

## EARLY FINE STRUCTURAL CHANGES IN HUMAN EPIDERMIS FOLLOWING APPLICATION OF CROTON OIL

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**Abstract.** Early epidermal changes in human subjects following application of 10% croton oil in acetone were studied by electron microscopy. The prominent changes observed at 1 and 5 hours were degeneration of epidermal cells and intercellular edema in the basal and spinous layers. One type of degeneration was homogenization and aggregation of the tonofilaments of keratinocytes at 1 hour, which may explain the presence of dyskeratotic cells at 5 hours. Another type was of a cytolytic nature as evidenced by severe perinuclear edema of keratinocytes and destruction of Langerhans' cells with outpouring of Birbeck's granules in the intercellular spaces.

**Key words:** Croton oil; Primary irritant contact dermatitis

Croton oil-induced epidermal injury has been studied as a model of primary irritant contact dermatitis (PICD). Under light microscopy, epidermal changes which occur 5 to 6 hours after application of 50% croton oil consists of cleft formation in the hair follicles and nuclear pyknosis of keratinocytes (1). There is, however, no intercellular edema with elongation or rupture of intercellular bridges. On the other hand, the electron microscopic study of 50% croton oil-induced PICD demonstrates that the noticeable changes at 5 to 6 hours are intercellular edema of the lower epidermis and cytolytic degenerative events (6). These varying results suggest that the epidermal changes induced by croton oil already occur to a variable degree at 5 to 6 hours. Using the electron microscope, we examined early epidermal changes of human skin induced by croton oil.

### MATERIALS AND METHODS

The application of croton oil was performed according to the method of Johnson et al. (3) with slight modification. Three healthy human subjects were patch tested under occlusion with 0.04 ml of 10% croton oil in acetone on the

extensor surface of the thigh (area, ca. 1×1 cm). Biopsy specimens were obtained at 1 hour from 3 subjects and at 5 hours from one subject after application of croton oil. Specimens from untreated skin and skin patch-tested with acetone only were also obtained from 2 subjects who served as controls.



**Fig. 1.** One hour after application of croton oil, intercellular spaces are slightly widened and contain granules resembling ribosomes in the spinous layer. Desmosome-tonofilament complexes are disrupted in many areas. Keratinocytes show varying degrees of electron density of cytoplasm and possess many vacuoles. ×8 100.

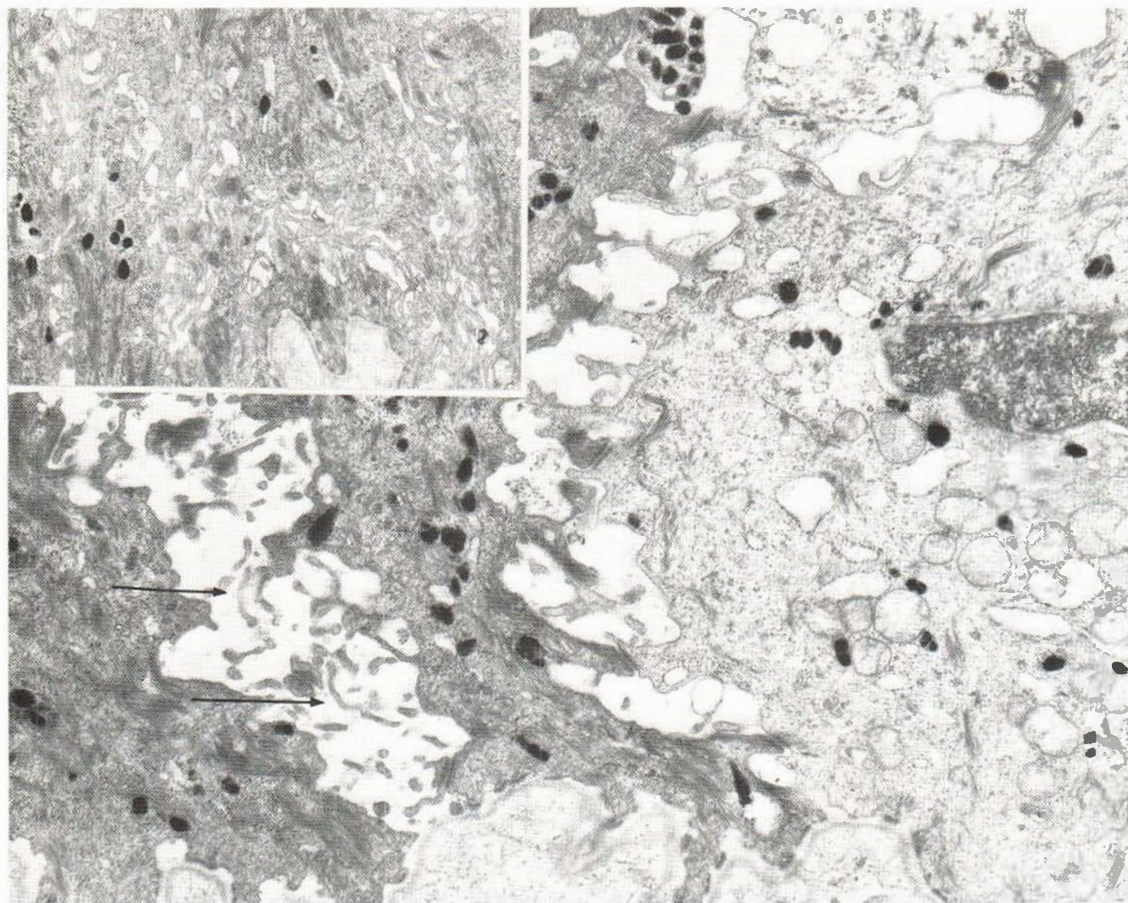


Fig. 2. Basal cells at 1 hour, showing dilatation of endoplasmic reticulum and alteration of mitochondria. Villous projections (arrows) are prominent.  $\times 15000$ . Inset: Vil-

lous projections of basal cells in no intercellular edema.  $\times 10000$ .

Each biopsy specimen was cut into small pieces. One was processed for light microscopy, while the others were fixed in phosphate-buffered 4% glutaraldehyde and 1% osmium tetroxide for 2 hours at 4°C, respectively. After the fixation, the specimens were dehydrated in graded alcohol and embedded in Epon 812. Thick sections were stained with 1% toluidine blue and studied optically for orientation. Thin sections were cut with glass knives on a Porter-Blum MT 2 microtome, mounted on carbon-coated Formvar and stained with uranyl acetate and lead citrate. Photos were taken with a Hitachi HU-11 D or HS-9 electron microscope.

## RESULTS

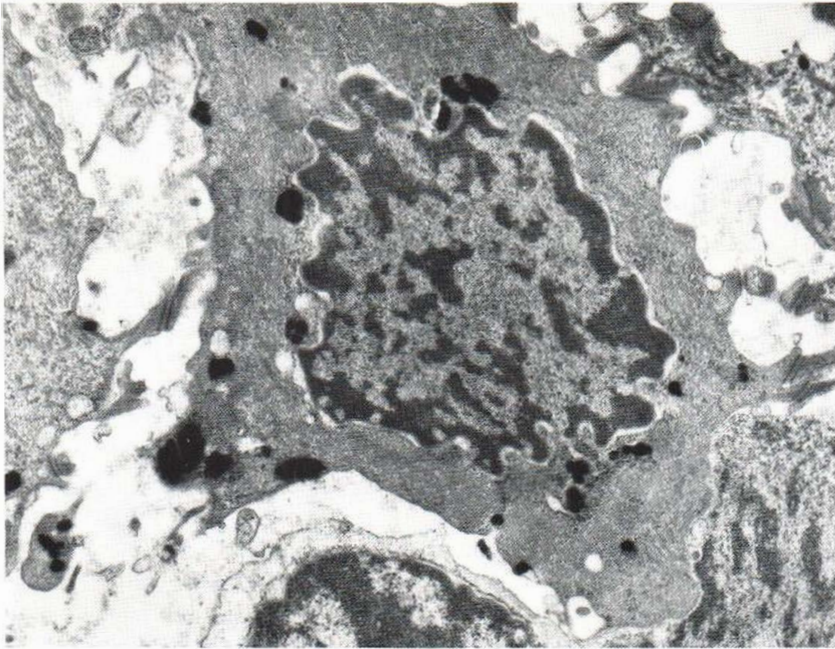
### Gross changes

No visible change was evident at the patch test sites 1 hour following application of croton oil. A mot-

tled, faint erythema first appeared at 5 to 6 hours in all subjects. At 24 hours, erythema was limited to the patch test sites which were studded with small papules, vesicles and few pustules. At 48 hours, pustules were more prominent. These changes represent the normal inflammatory responses to croton oil as defined by Johnson et al. (3).

### Light microscopical changes

At 1 hour, the epidermis showed mild intercellular edema and perinuclear edema of some keratinocytes in the basal and spinous layers. At 5 hours, a few dyskeratotic cells possessing pyknotic nuclei and eosinophilic cytoplasm were observed in addition to the augmentation of those changes seen at 1 hour.

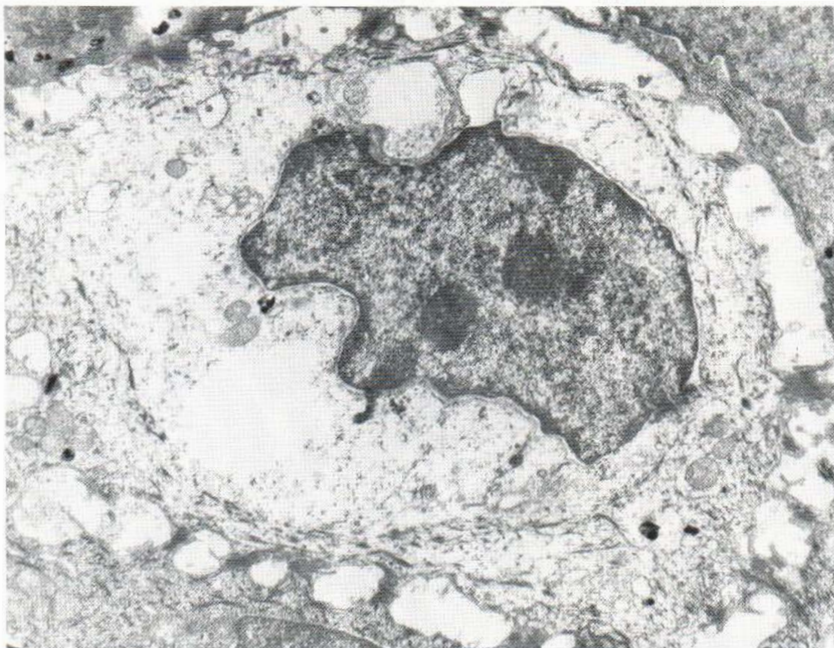


*Fig. 3.* Keratinocyte in the spinous layer at 1 hour, showing homogeneous aggregation of tonofilaments.  $\times 13\ 000$ .

#### *Electron microscopical changes*

In the control untreated skin or the skin patch-tested with acetone only, the ultrastructural details were similar to those seen in normal skin such as described by Breathnach (2).

One hour after application of croton oil, the intercellular spaces of the basal and spinous layers were widened to a varying degree and contained granules resembling ribosomes (Fig. 2). The intercellular bridges were stretched and, in some



*Fig. 4.* Keratinocyte in the spinous layer at 1 hour, showing marked perinuclear edema, and dilatation of endoplasmic reticulum and perinuclear cisternae. Tonofilaments are arranged into a ring at the cell periphery.  $\times 8\ 000$ .



Fig. 5. Outpouring of cytoplasmic organelles of Langerhans' cell at 1 hour in the spinous layer. Arrow: Birbeck's granules.  $\times 14\,000$ .

areas, disrupted proximal to the desmosomes, the structure of which was well preserved.

Most keratinocytes of the basal and spinous layers showed a varying degree of electron density of the cytoplasm and contained cytoplasmic vacuoles including membrane-free clear spaces and dilated endoplasmic reticulum and mitochondria (Figs. 1 and 2). Villous projections were prominent in the basal cells (Fig. 2 and Inset). In some keratinocytes, the tonofilaments were aggregated into irregular packets and lost their normal orientation (Fig. 3). The aggregates appeared homogeneous and individual filaments could not be easily distinguished. In other keratinocytes, perinuclear edema was severe and the thin bundles of tonofilaments appeared to be compressed into a ring at the cell periphery (Fig. 4).

Destruction of Langerhans' cells was often observed, and was independent of alterations to adjacent keratinocytes. Cytoplasmic organelles such as Birbeck's granules seemed to pour out into the intercellular spaces (Fig. 5). The melanocytes appeared structurally normal.

Five hours after application of croton oil, the ultrastructural changes were essentially the same but more severe than those seen at 1 hour. Typical dyskeratotic cells showing densely aggregated to-

nofilaments and pyknotic nuclei were observed (Fig. 6). Only a few mononuclear cells had infiltrated into the lower epidermis at 5 hours. The basement membrane was intact at 1 and 5 hours.

#### COMMENT

Epidermal changes in the skin as detected by light and electron microscopy were without noticeable gross alterations 1 hour after application of croton oil. The predominant ultrastructural changes at 1 and 5 hours were degeneration of epidermal cells and intercellular edema in the basal and spinous layers.

Severe damage by croton oil resulted in two types of degeneration of epidermal cells. One type, not so far reported in croton oil-induced PICD, was the appearance of dyskeratotic cells at 5 hours. Homogenization and aggregation of the tonofilaments in some keratinocytes at 1 hour seem to represent an early stage of formation of dyskeratotic cells. Another was of a cytolytic nature, as described by Metz (6). Keratinocytes showed severe perinuclear edema and, more than 5 hours following application, would be lysed, forming epidermal vesicles. Lysis of Langerhans' cells with outpouring of cytoplasmic organelles was another sign of cytolytic degeneration.

Intercellular edema was usually associated with

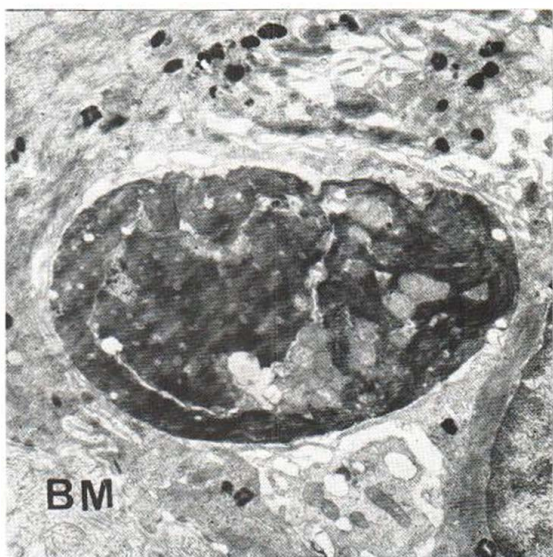


Fig. 6. Dyskeratotic cell at 5 hours. *BM*, basement membrane.  $\times 8\,800$ .

disruption of intercellular bridges proximal to the structurally normal desmosomes and retraction of the desmosome-tonofilament complexes towards one of the opposing cells. Most epidermal cells showed cytoplasmic vacuoles. These changes have been reported in early epidermal changes induced by various irritants such as acetone (4) and thermal burn (8). It is suggested that the epidermal cell damage elicited by various forms of injury is, in effect, stereotyped if injuries are of an appropriate type and degree.

A comparison of the present findings with those epidermal changes seen early after DNCB-induced PICD (5) reveals that inter- and intracellular edema and lysis of keratinocytes are the common features. However, lysis of Langerhans' cells is not observed in DNCB-induced dermatitis, thus suggesting that Langerhans' cells are more resistant than keratinocytes to DNCB (7). Dyskeratotic cells are also not evident in DNCB-induced dermatitis. This may reflect a difference in the chemical nature of the two irritants.

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