & Ofuji, S.: Ultrastructural studies of allergic contact dermatitis in man. Epidermal cell changes at 3, 6 and 12 hours after application of DNCB. Arch Dermatol Res 267: 275, 1980.
11. Komura, J., Oguchi, M., Aoshima, T. & Ofuji, S.: Ultrastructural studies of allergic contact dermatitis in man. Infiltrating cells at the earliest phase of spongiotic bulla formation. Arch Dermatol Res *267:* 283, 1980.
12. Lindberg, M., Forslind, B. & Johannesson, A.: The effect of occlusive treatment of human skin: An electron microscopic study on epidermal morphology as affected by occlusion and dansyl chloride. Acta Dermatovener (Stockholm) *62:* 1, 1982.
13. Lupulesco, A. P., Birmingham, D. J. & Pinkus, H.: An electron microscopic study of human epidermis after acetone and kerosine administration. J Invest Dermatol *60:* 33, 1973.
14. Medenica, M. & Rostenberg, A.: A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. J Invest Dermatol *56:* 259, 1971.
15. Metz, J.: Elektronenmikroskopische Untersuchungen an allergischen und toxischen Epicutan Testreaktionen des Menschen. Arch Dermatol Forsch *245:* 125, 1972.
16. Nagao, S., Stroud, J. O., Namada, T., Pinkus, H. & Birmingham, D. J.: The effect of sodium hydroxide and hydrochloride acid on human epidermis. Acta Dermatovener (Stock) *52:* 11, 1972.
17. Nix, T.: Ultraviolet induced changes in epidermis. *In* Ultrastructure of Normal and Abnormal Skin (ed. A. S. Zelickson). Lea & Febiger, Philadelphia, 1967.
18. Polak, L. & Turk, J. L.: Studies on the effect of systematic administration of sensitizers in guinea-pigs with contact sensitivity to inorganic metal compound. Clin Exp Immunol *3:* 245, 1968.
19. Polak, K., Turk, J. L. & Frey, J. R.: Studies on contact hypersensitivity to chromium compounds. Progr Allerg *17:* 145, 1973.
20. Samitz, M. H. & Katz, S.: A study of chemical reactions between chromium and skin. J Invest Dermatol *43:* 35, 1964.
21. Scheuplein, R. J.: Percutaneous absorption after twenty years: Or "Old wine in new wineskins". J Invest Dermatol *67:* 31, 1976.
22. Silberberg, I.: Studies by electron microscopy of epidermis after topical application of mercuric chloride. J Invest Dermatol *56:* 147, 1971.
23. Silberberg-Sinakin, I. & Thorbecke, G. J.: Contact hypersensitivity and Langerhans cell. J Invest Dermatol *75:* 61, 1980.
24. Skog, E. & Wahlberg, J.-E.: Sensitization and testing of guinea-pigs with potassium bichromate. Acta Dermatovener (Stockholm) *50:* 103, 1970.
25. Skoog, M.-L.: The Cellular Infiltrate in Experimental Contact Dermatitis. Linköping University Medical Dissertations No. 113, 1981.
26. Stingl, G., Tamaki, K. & Katz, S. I.: Origin and function of epidermal Langerhans' cells. Immunol Rev *53:* 149, 1980.
27. Takigawa, M., Komura, J. & Ofuji, S.: Early fine structural changes in human epidermis following application of croton oil. Acta Dermatovener (Stockholm) *58:* 31, 1978.
28. Takigawa, M. & Ofuji, S.: Early changes in human epidermis following thermal Burn: An electron microscopic study. Acta Dermatovener (Stockholm) *57:* 187, 1977.
29. Trump, B. F., Goldblatt, P. J. & Stowell, R. E.: An electron microscopic study of early cytoplasmic alterations in hepatic parenchymal cells of mouse liver during necrosis in vitro (Antolysis) Lab Invest *11:* 986, 1962.
30. Weedon, D., Searle, J. & Kerr, J. F. R.: Apoptosis. Its nature and implications for dermatopathology. Am J Dermatopathol *1:* 133, 1979.
31. Wyllie, A. H., Kerr, J. F. R. & Currie, A. R.: Cell death: The significance of apoptosis. Int Rev Cytol *68:* 251, 1980.

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