

THE ACTION OF AN EXTRACT OF NAPHTHALAN OIL ON THE
EPIDERMIS OF THE EMBRYO AND ITS EFFECT ON THE
TAKE-UP OF RADIOSULFUR (S^{35} -METHIONINE)

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Investigation of the problem of atypical proliferation of the epithelium and the search for methods of stimulation of wound healing have led to detailed studies, being made at the present time, of the action of exogenous factors of various chemical natures on the epidermis of mammals and man. However, the literature on this subject contains no reference to histological investigations of the reactive changes of the epidermis in response to chemical irritants in the period of embryonic histogenesis. Such investigations are necessary both from the point of view of analysis of the problems which we have mentioned above and of elucidating the biological properties of the normal epidermis.

In the present research, we investigated the reactive changes in the epidermis of white rats in the period from the 17th to the 20th day of intrauterine development.

EXPERIMENTAL METHOD

The irritant used was an alcoholic extract of naphthalan oil which, according to S.K. Rozental' [5], I.I. Kopzon [4] and others, causes the typical picture of hyperkeratosis in adult animals. By means of a specially devised technique (A.K. Dondua and A.A. Zavarzin [2]), we introduced powdered carmine soaked with naphthalan oil into the epidermis of rat embryos on the 17th day of development. The reactive changes in the epidermis were studied 24, 48 and 72 hours after the operation. Material from 30 embryos was studied.

Application of an irritant stimulating the process of keratinization made it necessary not only to study the morphological features of the reactive changes in the epidermis of the embryos, but also to compare them with the changes in the intensity of metabolic processes specifically connected with keratinization. A characteristic feature of the latter is the accumulation in the epithelial cells of proteins containing sulfur.

After administration of amino acids labeled with S^{35} to the animals, their selective accumulation in epithelial cells preparing to undergo keratinization and their subsequent transposition to the stratum corneum was revealed by the method of autoradiography [6, 7].

Thus, by the rate of incorporation of radiosulfur in the various layers of the epidermis, it was possible to judge the intensity of metabolism of sulfur-containing proteins connected with keratinization. Accordingly, female rats, after undergoing the operation, were given injections of methionine, labeled with S^{35} , in a dose of $0.5 \mu\text{C/g}$ body weight at different times before the material was taken for examination (4, 24 and 48 hours).

Besides the ordinary histological preparations, from the fixed material were prepared tracer and contrast autoradiographs on type "P" emulsion, supplied by the Research Institute of Cinematography.

The changes in the epidermis observed in the region of injection of the irritant were compared with the histological structure of the normal epidermis at corresponding stages of development of the embryo and with the intensity of inclusion of radiosulfur in the epidermis.

EXPERIMENTAL RESULTS

The research of Hansen [8] and T.F. Grenberg [1], and also our own findings, have shown that the epidermis of rat embryos on the 17th day of development consists of three layers of cells: basal, intermediate and peridermal. Later on, a thickening of the epidermal layer takes place on account of proliferation of the basal and intermediate cells, and, on the 18th day of development, the latter are arranged in several layers, while in areas of this tissue adjacent to the periderm these cells have a distinctive, flattened shape. In the lower areas of this tissue, prickly cells may already be seen, at this time.

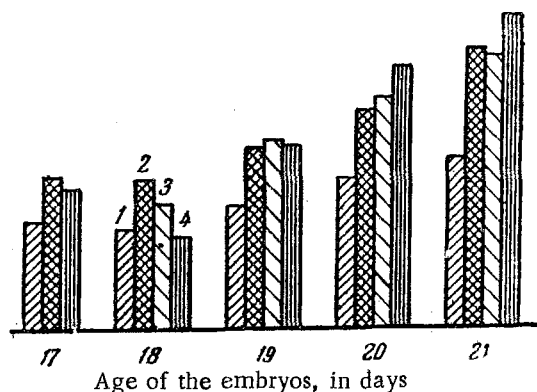


Fig. 1. Intensity of incorporation of S³⁵ in the epidermis and connective tissue. Embryos on the 17th, 18th, 19th, 20th and 21st days of development. 1) Connective tissue; 2) basal layer; 3) prickle-cell layer; 4) superficial granular layer of the epidermis (methionine, labeled with S³⁵, injected 24 hours before fixation).

When the tracks were being counted, for each age, the average number on 1 square of the grid of the ocular micrometer was calculated ($100 \mu^2$ area of section) from 400 such squares on the tracer autoradiographs from two different embryos.

The results obtained are shown in the form of a diagram (Fig. 1). Analysis of these results showed that at the beginning of the period under scrutiny, the intensity of inclusion of S³⁵ was maintained at a comparatively low level in all the layers of the epidermis.

With the appearance in the epidermis of the first signs of keratinization, the intensity of inclusion of S³⁵ increased (in embryos on the 19th day of development by 30%, 20th day by 50-80%, and 21st day by 80-120%), although the intensity of accumulation of S³⁵ in the different layers of the epithelium was not the same. It took place more intensively in the stratum spinosum and stratum granulosum, presumably on account of the increase in the processes of synthesis and the accumulation of sulfur-containing proteins here. A regular connection between the intensity of accumulation of S³⁵ in the epidermis and its differentiation became apparent under these experimental conditions, during artificial stimulation of keratinization.

Injection of the irritant into the thickness of the epithelial layer of the embryo on the 17th day of development caused stimulation of cell proliferation, and only 24 hours later, the thickness of the epithelial layer in the center of the injured area was 3-4 times thicker than the normal epidermis (Fig. 2, 1a). Thickening of the epidermis also took place in neighboring areas. In the hypertrophic epidermis, basal, prickly cell, superficial

The first morphological signs of keratinization appeared in the cytoplasm of the superficial cells in the form of tiny granules of keratohyalin, on the 19th day of development. Later, these cells became filled with large, oxyphilic inclusions, and stood out sharply among the basophilic peridermal and underlying superficial cells.

On the 21st day of development, the epidermis had now acquired all the signs of a multilayered, keratinizing epithelium, and consisted of stratum basale, stratum spinosum, stratum granulosum and stratum corneum.

The morphological observations were compared with the results of a study of the intensity of inclusion of S³⁵ in the stratum basale, stratum spinosum and the superficial stratum granulosum of the developing epidermis. In order to check the reliability of the results, methionine in the same dilution was injected at the same time into female rats at various periods of pregnancy. Sections of the skin of embryos of different ages were placed on the same glass slide.

and peridermal layers were well distinguished. A feature of the traumatized area was the gross thickening of the superficial and prickle-cell layers, and also the border between these cells, which was clearer than normal, and the rounding of the peridermal cells, forming characteristic protrusions into the lumen of the amniotic cavity.

The layer of superficial cells was distinguished in the epidermis by the intensity of staining of its cytoplasm. Among the ordinary cells in the center of the traumatized area were encountered isolated cells, the cytoplasm of which stained with acid dyes. Their appearance gave evidence of the onset of mosaic keratinization of the superficial cells, the full development of which took place in the course of the second day after operation.

In the center of the traumatized area, at a point adjoining the periderm of superficial cells, a layer of keratinizing cells was formed, which gradually thinned out toward the periphery, changing into a layer of keratin scales (Fig. 2, IIa).

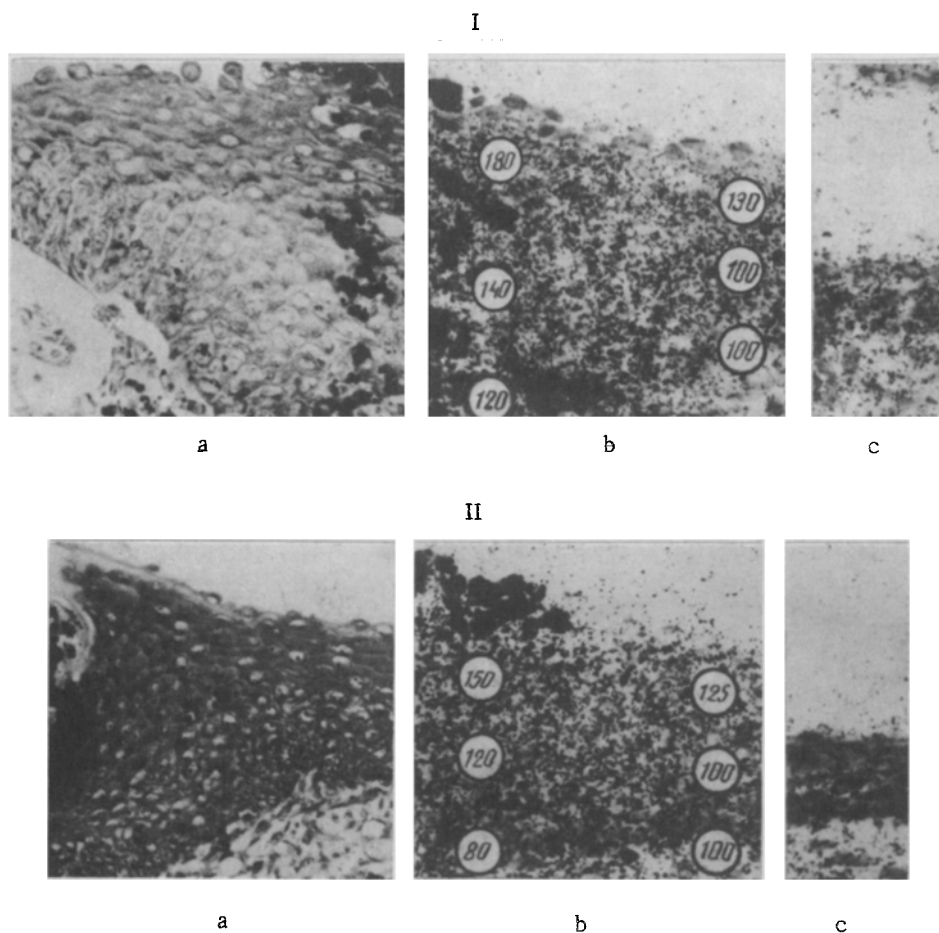


Fig. 2. Changes in the epidermis in the region of administration of extract of naphthalan oil.

I) 24 hours after injection. a) Hypertrophic epidermis in the region of the injury. Stained by eosin-azure. Objective 60. Ocular 5; b) contrast autoradiograph of the hypertrophic epidermis. Methionine, labeled with S^{35} , injected 24 hours before fixation. Objective 20. Ocular 7; c) contrast autoradiograph of the normal epidermis of an embryo on the 17th day of development. II) 48 hours after administration. a) Hypertrophic epidermis in the region of the injury. Stained by eosin-azure. Objective 20. Ocular 7; b) contrast autoradiograph of the hypertrophic epidermis. Methionine, labeled with S^{35} , injected 48 hours before fixation. Objective 20. Ocular 7; c) contrast autoradiograph of the normal epidermis of an embryo on the 18th day of development.

(continued)

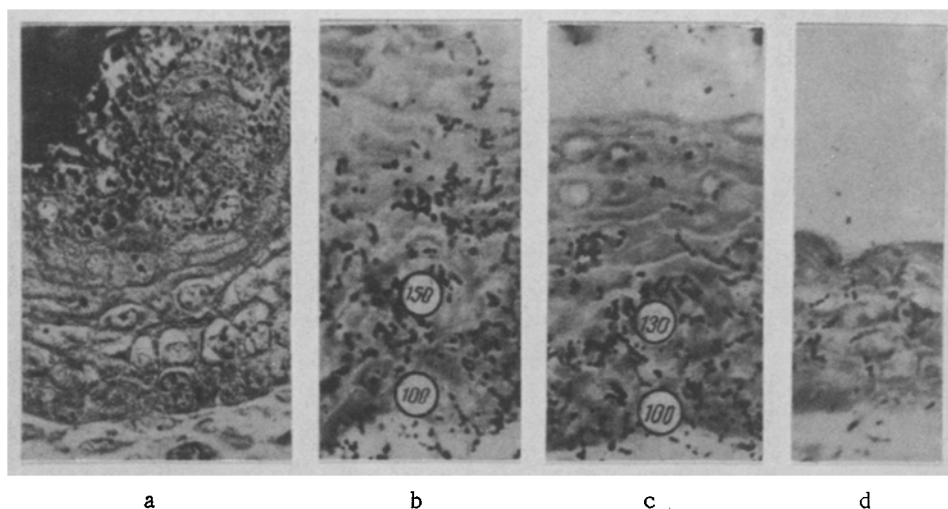


Fig. 2. Changes in the epidermis in the region of administration of extract of naphthalan oil (continued). III) 72 hours after administration. a) Hypertrophic epidermis in the region of the injury. Stained by Heidenhain's hematoxylin. Objective 90. Ocular 7; b) tracer autoradiograph of the hypertrophic epidermis. Stained by eosin-azure. Methionine, labeled with S^{35} , injected 4 hours before fixation. Objective 90. Ocular 7; c) tracer autoradiograph of the normal epidermis of an embryo on the 19th day of development. Methionine, labeled with S^{35} , injected 4 hours before fixation. Objective 90. Ocular 7; d) contrast autoradiograph of the normal epidermis of an embryo on the 19th day of development.

A study of the intensity of incorporation of radiosulfur in the different layers of the traumatized epithelium in the course of the first 48 hours after injection of the oil provided a much clearer explanation of the morphological findings.

The results of the track counts on serial autoradiographs are shown in the form of the appropriate numbers on the microphotographs of the contrast autoradiographs (Fig. 2, Ib, and IIb). Each of the numbers given indicates the relative amount of incorporated radiosulfur as a percentage of its amount in the corresponding layer of the normal epidermis.

Analysis of these results shows that the greatest changes in the intensity of incorporation of radiosulfur, both as regards amount taken up and area of distribution, were observed in the superficial layer of the injured epidermis. 24 hours after operation (Fig. 2, Ib), the intensity of incorporation of radiosulfur was 80% above normal in the central areas and 30% above normal in the peripheral areas of the traumatized area, and after 48 hours (Fig. 2, IIb) – 50 and 25% above the level of accumulation of radiosulfur in the superficial cells of the normal epidermis.

At the end of the second day after injection of the irritant, there was a sharp fall in the power of the superficial cells of the directly injured epidermis to accumulate radiosulfur (80% below normal when methionine was given 4 hours before fixation of the material). In the superficial cells of the peripheral areas of the hypertrophied epidermis, the intensity of incorporation of radiosulfur at the end of the second day was still 25% above normal.

In spite of the considerable increase in the number of cells in the stratum spinosum of the traumatized epidermis, the increase in the intensity of incorporation of radiosulfur in these cells was very slight and was confined to the area directly adjoining the foreign body. For instance, 24 hours after operation (Fig. 2, Ib) the take-up was increased by 40%, and after 48 hours (Fig. 2, IIb) by 20%. Only at the end of the second day was an increase observed in the incorporation of radiosulfur in the prickle-cells of the peripheral areas of the hypertrophied epidermis (25% above normal when methionine was given 4 hours before fixation of the material).

In the course of the third day after operation, the changes described above in the superficial layer usually came to an end, and processes of differentiation of the cells of the injured epidermis into succeeding generations came into the foreground. In typical cases, a layer of normal granular cells was formed here (Fig. 2, IIIa) and the injured epidermis of the embryo on the 20th day of development became similar in structure to the epidermis of embryos on the 21st day of development.

An autoradiographic examination of the changes in the traumatized epidermis at this stage revealed that (Fig. 2, IIIb), when methionine was given 4 hours before fixation, the take-up of radiosulfur by the prickle cells was observed to increase by 50% in the center of the traumatized area and by 30% in the peripheral portion.

A similar relationship was found in the intensity of incorporation of radiosulfur by the uppermost prickle cells preparing to undergo keratinization, when methionine was given 24 hours before fixation (40% above normal). This intensification of the metabolism of sulfur-containing proteins in the prickle cells of the injured epidermis agreed with the morphological findings and was characteristic of an intensively keratinizing epithelium.

It follows from a comparison of the morphological and autoradiographic findings that the reactive changes in the epidermis in the period under review were connected with a considerable increase in the volume of cellular material in the traumatized area and with fundamental changes in the morphological and biochemical differentiation of the superficial layer of the epidermis. Under these circumstances, there was not only an acceleration of the process of keratinization, but an increase in its intensity on account of the larger number of cells taking part in the process.

The increase of 50-80% above normal in the incorporation of S^{35} , observed in the traumatized epidermis, cannot, evidently, be regarded as an indication of the abnormal intracellular metabolism of sulfur-containing proteins.

When these results are analyzed, it has to be remembered that, in the course of the normal histogenesis of the epithelium an increase in the incorporation of radiosulfur takes place on the 19th-21st day, especially in the prickle cell and superficial layers.

The traumatized area of the epidermis corresponded in its structure to the epidermis of later embryos which had not been exposed to the action of an irritant and advanced the development of the epidermis by two or three days. For this reason, the increased level of metabolism of sulfur-containing proteins which was found in the traumatized epidermis could largely be attributed to the acceleration of its biochemical differentiation.

Comparison of all the findings shows that certain general relationships lie at the basis of the normal histogenesis and the reactive changes in the embryonic epidermis. Infliction of trauma, in the main, merely accelerates its normal morphological and biochemical differentiation.

In the course of development of the reactive process, there is, first and foremost, a speeding up of the differentiation of the superficial cells. The biochemical differentiation of the prickle cells in the traumatized epidermis becomes apparent later.

The character of the keratinization of these cells is identical with that of the epidermis at later stages of histogenesis, differing from it only by the slightly greater number of cells taking part in the process of keratinization and by the more rapid conversion of granular cells into keratin scales.

SUMMARY

Experiments were performed on rat's fetuses from the 17th to the 20th day of their development. The author studied the normal histogenesis and reactive changes occurring in the epidermis by injection of an extract of naphthalan oil into it.

Morphological observations were compared with the study of the intensity of S^{35} incorporation during the course of normal histogenesis and development of the reactive process in the epithelium.

The comparison of morphological and autoradiographical data demonstrated a conformity between the initial stage of the keratinization of the stratum and a selective increase of the intensity of S^{35} incorporation in the keratinizing cells and those which are preparatory to keratinization. An analogous regularity has been revealed, experimentally, in artificial stimulation of processes of keratinization in the epidermis. The increase of S^{35} incorporation intensity in the traumatized epithelium does not exceed the level of its accumulation by the normal epidermis of the more mature embryos. A suggestion is made that normal histogenesis and reactive changes of the embryonic epithelium are based on general regularities.

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* See English translation.