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HISTIDINE AND KERATOHYALIN GRANULES*

ALVIN J. COX, M.D. AND EVE P. REAVEN, PH.D.

The distribution of histochemically-identifiable histidine in the epidermis is distinctive and striking. Histidine, bound in an insoluble form, is localized in the region of transition to the stratum corneum. In this narrow zone of epidermis, which includes the stratum granulosum, histidine can easily be demonstrated by means of the Pauly reaction with diazotized sulfanilic acid after a variety of methods of tissue fixation (1). Fukuyama, Nakamura and Bernstein (2) found that histidine, administered as a radioactive isotope, was rapidly incorporated into the upper malpighian portion of the newborn rat epidermis to a greater extent than in the multiplying basal cells. Subsequently, the radioactive label appeared to move into the stratum corneum.

The significance of this peculiar localization of histidine in the epidermis has not been determined, although a specific relationship to keratohyalin granules and to the process of keratinization has been suspected. In the study to be reported, radioautography and electron microscopy were used to provide a more detailed analysis of the epidermal deposition and subsequent behavior of administered histidine.

MATERIAL AND METHODS

Three experiments with littermate newborn Swiss mice were conducted at different times. Each animal was injected intraperitoneally within 24 hours of birth with 0.1 ml of physiological saline solution containing histidine uniformly labeled with tritium; this was obtained from Calbiochem Corporation. Each mouse received 50 microcuries of the isotope. From two of the groups the injected mice were sacrificed serially by decapitation at 2 hours, 5 hours, 24 hours and 3 days after administration of the labeled histidine. In one of these groups 12 hour and 7 day intervals were also represented. The third group provided animals for study only after 24 and 48 hours.

Discs of skin 0.5 mm in diameter were excised from the anterior back and were fixed at refrigerator temperature for 2 hours in 1% buffered osmic acid solution. They were embedded in Vestopol according to a standard method (3). Pale gold sections were mounted on formvar-covered copper grids and were overlaid by a thin gelled film of Ilford L-4 photographic emulsion by the method of Caro and van Tubergen (4). After exposure times of 1, 2 or 3 months representative preparations were developed in Microdol X, fixed in Kodak Rapid Fixer, and washed in water. They were examined with a Phillips Electron Microscope 75 without further treatment. Multiple low-power electron micrographs were prepared to represent fields selcted at random from one or two sections of each specimen. Radioautograph prints with a final magnification of 5000× were used for counting the numbers of reduced silver grains in the different parts of the epidermis, which could readily be divided into four zones: an outer, more electron-dense, cornified layer; a less dense, inner cornified layer; the stratum granulosum; and the remaining deep half of the epidermis. The limits of the zones were marked on each micrograph and the area of each outlined portion was measured with a planimeter. Each zone was represented by 10–20 micrographs, and counts of the characteristic filamentous structures formed by specific reduction of silver particles permitted an expression of the number of recorded electron emissions relative to a standard micrograph area for each zone. A few reduced silver grains were situated over nuclei; these were not included in the counts. An arbitrary index of the amount of bound histidine isotope in each epidermal zone for each animal has been expressed as the number of counted silver particles per 100 sq cm of micrograph magnified 5000×.

RESULTS

Inasmuch as the findings were similar in the different groups of animals, these will be considered together. Histidine, identified by the presence of reduced silver grains in radioautographs, reached the epidermis rapidly after intraperitoneal injection into newborn mice. Even though its route must have traversed the deep layers of the epidermis, there was a striking localization of bound histidine in the stratum granulosum after two hours (Fig. 1) as was found by Fukuyama, Nakamura and Bernstein using light microscopy (2). Many of the reduced silver foci were superimposed upon keratohyalin granules, indicating a localization of histidine at

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* From the Department of Dermatology, Stanford University School of Medicine, Palo Alto, California.

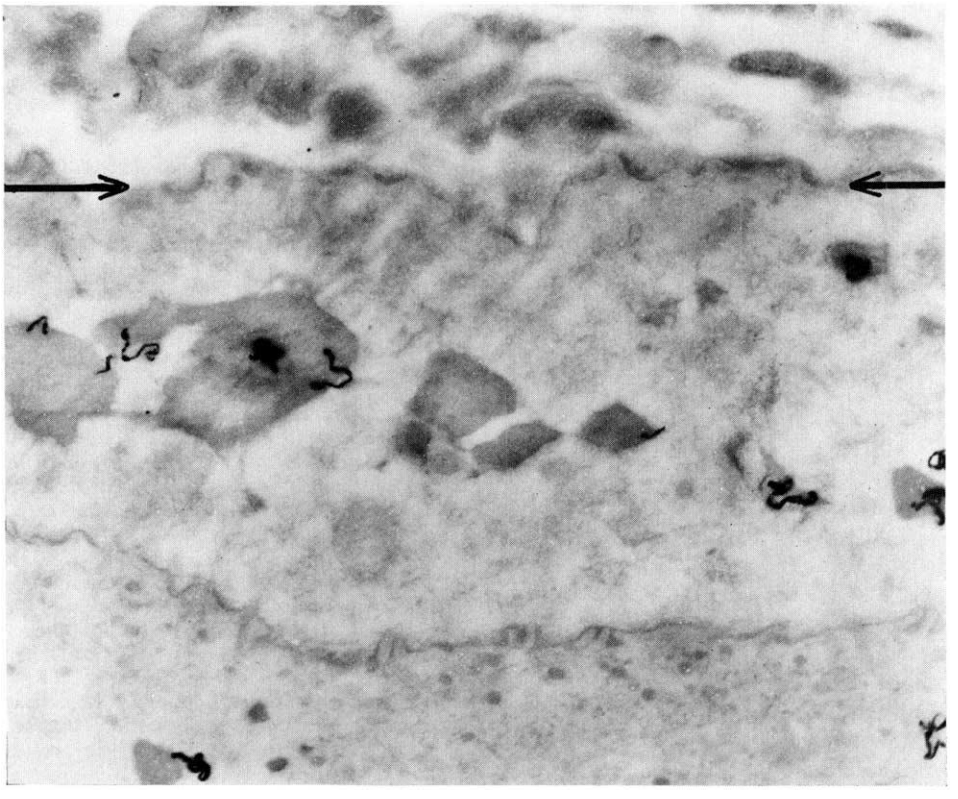

FIG. 1. Radioautograph at the junction (arrows) between stratum granulosum and stratum corneum of newborn mouse epidermis 2 hours after intraperitoneal injection of Histidine-H^3. Portions of 2 cells contain keratohyaline granules (irregular gray bodies), upon some of which there are superimposed filamentous foci of reduced silver. Several silver particles do not correspond to keratohyalin granules, but none are seen in the stratum corneum (above the arrows). (× 8,300)

the sites of these structures. The proportion of silver grains in this location has been found to be more than 50%. Measurements of the volume of the keratohyalin granules proportional to that of other cytoplasmic structures by the point sampling technique of Chalkley (5) have shown a ratio of from 1:8 to 1:33 depending upon which level of the stratum granulosum was being measured. It is apparent from these figures that the attachment of labelled histidine to keratohyalin granules occurred roughly 10 to 20 times more frequently relative to their volume than deposition in the remainder of the cytoplasm of the granular cells.

The administered histidine was bound to both deep and superficial granules of the thick stratum granulosum in the newborn mice. Large granules were as frequently labeled as small granules.

The sequential studies showed changes indicating movement of the bound histidine corresponding to that of the cells of the granular layer as they became transformed into elements of the stratum corneum. Beginning at 5 hours, but most prominently at 24 hours, labeled isotope could be found in increased concentration in the inner transitional portion of the stratum corneum (Fig. 2). The amount in the stratum granulosum diminished progressively after the initial peak at 2 hours. By 3 days the major portion of the label was in the outer portion of the stratum corneum, although radioactivity was still present in the inner portion. At 7 days the latter had disappeared, while some labeled material still persisted in the superficial stratum corneum. Fig. 3 shows the sequential changes in the different epidermal zones as judged by radioautographs.

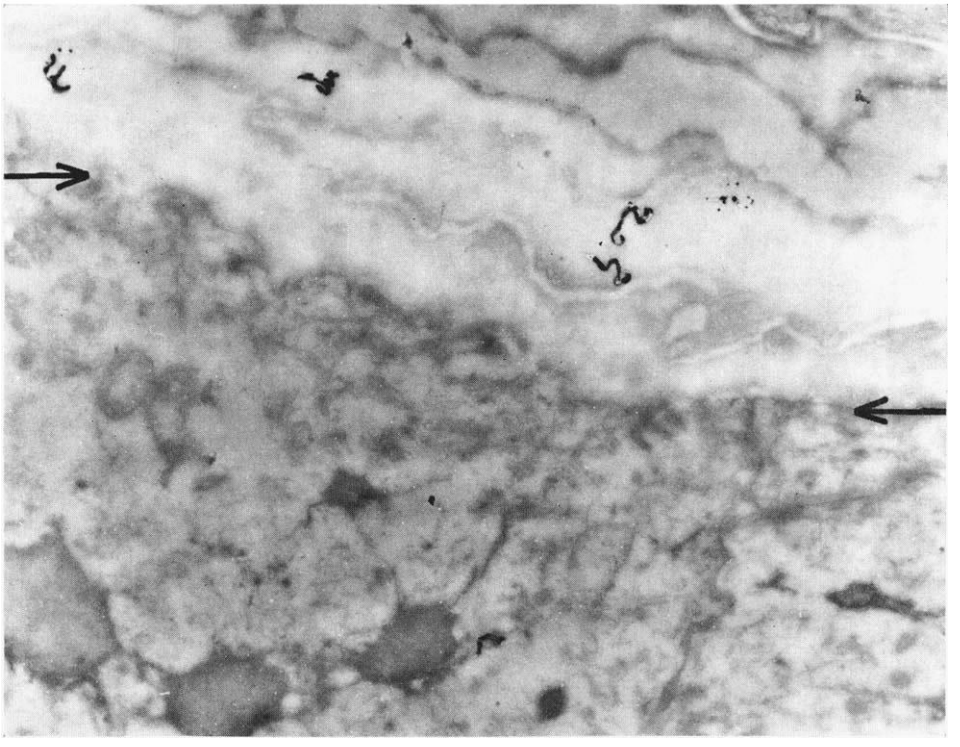

FIG. 2. Radioautograph at the junction (arrows) between stratum granulosum and stratum corneum of newborn mouse epidermis 24 hours after intraperitoneal injection of Histidine-H^3. The radioactive label has now appeared in the inner portion of the stratum corneum, and is decreased within the granular cells (below arrows). Keratohyalin granules are visible at the lower left. (× 8,300)

DISCUSSION

The quantitative data presented in Fig. 3 are subject to some uncertainties inherent in the character of the study. The absolute numbers of counted silver grains have little importance, but comparative differences between epidermal zones of the same animal should be subject to no major error inasmuch as photomicrographs representing the different zones were always obtained from each section that was chosen for quantitative study. Since the sequential changes in the different groups of animals were all of the same character, it is unlikely that they were significantly distorted by the fact that different animals were used for the different observations. The changes shown in Fig. 3 conform to the differences that were apparent on ultramicroscopical inspection of many fields from larger numbers of thin sections.

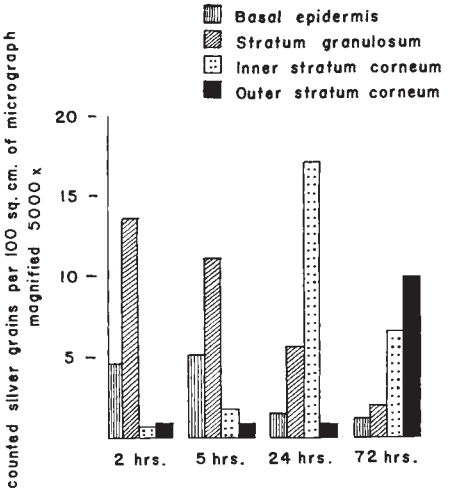

FIG. 3. Sequential changes in relative distribution of radioactivity in newborn mouse epidermis following administration of histidine-H^3 (Series 1).

It should be recognized that the radioactive substance identified in these experiments included only bound histidine or related material that was not extracted by the aqueous media, acetone and Vestopol employed in the prepara-

tion of the ultrasections. That the labeled material bound in the stratum granulosum was unaltered histidine is supported by the fact that chemically recognizable bound histidine is concentrated in the same epidermal zone (1, 6). It does not necessarily follow, however, that the bound labeled material in progressively more superficial parts of the keratinizing epidermis was unchanged histidine. Histidine is transformed to urocanic acid in the epidermis by the enzyme, histidase, which has been found concentrated in the vicinity of the stratum granulosum (7). Whether this enzyme can alter bound histidine, however, is not certain.

The nature of the histidine bond has not been identified by our observations, though the failure to find any pronounced difference in the binding of radioactive material to keratohyalin granules of different sizes or in different levels of the stratum granulosum indicates that the incorporation occurred at different stages of development of the granules, and is consistent with the view that this histidine is not a basic element of the granule structure, but instead has a functional significance. Of importance is the work of Hoober and Bernstein (6) which suggests that much bound histidine in the epidermis may take part in the formation of a unique polypeptide in which glycine and histidine are prominent components. Further understanding of the chemical relationships will doubtless clarify the significance of the histidine binding.

We have not determined whether the binding of radioactive material outside of recognizable keratohyalin granules has any relationship to the presence of labeled molecules in contact with granules. Histidine may have been bound to granules that were too small for identification, or to precursor material. Inasmuch as some deposition occurred in cells of the basal portion of the epidermis, however, it seems probable that a part of the administered histidine was bound in constituents of the cytoplasm not specifically related to keratohyalin granules.

The possibility that a significant amount of bound histidine may be re-utilized after it has been incorporated into keratohyalin granules receives no support from our findings. There was a continued rapid decrease in the amount of isotope localized in the keratohyalin granules as the labeled material progressed outward to the superficial stratum corneum.

SUMMARY AND CONCLUSIONS

Intraperitoneally injected histidine labeled with tritium and followed electron-microscopically in radioautographs of newborn mouse epidermis, passed rapidly into the stratum granulosum, where much of it was bound to keratohyalin granules. Over the next 3 days the bound labeled material moved progressively toward the keratin surface. The findings suggest that keratohyalin granules are not inert structures but possess activity related in some way to the presence of bound histidine.

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