THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 55, No. 1

*Printed in U.S.A.*

# FURTHER OBSERVATIONS ON EPITHELIALIZATION OF SMALL WOUNDS*

## AN AUTORADIOGRAPHIC STUDY OF INCORPORATION AND DISTRIBUTION OF $^3$H-THYMIDINE IN THE EPITHELIUM COVERING SKIN WOUNDS

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## ABSTRACT

Linear incisions were made on the back skin of rabbits and $^3$H-thymidine was injected intravenously during various stages of epithelialization of the wounds. Biopsy specimens were taken 30 minutes and one to three days after injection of the tracer.

In the migrating epithelium, the nuclei of basally located cells were labeled thirty minutes after the introduction of $^3$H-thymidine. The cells located at higher levels did not incorporate the tracer. One or two days later, labeled nuclei also appeared in the upper layers.

After wound closure, nuclei were labeled in the basal layer of the epithelium 30 minutes after the introduction of $^3$H-thymidine. One day later, labeled nuclei appeared in both basal and suprabasal layers, and after three days the nuclei of parakeratotic surface cells were also labeled.

These observations indicate that the cells which emigrate from the epidermis divide and differentiate while they move over the wound surface. Thus, mitosis and differentiation are not incompatible with migration as it has been postulated by previous workers. It is concluded that both mitotic and differentiating cells participate in formation of a protective cover over skin wounds.

Repair of skin wounds has been extensively studied in the past and most investigators found that epithelialization began with an increase of mitotic activity in the epidermis at the wound edge, followed by migration of newly formed cells over the wound surface (1–8). Dividing or differentiating cells were not noted in the migrating epithelium and, therefore, it was thought that proliferation and differentiation were incompatible with migration (9–15).

In our previous study we noted that formation of a new epithelium is a complex process involving migration, mitosis, and differentiation rather than a simple migration of cells from the epidermis surrounding the wound. Divisions within the migrating epithelium were observed by arresting cells in mitosis with colchicine or labeling cells with tritiated thymidine. Differentiation was revealed by the presence of keratohyalin granules in cells forming the upper part of the migrating epithelium (16).

It is the purpose of this study to investigate in more detail the role of cell proliferation and differentiation in formation of the epithelium that covers small skin wounds. To study such activities of migrating epidermal cells, tritiated thymidine was utilized. It is well known that this tracer is incorporated into the nuclei of epidermal cells during the premitotic period when DNA is synthesized (6). Thus, by short-term autoradiographic studies, information can be obtained with this tracer about the site of mitotic cells in the migrating epithelium covering the wound. It is also known that the labeled DNA, containing tritiated thymidine, is portioned out to the daughter cells and that DNA is stable throughout the life of those daughters. Such cells of course do not divide further, but enter the course of differentiation

This investigation was supported by Research Grant AM 05779 from the National Institute of Arthritis and Metabolic Diseases, United States DHEW.

Received December 22, 1969; accepted for publication February 9, 1970.

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(17). Accordingly, by long-term autoradiographic studies, the displacement of newly formed cells can be followed, and the site of differentiating cells identified. Observations made in such short- and long-term studies during various stages of epithelialization of small skin wounds are presented in this publication.

## MATERIAL AND METHODS

White rabbits weighing two to three kg were clipped on the back and linear incisions 0.5 mm in depth were made with a guarded surgical knife as described in a previous study (16). Experiments were conducted in duplicate as outlined in Table I.

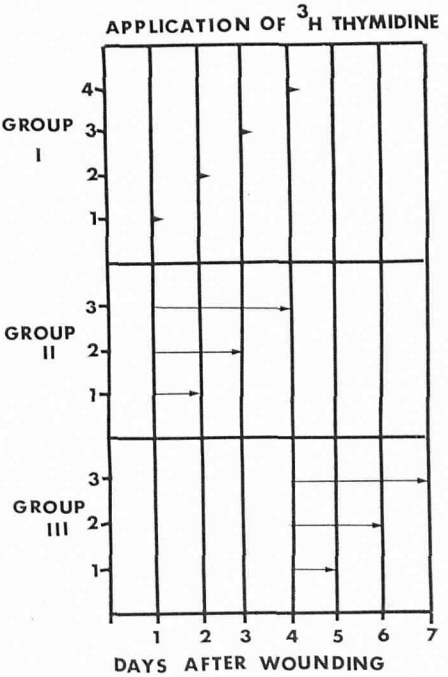
*Group I. Short-term experiments.* A short linear incision was made daily on the back of the rabbit and on the fourth day tritiated thymidine was injected. Thirty minutes later biopsy specimens were obtained from 1, 2, 3 and 4-day-old wounds.

*Group II. Long-term experiments.* A long incision was made on the back of the rabbit and one day later tritiated thymidine was injected. Biopsy specimens were taken on the 2nd, 3rd and 4th days after wounding, e.g., 1, 2 and 3 days after the use of the tracer.

*Group III. Long-term experiments.* A long incision was made on the back of the rabbit and four days later, when the wound was closed, tritiated thymidine was injected. Biopsy specimens were taken on the 5th, 6th and 7th days after wounding, e.g., 1, 2 and 3 days after the use of the tracer.

Tritiated thymidine was injected intravenously into the rabbits in 1 mc/kg body weight amounts, the specific activity of the tracer was 6,700. Biopsy specimens were fixed in Helly's fluid and embedded in paraffin by routine histological methods; sections were stained with hematoxylin-eosin. Autoradiographs were prepared by dipping sections into Kodak bulk emulsion NTB, and were developed six to eight weeks thereafter. A nucleus was considered labeled if it was covered by five or more grains. In background grain counts, less than one grain was found per cell in a section.

TABLE I




## RESULTS

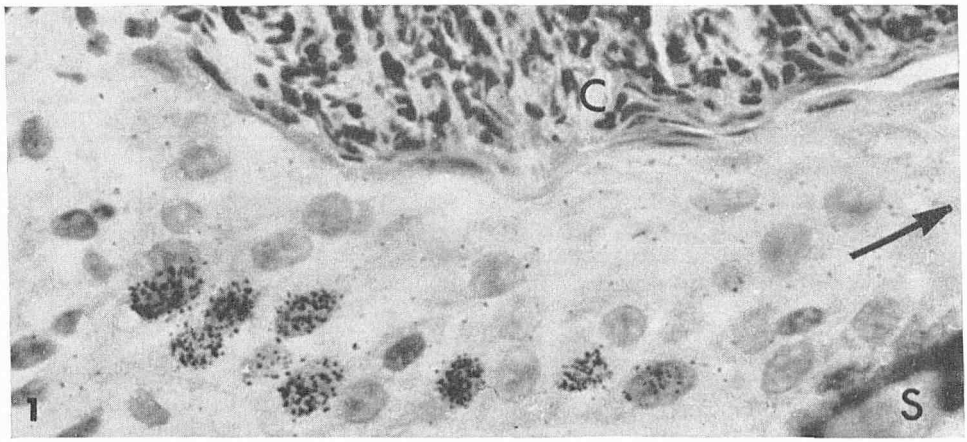
### *Sequential Events of Epidermal Repair*

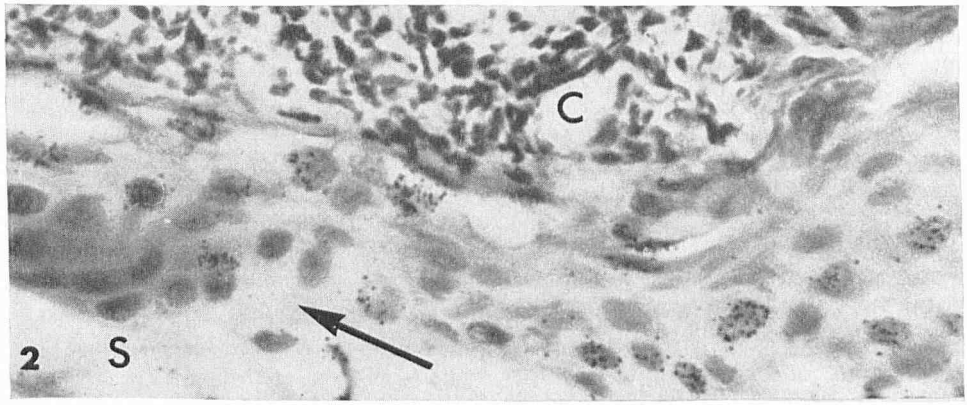
Examination of sections prepared from the biopsy specimens taken daily after wounding shows that epidermal repair proceeds as follows: During the first day, the epidermis is hypertrophic around the wound, and after a day, a wedge shaped epithelial mass consisting of a few cells appears at the wound edge. On the second day, an epithelial tongue, two to four cells thick with a wedge shaped tip, covers part of the wound (Fig. 1). On the third day, most of the wound surface is covered by a four- to six-cell-thick epithelium (Fig. 2), and on the fourth day, the surface is completely covered by an eight- to twelve-cell-thick epithelium which possesses a parakeratotic surface layer (Fig. 3). During the subsequent three days, the epithelium does not thicken further, but the surface layer reveals extensive scaling (Fig. 6).

### *Autoradiography of the Migrating Epithelium*

Autoradiographs prepared from specimens obtained 30 minutes after the injection of tritiated thymidine (Group I) show that the nuclei of basally located cells are exclusively labeled in the epithelium migrating over one, two, or three-day wounds. The nuclei of cells forming the higher layers are not labeled. Labeled basal cells seen over such wounds are demonstrated in Figure 1.

One or two days after the injection of the tracer (Group II), some of the labeled cells are displaced to higher levels. Labeled nuclei are seen scattered all over the migrating epithelium over two- or three-day-old wounds. An



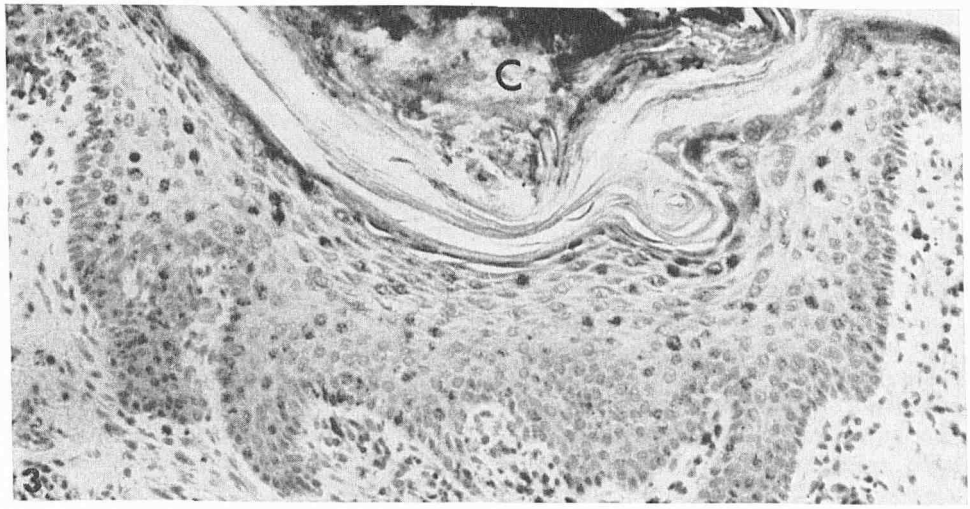
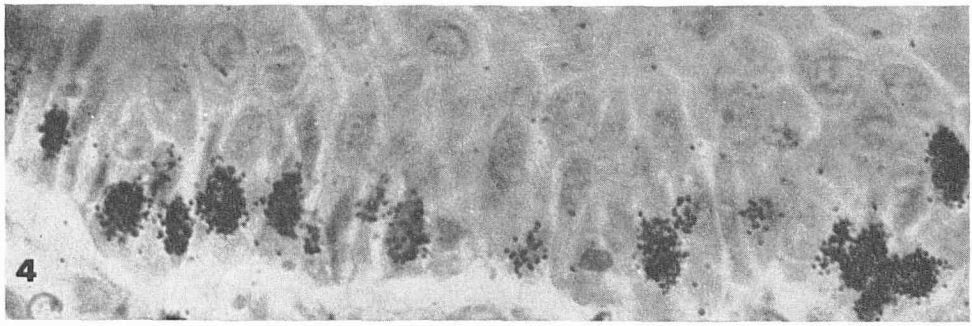
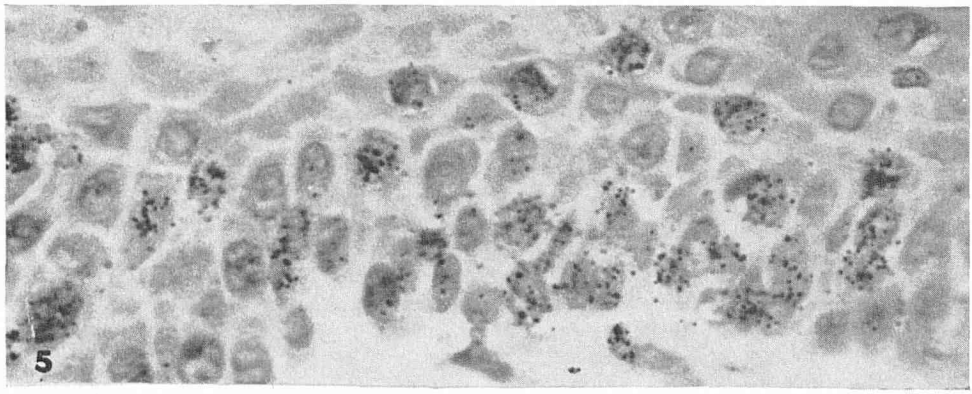




FIG. 1. Epithelium migrating over the surface (S) of 2-day wound showing labeled nuclei in the basal portion 30 minutes after the introduction of $^{3}$H-thymidine. Epithelium is covered with crust (C), direction of migration is indicated by arrow (Group I, 2).

FIG. 2. Epithelium migrating over the surface (S) of 3-day wound showing labeled nuclei in all layers two days after the injection of $^{3}$H-thymidine. Epithelium is covered with crust (C), arrow shows direction of migration (Group II, 2).

FIG. 3. Epithelium closed over 4-day wound showing multiplicity of labeled nuclei in the upper part, and paucity in the lower part 3 days after $^{3}$H-thymidine has been injected. Some crust (C) is still visible over the parakeratotic surface layer (Group II, 3).



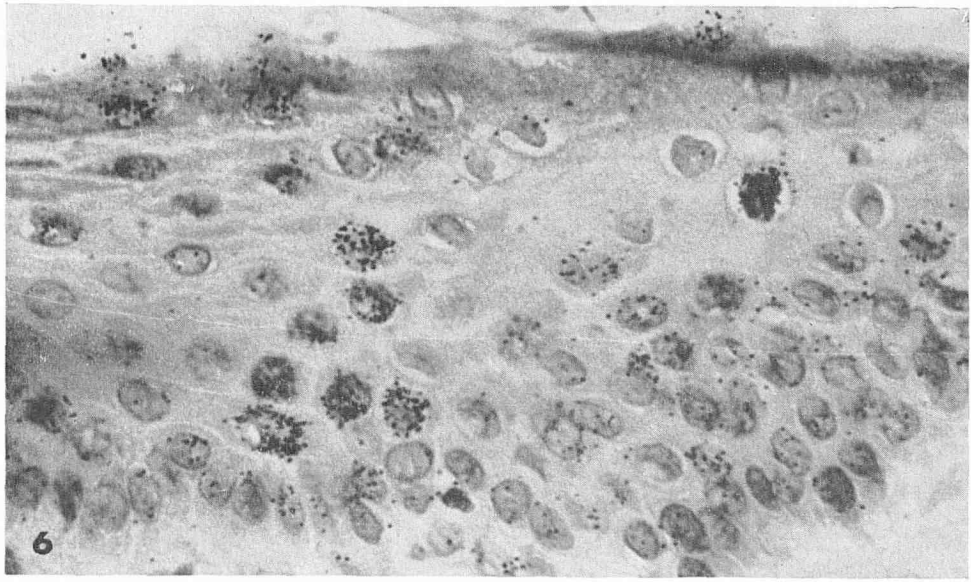




FIG. 4. Lower part of the epithelium that closed over 4-day wound revealing labeled nuclei in the basal layer 30 minutes after the injection of $^{3}$H-thymidine (Group I, 4).

FIG. 5. Lower part of the epithelium over 5-day wound showing labeled nuclei in both basal and suprabasal layers one day after the introduction of $^{3}$H-thymidine (Group III, 1).

FIG. 6. Epithelium over 7-day wound revealing labeled nuclei scattered over the entire epithelium three days after the injection of $^{3}$H-thymidine (Group III, 3).

autoradiograph revealing labeled nuclei in the various layers of the migrating epithelium is shown in Figure 2.

Three days after the introduction of the tracer (Group II) labeled nuclei are numerous in the upper part of the epithelium that had closed over four-day-old wounds. In the lower part of the epithelium, only a few nuclei reveal labeling. The distribution of labeled cells at this stage of repair is illustrated in Figure 3.

### *Autoradiography of Non-Migrating Epithelium Seen After Wound Closure*

The epithelium over four-day-old wounds obtained 30 minutes after the injection of tritiated thymidine (Group I), shows incorporation of the tracer into the nuclei of basal cells. The nuclei of cells located at higher levels are not labeled. Labeled nuclei of basal cells over such wounds are shown in Figure 4.

During subsequent days, many cells are displaced toward the surface (Group III). The number of labeled basal cells is reduced, and the label in the nucleus is diluted, as evidenced by fewer grains. One day after the injection of tritiated thymidine, labeled nuclei appear scattered in the lower part of the covering epithelium of five-day-old wounds. Two days later, labeled nuclei occur in all layers of the epithelium covering six-day-old wounds excepting the parakeratotic surface layer. Three days later, the nuclei of parakeratotic surface cells covering seven-day-old wounds are also labeled. The gradual displacement of labeled cells toward the surface of the newly formed epithelium is illustrated in Figures 5 and 6.

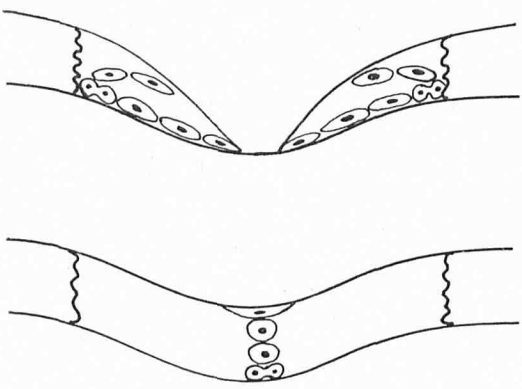


FIG. 7. Schematic illustration of direction of cell movement during epithelialization of small wounds as revealed by the autoradiographic studies. Note that the newly formed cells are displaced, both in upward and forward directions, in the migrating epithelium as shown in the upper drawing. After wound closure, cells are displaced toward the surface of the non-migrating epithelium, as indicated in the lower drawing.

## DISCUSSION

Incorporation, transfer and dilution of tritiated thymidine, as revealed by autoradiography, provides valuable information about proliferative and differentiative activities of cells participating in reestablishment of the continuity of the epidermis after wounding. The short-term experiments reveal that DNA is synthesized exclusively by those cells which are close to the wound surface, thus it may be concluded that basally located cells of the migrating, as well as the non-migrating epithelium are mitotically active; the cells located at higher levels do not incorporate the DNA precursor, so presumably they do not enter division cycles. Some of the long-term experiments (Group II) indicate that in the migrating epithelium, cells are continuously displaced in both upward and forward directions after division (Fig. 7). This is revealed by the appearance of labeled nuclei in both basal and suprabasal parts of the migrating epithelium as soon as one day after the introduction of the label. This is also demonstrated by the multiplicity of labeled cells in the upper part, and the paucity of labeled cells in the lower part of the epithelium at a later time when the epithelium closes over the wound. Apparently the label is retained by the upper cells which enter the course of differentiation, and is gradually lost by dilution in the lower cells which frequently divide. Other long-term experiments (Group III) reveal that after cessation of migratory activity, many newly formed cells enter the course of differentiation and are displaced toward the surface within a period of three days (Fig. 7). Thus, turnover of the newly formed epithelium is fast, and little time is left for the cells to differentiate; the surface of the epithelium becomes covered by parakeratotic cells.

These observations lead to the conclusion that neither proliferation nor differentiation is incompatible with migration. Epidermal cells are capable of dividing or differentiating while

they migrate over the wound surface. Thus, the cells which emigrate from the epidermis do not abandon their original activities. Movement of the newly formed epithelium seems to be elaborated by both "active" and "passive" movements of the cells, i.e., by migratory activity and displacement after division.

Cell proliferation within the migrating epithelium provides new cells *in situ* for wound coverage. Hence, wound coverage does not depend entirely upon those cells which emigrate from the intact epidermis, as many new cells spread over the wound surface originating from the migrating epithelium. The efficiency of wound coverage is considerably enhanced by mitotic cells of the migrating epithelium.

Differentiation of cells within the migrating epithelium also is of significance because it results in the formation of protective surface cells. Differentiation is accelerated and incomplete during epithelialization as revealed by this study. Parakeratotic surface cells, however, may provide an adequate temporary cover for the wound. It has been shown that parakeratotic surface cells formed after stripping of the stratum corneum, prevent the loss of water from the underlying tissues, to a relatively large degree (18). Parakeratotic surface cells may be considered capable of preventing desiccation of the migrating epithelium and thereby securing an environment that is suitable for actively migrating and dividing cells.

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