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INVESTIGATIVE REPORT

An Ultrastructural Study of Chronic Chromate Hand Dermatitis

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Occupational chromate dermatitis is one of the most common occupational diseases, predominantly causing hand eruptions. The ultrastructural manifestations of this condition have not been previously described. In this study, 7 cases of chronic occupational chromate hand dermatitis were investigated. Biopsies were taken from palmar skin and examined using light and electron microscopy. The ultrastructural features of chronic chromate dermatitis are similar to those of acute inflammatory dermatoses, even in the absence of clinical or histological features of an acute inflammatory process. Most changes are probably mechanical in nature and are a result of increasing intercellular oedema. Several features of chronic chromate dermatitis are common to other inflammatory dermatoses, including the presence of marked intercellular oedema of the lower epidermal keratinocytes, the formation of intracellular vacuoles in cells of the lower epidermis and the presence of milder ultrastructural changes in the mid-epidermis. The study has documented the presence of dendritic, spindle-shaped granular cells in the upper dermis, which have not previously been described in chromate dermatitis. The epidermis in chromate dermatitis appears to have fewer desmosomes when compared with other forms of dermatitis. ***Key words: electron microscopy; chromate; dermatitis.***

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The ultrastructural manifestations of the early stages of acute dermatitis and experimentally induced acute dermatitis were described in the 1960s and 1970s (1, 2). To date, studies have described changes in animal and human skin within the first few hours of application of a potent contact allergen. Most occupational dermatitis, however, tends to be chronic in nature and often persists for months or years. There is a paucity of ultrastructural data on the chronic spongiotic dermatoses.

Chromate is a common occupational allergen and is known to cause dermatitis in a wide range of professions, including metal workers and construction workers. The usual symptom is of a persistent hand dermatitis, which is often debilitating. This study investigates subjects with chronic hand dermatitis attributable to occupational chromate exposure. The specific aim of our study was to document the range of ultrastructural appearances of occupational chromate dermatitis and to compare these data with those of other spongiotic dermatoses. These changes were examined to determine whether they correlated with the clinical severity of the disease.

MATERIAL AND METHODS

Patients

The study was approved by the South Sheffield Ethics Committee. Patients were carefully selected in order to identify those with dermatitis related directly to occupational chromate exposure. Chromate allergy was confirmed by a strongly positive allergic patch test reaction to potassium dichromate. Patients with a dubious or possible irritant patch test reaction were not included in the study. Each patient was individually assessed to confirm the importance of the chromate allergy. The following factors were taken into account to assess the relevance of chromate allergy: (1) the degree of exposure to chromate during the course of the individual carrying out his occupation; (2) the characteristics of the eruption being compatible with those of a contact dermatitis; (3) the distribution of the dermatitis being such that it would be compatible with the pattern of occupational exposure; and (4) the exclusion of other factors that could be responsible for a dermatitis of the appearance and distribution to that seen, or the likelihood that any such factors were of lesser importance than exposure to the allergen. Although chromate allergy was thought to be of over-riding importance in all patients, the co-existence of irritants and other allergic factors was noted.

Seven patients, all men, fitting the study criteria were recruited. A full spectrum of clinical disease was studied, ranging from clinically mild chronic dermatitis to acute exacerbations on a background of chronic hand dermatitis. Four patients had clinically chronic hand dermatitis with no recent acute flare of their disease. A further 3 patients had a clinically active acute exacerbation of their chronic dermatitis at the time of the study. Clinical characteristics and occupational details for all patients are summarized in Table I.

The control specimens were inflammatory dermatoses consisting of a patch test reaction showing acute chromate dermatitis, chronic hand dermatitis with multiple patch test positives but none to chromate, chronic irritant dermatitis (patch test negative), chronic hand and foot dermatitis (patch test negative), and acute-on-chronic hand and foot dermatitis (chromate-allergic patient, also allergies to cobalt and nickel).

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Table I. *Clinical characteristics and occupational data from 7 chromium-allergic male patients*

Patient	Age (years)	Extent of dermatitis	Duration of disease (years)	Duration of occupational chromate exposure (years)	Source of chromate exposure	Occupation	Other positive patch test results	Time off work due to dermatitis	Assessed industrial dermatitis
1	30	Hands, forearms, wrists, knees	5	9.5	Cement, metal cutting	Cement bagger (previously metal grinder)	Nil	No	No
2	55	Hands, forearms	20	38	Cement, grouts	Wall-floor tiler	Epoxy resin, rubber	Yes	Yes
3	54	Hands, lower legs	2	30	Cement	Builder	Nil	No	No
4	47	Hands, arms, lower legs	29	31	Metal grinding	Universal miller	Nickel, cobalt	No	No
5	43	Hands	6	32	Metal smelting	Metal smelter	Nil	Yes	No
6	63	Hands	41	30	Cement	Retired miner	Balsam Peru, fragrance mix, wood tar mix	Yes	Yes
7	66	Hands, face, forearms, trunk	6	50	Cement, grouts	Decorator	Balsam Peru, epoxy resin, neomycin, wool alcohols, tixocortol pivalate, phenol-f-resin	No	No

Methods

A punch biopsy of skin measuring 4 mm in diameter was taken from the hypothenar region of an affected hand. The biopsies were immediately divided for light and electron microscopy, care being taken to limit mechanical damage and to prevent desiccation.

Specimens for light microscopy were fixed in 10% buffered formalin and processed into paraffin wax according to normal histopathology protocols; 5 μm sections were taken and stained with haematoxylin with an eosin counter-stain.

Specimens for electron microscopy were fixed in half-strength Karnovsky's fixative (3) for 4 h at room temperature or overnight at 4°C. Specimens underwent secondary fixation of 1.3% osmium tetroxide, dehydrated through a graded series of alcohols and embedded in Epon 812 epoxy resin (TAAB Ltd., Aldermaston, UK). Semithin sections of 0.5 μm were taken for light microscopy and stained according to Richardson et al. (4). Ultrathin sections of 70 nm were taken using a Reichert Ultracut ultramicrotome. These were then stained with saturated uranyl acetate in 50% ethanol for 40 min and Reynold's Lead Citrate (5) for 10 min. The sections were observed using a Philip's EM 400 transmission electron microscope.

RESULTS

Light microscopy of patients' biopsies

Patients 1 to 4 (see Table I) presented a history of chronic persistent hand dermatitis with no recent acute clinical exacerbations of their disease. The light microscopy demonstrated histological features of chronic dermatitis, but little evidence of acute features. Acanthosis was present with a chronic inflammatory cell infiltrate in the upper dermis. In particular, there was no significant evidence of spongiosis or microvesicles affecting the epidermis and little or no upper dermal oedema. These features were seen on both the paraffin-embedded and resin sections. In all 4 patients, spindle-shaped cells were evident in large numbers below the basement membrane. These cells were seen in the resin sections but were not evident in the haematoxylin and eosin-stained paraffin sections. The cells contained metachromatic granules that were demonstrated by the toluidine blue of the Richardson's stain. Patients 5 to 7 clinically had chronic dermatitis with recent acute exacerbations. Histological features on light microscopy showed acute features of dermatitis in all 3 cases. The degree of spongiosis varied. Intercellular oedema varied from lower keratinocyte separation to frank vesicle formation.

The spindle cells were evident in all the patients. There were increased numbers of cells in the upper and mid-dermis especially around blood vessels. Numbers did not appear to vary significantly with the clinical stage of dermatitis. For all patients, there was a mean of 14 spindle cells per 100 basal keratinocytes.

Electron microscopy

Ultrastructural changes differ according to the clinical state of disease but specific ultrastructural changes are consistent in all patients.

Changes in the lower epidermis. The most consistent feature found in all patients was the marked intercellular oedema of the lower epidermal keratinocytes. This finding was visible in all biopsy sections, even on low power under the electron microscope. Intercellular oedema occurred initially between the lower epidermal keratinocytes. This led to elongation of the intermediate filaments but no damage to the desmosomal plaque unit. These early changes were not evident on light microscopy. Changes were often localized, restricted to an area of only a few cells with adjacent cellular architecture being normal. As the intercellular oedema increased the intermediate filaments broke down, probably due to the mechanical forces being applied, resulting in keratinocyte separation. The desmosomal plaque units remained intact and were demonstrated in the intercellular space (Fig. 1*a*).

Other ultrastructural changes were noted in relation to the increasing intercellular oedema. Intracellular vacuole formation occurred within keratinocytes, and was most marked in the cells of the lower epidermis. Vacuoles were found throughout the cell cytoplasm but were more common around the cell periphery when the intracellular oedema was mild (Fig. 1*b*). As intracellular oedema increased, vacuoles coalesced to form larger intracellular spaces. Cell separation resulted in the breakdown of the intermediate filaments. These filaments were occasionally found aggregating around the cell periphery (Fig. 1*c*).

Ultrastructural changes became more dramatic as the keratinocytes separated and began to become damaged. In patients with an acute flare of chronic chromate dermatitis the histological and ultrastructural changes were more dramatic, with vesicle formation in the epidermis. The keratinocytes had separated, producing intraepidermal vesicles in which inflammatory cells were found. Although keratinocyte damage was severe, the desmosomal plaque units remained intact. These were found freely in the intercellular space (Fig. 1*d*). Free filaments were found in the extracellular space, some of them attached to the desmosomal plaque units (Fig. 1*a*).

Changes in the mid-epidermis. In patients with chronic, low-grade indolent dermatitis, the ultrastructural changes were often localized, with areas of intercellular oedema adjacent to normal areas of cells in the lower and mid-epidermis. Again, as the intercellular oedema progressed, keratinocytes acquired intracellular vacuoles. In clinically chronic dermatitis with no acute flare, the cell separation was milder in the mid-epidermis than the lower epidermis. However, the more severe the clinical state of dermatitis, the more marked the cell separation in both the lower and mid-epidermis.

The upper dermis. Few abnormalities were seen in the upper dermis. Whilst there was a degree of dermal oedema on histology, this was not readily demonstrated on electron microscopy. The striking feature demonstrated in all patients was the presence of numerous spindle-shaped granular cells. These cells stained metachromatically under light microscopy with toluidine blue stain. They were elongated, appeared to lie close to the basement membrane, had filamentous processes, and contained electron-dense, membrane-bound granules. In some sections, the granules were present in the surrounding dermis, suggesting cellular extrusion. Using high-power electron microscopy, it was possible to see the typical whorled appearance of the granules, suggesting the cells were mast cells (Fig.2).

Control specimens

Seven control specimens of normal palmar skin and inflammatory dermatoses were taken for analysis. The clinically normal control skin showed no ultrastructural abnormalities (Fig. 1*e*). The positive patch test reaction to chromium represented a true, acute allergic reaction. Histological changes showed a severe, acute dermatitis with marked spongiosis and vesicle formation. Ultrastructural changes were similar to those seen at the more severe acute-on-chronic end of the chromate dermatitis spectrum. Control biopsies of chronic dermatitis (patch test negative to chromate) failed to demonstrate spindle-shaped cells. In all cases the desmosomes were present in normal numbers and only slight keratinocyte separation was evident (data not shown).

DISCUSSION

Early ultrastructural work on inflammatory dermatoses demonstrated that the features of acute experimentally induced dermatitis were non-specific. Intercellular oedema, intracellular vacuole formation and intermediate filament clumping were reported (1). Croton oil dermatitis was studied as an example of a pure irritant dermatitis (2). The changes seen were widening of the intercellular spaces of the basal and spinous layers, and stretching of intercellular bridges. In acute radiation dermatitis, there is a decreased number of desmosomes with the formation of cytoplasmic vacuoles and perinuclear aggregation of intermediate filaments (6).

The ultrastructural effects of simple occlusion with distilled water as a vehicle on the skin show similar ultrastructural manifestations (7), with changes in the stratum spinosum and to a lesser extent in the basal layer and stratum granulosum. Also noted were perinuclear vacuole formation, a widening of the intercellular spaces and, in more severe cases, breakdown of intercellular contacts near the desmosomes. When the experiments were repeated with chromate as the vehicle, the morphological picture was similar.

More recent work on cutaneous inflammation has confirmed the importance of the mast cell in the early

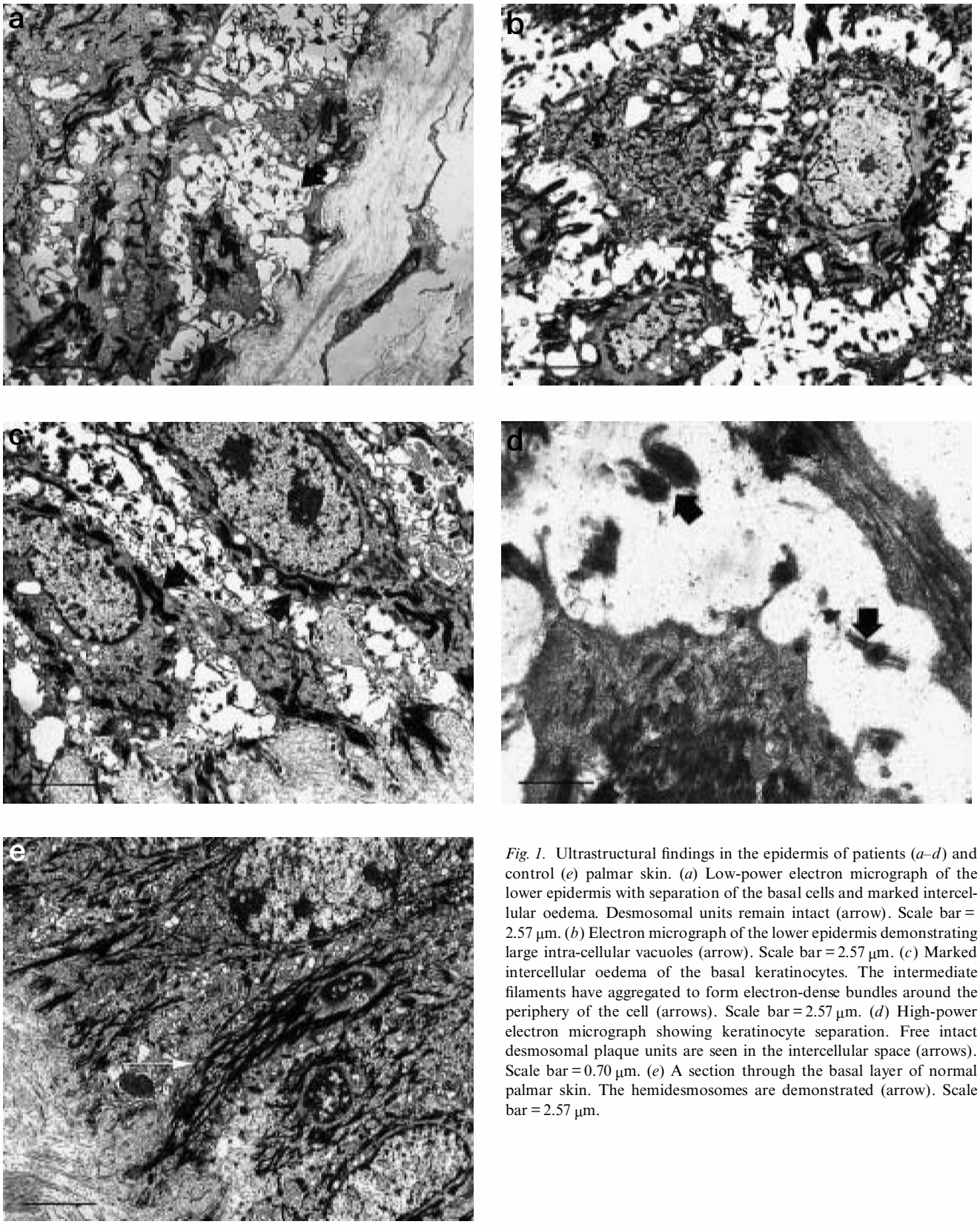

Fig. 1. Ultrastructural findings in the epidermis of patients (*a–d*) and control (*e*) palmar skin. (*a*) Low-power electron micrograph of the lower epidermis with separation of the basal cells and marked intercellular oedema. Desmosomal units remain intact (arrow). Scale bar = 2.57 μm. (*b*) Electron micrograph of the lower epidermis demonstrating large intra-cellular vacuoles (arrow). Scale bar = 2.57 μm. (*c*) Marked intercellular oedema of the basal keratinocytes. The intermediate filaments have aggregated to form electron-dense bundles around the periphery of the cell (arrows). Scale bar = 2.57 μm. (*d*) High-power electron micrograph showing keratinocyte separation. Free intact desmosomal plaque units are seen in the intercellular space (arrows). Scale bar = 0.70 μm. (*e*) A section through the basal layer of normal palmar skin. The hemidesmosomes are demonstrated (arrow). Scale bar = 2.57 μm.

stages of contact dermatitis (8). In recent ultrastructural studies on eczema the role of cutaneous nerves (9) and activation of Langerhans' cells have been examined (10). The complex interaction between these various factors is now better understood. Dendritic mast cells have been demonstrated in the lesional dermis of prurigo

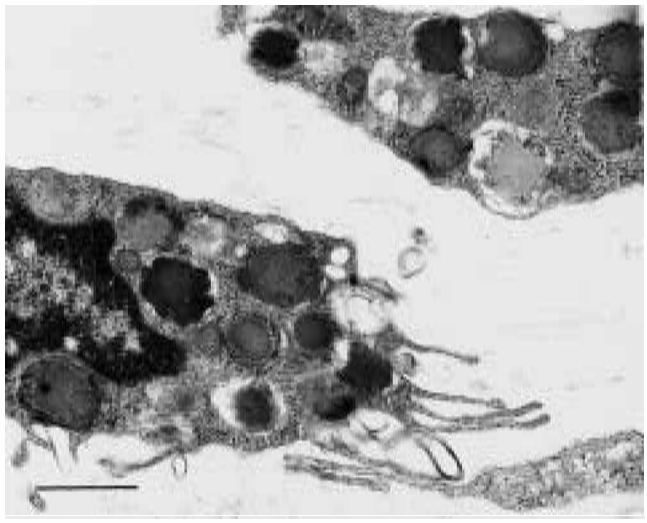

Fig. 2. High-power view of 2 typical spindle-shaped cells in the upper dermis. The dendritic processes of one cell are demonstrated. The whorled appearance of the intra-cellular granules is also seen. Scale bar = 0.56 μm.

nodularis skin (11). No statistically significant correlation has been found between clinical score and the number of mast-cell profiles per square millimetre (12).

The ultrastructural manifestations of chronic occupational chromate dermatitis have not previously been described. The changes can be followed throughout the clinical spectrum of disease. The earliest changes occurred in the lower epidermis with keratinocyte separation. Initially, there was little cell separation and no damage to the intercellular connections. It is probable that the events of cell separation were mechanical in origin due to increased intercellular oedema. With increased intercellular oedema, the keratinocytes separated further, resulting in damage to the intercellular connections. It was found that it was the intermediate filament part of the desmosome that was damaged, while the desmosome plaque units stayed intact in all cases and these were often found freely in intercellular spaces.

Increased clinical severity of the dermatitis produced increasingly pronounced ultrastructural changes in the mid- and upper epidermis. As a severe end stage of the disease, frank vesicle formation occurred within the epidermis, and this was readily visible both clinically and histologically. Ultrastructurally, the keratinocytes were severely damaged. Numerous desmosomal plaque units were found within the massively widened intercellular spaces along with cellular debris and intermediate filaments. Migrating inflammatory cells were also present in large numbers.

One new feature described that was consistent throughout all stages of chronic chromate dermatitis was the presence of numerous spindle-shaped cells in the upper dermis. These cells were closely opposed to the dermo-epidermal junction, with numbers not appearing to vary significantly according to the stage of dermatitis. Unfortunately, it was not possible to confirm the nature of these cells retrospectively by immunocytochemistry. However, the typical ultrastructure of the villous processes and granules along with the positive cell staining by toluidine blue strongly points to the cells being mast cells.

Boehncke et al. (13) described similar dermal spindle cells in psoriatic skin. In this study, results of immunocytochemical tests suggest that the cells belonged to a macrophage subpopulation. The cells were found in large numbers in lesional psoriatic skin but were also found to a lesser extent in atopic dermatitis skin. Almost none were found in normal skin. Wollenberg et al. (14) identified a new inflammatory dendritic cell in lesional skin of atopic eczema. However, these cells had the ultrastructural features of Langerhans' cells and were found in the epidermis.

Ultrastructural analysis of normal control skin in our study has shown that the electron microscopic processing did not produce any significant artefacts. Analysis of the control specimens of patients with inflammatory dermatoses has shown differences from specimens of chromate-allergic patients. Whilst many changes are similar and relate to increasing epidermal oedema and mechanical separation of keratinocytes, there were significantly fewer desmosomes in the chromate-allergic sections. In addition, the ultrastructural changes in the chromate-allergic patients were more severe than for a comparable clinical stage of non-chromate dermatitis.

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