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# THE SYNTHESIS OF KERATINOSOMES DURING EPIDERMAL WOUND HEALING

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Employing suction-induced subepidermal blisters as a model of epidermal wound healing, the formation of keratinosomes in a differentiating epidermis has been studied. Keratinosomes appear at 24 hr after wounding. They are more fully developed at 36 hr, preceding the formation of the horny layer at 48 hr. At that time a horny layer becomes visible and keratinosomes can be seen both intra- and extra-cellularly at the granular horny cell interface.

Keratinosomes are subcellular lamellated organelles of the epidermis, which are seen intracellulary in upper-level keratinocytes, and extracellularly at the junction of the granular and horny layers [1-17]. While the complete biochemical composition and exact function of the keratinosomes is as yet unknown, preliminary histochemical evidence suggests that they may play an important role in epidermal physiology and formation of the horny layer [6,12,14].

This report presents ultrastructural observations made during epidermal repair of young mouse skin in a model wound-healing system [18]. In the sequential de novo development of the epidermis, keratinosomes could be observed prior to the formation of the stratum corneum.

## MATERIALS AND METHODS

Suction blisters were induced in the dorsal thoracic skin of 1- to 2-day-old mice of the Webster strain (Harvard Medical School, Department of Infectious Diseases) [18]. Intact subepidermal blisters were biopsied at 0, 24, 36, and 48 hr after wounding, and were fixed for 1 hr at room temperature in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Following primary fixation and a buffer rinse, the tissues were postfixed in phosphate-buffered 1% osmium tetroxide, stained en bloc with uranyl acetate [19], and rapidly dehydrated and embedded in Epon [20]. Thick sections were cut on a Porter-Blum MT-1 ultramicrotome and stained with borate-buffered toluidine blue. Thin sections, cut on a Porter-Blum MT-2 ultramicrotome and collected on uncoated grids, were stained with uranyl acetate and lead citrate [21] and observed in a Zeiss/EM 9A electron microscope.

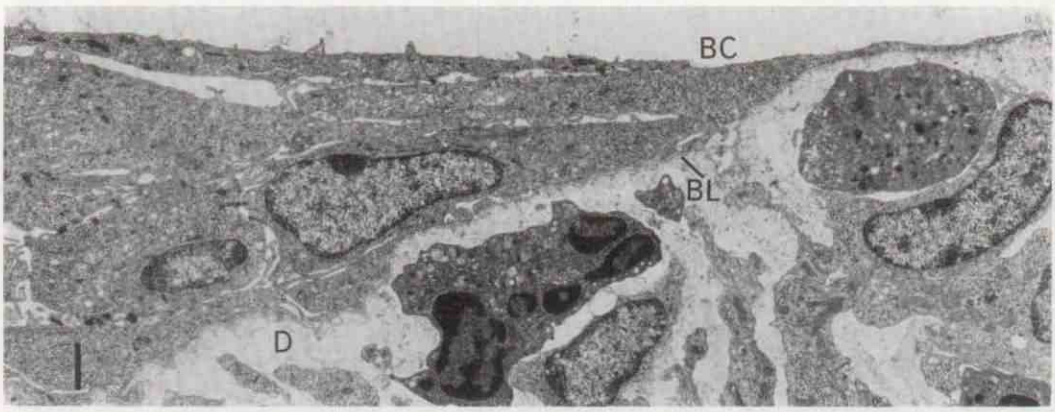


FIG. 1. Twelve hours after wounding, a wedge of elongated keratinocytes moves forward to cover the denuded dermis. No keratinosomes are seen at this stage of epidermal wound healing. *D*, dermis; *BL*, basal lamina; *BC*, blister cavity (Epon, × 5,185).

## RESULTS

Employing a suction device [18], it is easy to separate the epidermis from the dermis, thereby

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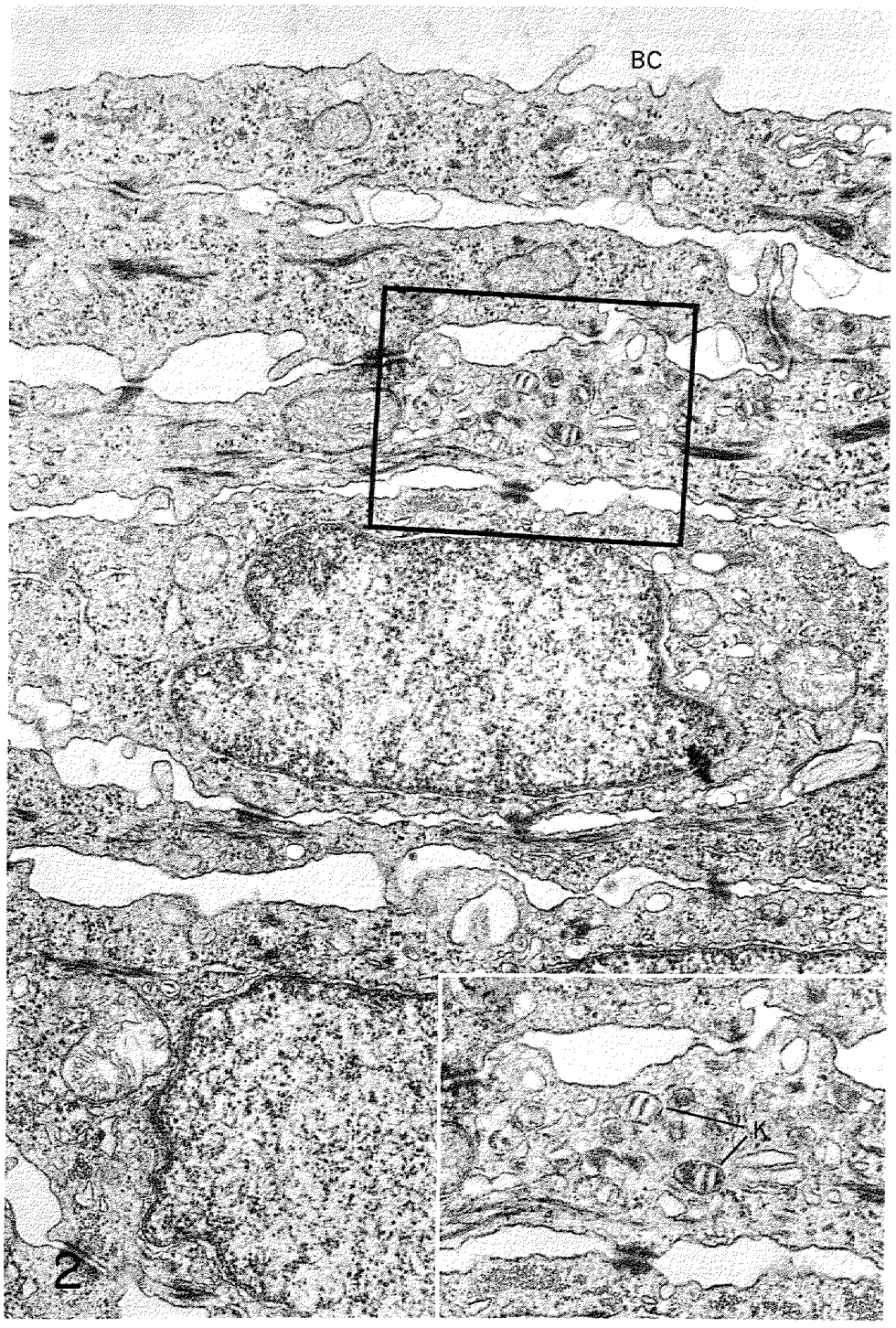


FIG. 2. At 24 hr, migration of epidermal cells has ceased. The newly established basal cells have begun to differentiate into regenerated epidermis, which is 3 to 4 cell layers thick. No horny layer is formed as yet, but keratinosomes are present intracellularly in the upper layers of the regenerating epidermis. *BC*, blister cavity (Epon, × 29,400). *Inset:* Under higher magnification, keratinosomes (*K*) appear somewhat different from those seen in unwounded skin (Epon, × 40,800).

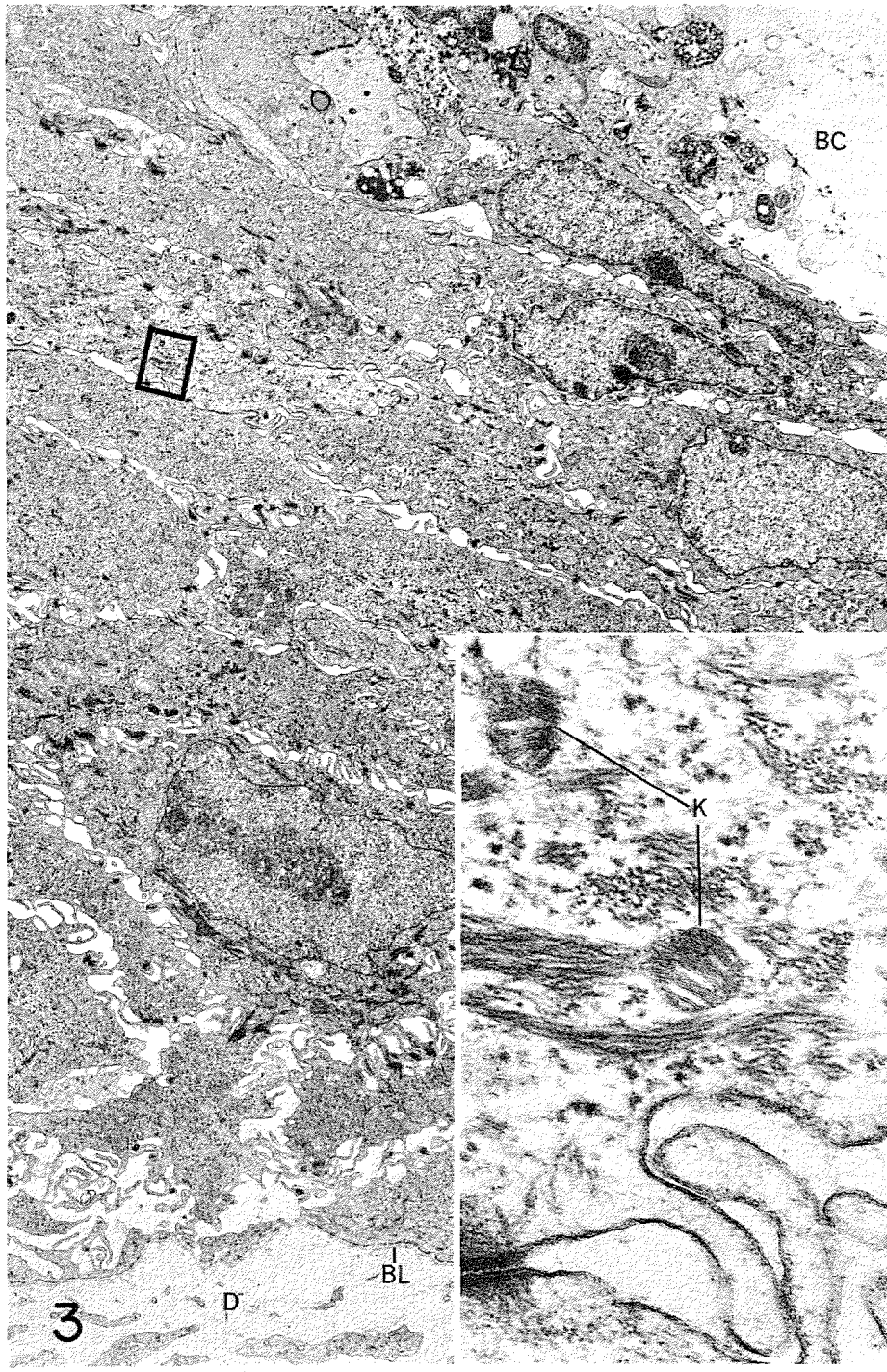


FIG. 3. At 36 hr, the epidermis is further developed, but still lacking a horny layer. Keratinosomes are seen more frequently. *BC*, blister cavity; *D*, dermis; *BL*, basal lamina (Epon, × 6,120). *Inset:* Keratinosomes (*K*) can be seen with features more closely resembling those seen in unwounded skin. Their lamellation is more regular and more dense than at 24 hr (Epon, × 75,600).

inducing a subepidermal blister. Biopsies of intact subepidermal blisters taken immediately after induction show that the electron-dense portion of the basal lamina remains at the superior border of the dermis. Sequential observations made after wounding show that epidermal restoration is a result of epidermal cell movement from the hair follicle root sheaths and the lateral intact epidermis. At 12 hr following wounding, undifferentiated epidermal cells can be observed moving from the root sheaths in a wedge-shaped formation (Fig. 1). Close observation of these migrating cells does not reveal any keratinosomes (Fig. 1). At 24 hr after wounding, the epidermal hiatus has been closed by a de novo formed epidermis, which was noncornified and 3 to 4 cell layers deep (Fig. 2). At this time, keratinosomes can be seen within the uppermost epidermal cells. The latter had differentiated from the basal layer after the migration of keratinocytes had been completed to fully cover the previously denuded dermis. However, no keratinosomes are observed extracellularly at 24 hr after wounding. The keratinosomes appearing at 24 hr did not show the regular and dense lamellation that can be seen in keratinosomes later on in the repair process (*Inset*, Fig. 2). In this model system it is not possible to establish whether keratinosomes originate in the Golgi region, as in normal and psoriatic skin [2,10].

At 36 hr after wounding, greater numbers of keratinosomes can be seen intracellularly (Fig. 3). The regularity and density of their lamellation is better established than at 24 hr (*Inset*, Fig. 3). No keratinosomes are seen at 36 hr in the extracellular region, and a horny layer has not yet formed.

At 48 hr, a horny layer is present and keratinosomes are observed intracellularly as well as extracellularly at the granular–horny cell interface (Fig. 4).

## DISCUSSION

Although there are a variety of experimental epidermal wound-healing systems, the discussion will be confined to the model wound-healing system employed in this study. At 12 hr after wounding, keratinocytes could be observed migrating over the basal lamina, which had remained intact during this type of blister formation on the uppermost layer of the dermis. In migrating keratinocytes, keratinosomes could never be observed. This observation is of importance as it allows the

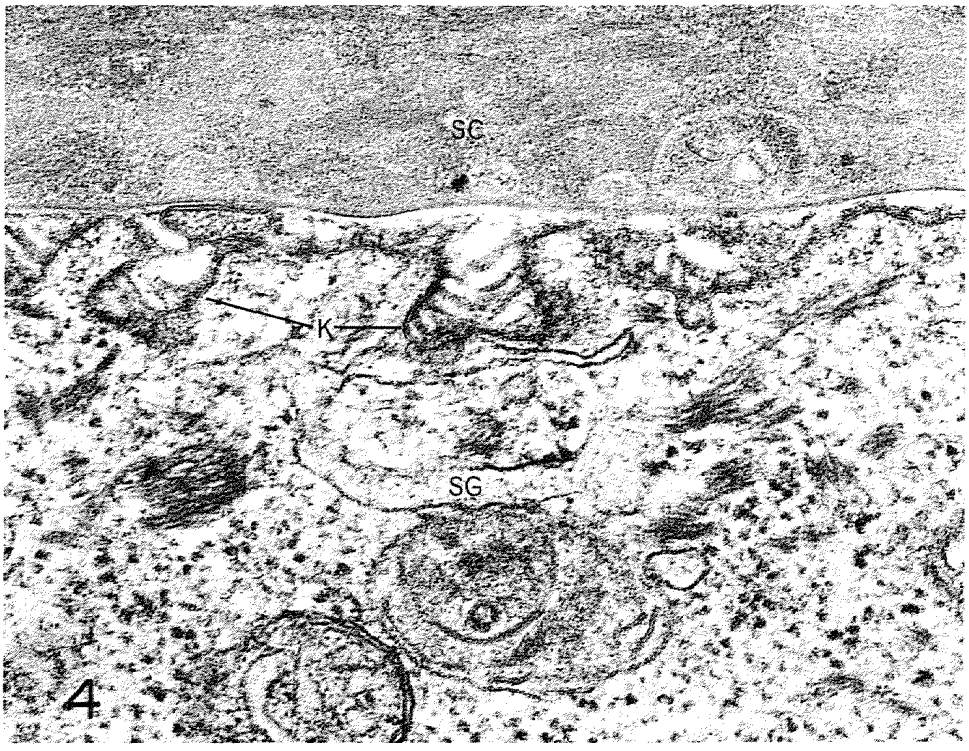


FIG. 4. At 48 hr, differentiation of the epidermis has been completed with a de novo formed horny layer. Keratinosomes can be seen intra- as well as extra-cellularly in a normal fashion. *K*, extracellular keratinosomes; *SC*, stratum corneum; *SG*, stratum granulosum (Epon, × 90,900).

conclusion that the appearance of keratinosomes later on in the differentiation of the dermis must have come about by de novo synthesis. At 24 hr after wound induction, keratinocytes had completely reepithelialized the previously denuded dermis in the form of a noncornified epidermis. Keratinosomes could now be observed intracellularly, but were not present in the extracellular space. They were seen intracellularly in greater numbers at 36 hr, well before the horny layer appeared approximately 48 hr after wounding. In other words, the morphogenesis of keratinosomes preceded the appearance of a horny layer.

There are two possible interpretations of this finding. First, one could assume that the appearance of keratinosomes prior to the formation of the horny layer is coincidental and unrelated to the conversion of granular into horny cells. A second interpretation would support the concept that the appearance of keratinosomes prior to the formation of the horny layer is an important step in the conversion of granular cells into horny cells, that keratinosomes are necessary for this conversion, and that they are necessary for the formation of the horny layer and eventually for desquamation.

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