AN INVESTIGATION OF THE PHYSIOLOGICAL REGENERATION OF THE EPIDERMIS AND HAIR AT DIFFERENT AGES BY THE METHOD OF AUTORADIOGRAPHY

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The distribution of labeled sulfur in the amino acids of keratinizing and nonkeratinizing epithelia of adult animals and animals in various stages of postnatal development has been determined in research by L. F. Belanger [5], L. N. Zhinkin [1, 2] and L. I. Chekulaeva [3, 4]. The most intensive accumulation of radioactive sulfur S^{35} in keratinizing epithelium during the first few hours after injection of the isotope was observed in the stratum spinosum, less so in the stratum basale and the stratum granulosum, and a quite negligible amount in the stratum corneum. The character of the distribution is connected with the formation of keratin and with the further keratinization of the cells. The cells of the stratum spinosum, labeled with radioactive sulfur, were transposed into the stratum corneum, still retaining their radiosulfur, which enabled the intensity of physiological regeneration to be judged by the rate of replacement of the stratum corneum. On this fact was based the study of the rate of physiological regeneration of the various components of stratified epithelium, differing in their structure. In the epithelium of the dorsal skin of the rat, for instance, it amounted to 3.3μ, and in the skin of the sole about 10μ per day. In the nonkeratinizing epithelium of the cornea, soon after administration of methionine the maximum inclusion of S^{35} was found in the basal cells, and it diminished towards the surface, thus reflecting the general level of protein metabolism. In this case too, by transposition of the cells it was established that the two superficial layers are replaced in the course of 48 hours [3].

These findings served as the basis for the study of the rate of physiological regeneration of the epithelium of the dorsal skin of rats at various stages of postnatal development, depending on the special features of the structure of the epidermis at different ages.

TABLE 1

The total Thickness of the Epidermis and of Its Individual Strata in Rats at Various Ages

Age of rats	Thickness of strata, μ			
	total	germinativum	granulosum	corneum
Newborn	65	22	20	23
5-days old	60	20	10	30
10-days old	52	14	8	30
20-days old	30 – 32	14	4 – 6	12
30-days old	34	20	4	6 – 10

TABLE 2

Number of S^{35} Tracks on the Epithelium of the Dorsal Skin of a Newborn Rat in Autoradiographs Exposed for 24 Hours

Time since injection of S^{35}	Stratum			
	basale	spinosum	granulosum	corneum
2 hours	0.44	0.6	0.34	0.02
24 hours	0.5	0.64	0.26	0.12
3 days	0.36	0.46	0.32	0.32

TABLE 3

Number of S^{35} Tracks on the Epithelium of the Dorsal Skin of 5- and 10-Day Old Rats in Autoradiographs Exposed for 24 Hours

Time since injection of S^{35}	Stratum							
	basale		spinosum		granulosum		corneum	
	Rats							
	5-days old	10-days old	5-days old	10-days old	5-days old	10-days old	5-days old	10-days old
1 hour	0.64	0.58	0.94	0.80	0.26	0.22	0.02	0.08
24 hours	0.7	0.6	1.26	0.84	0.74	0.54	0.18	0.22
3 days	0.58	0.46	0.64	0.66	0.48	0.84	0.22	0.62
5 days	0.36	—	0.34	—	0.66	—	0.86	—

EXPERIMENTAL METHOD

Fifty white rats were used in the experiments. The structural features of the dorsal skin were studied by the ordinary histological methods. The rate of physiological regeneration of newborn, 5- and 10-day old rats was investigated by the method of autoradiography. The experimental rats were injected subcutaneously with methionine, labeled with radioactive sulfur, in a dose of 0.5 μC/g body weight. The material was fixed at intervals of 1, 2, 4, 6, 12 and 24 hours, and 2, 3, 5 and 10 days after injection of the radioactive preparation. Autoradiography was carried out on a type P emulsion produced by the Research Institute of Cinephotography.

EXPERIMENTAL RESULTS

The epidermis of the dorsal skin of newborn rats within a few days of birth consisted of well developed strata germinativum, granulosum and corneum (Table 1). A characteristic feature of the epidermis at this period was the structure of the stratum granulosum, which was distinguished by the thickness and the morphological features of the cells of which it was formed. This layer accounted for about $\frac{1}{3}$ of the thickness of the epidermis. Its cells, arranged in 4-5 rows, were flattened and contained granules of keratohyalin, which differed in their staining, size and number. The staining of the rows of cells bordering on the stratum corneum was lighter. In their cytoplasm were found oxyphil granules, the number and size of which increased as the stratum corneum was approached. The stratum corneum of the epidermis of newborn rats was formed of keratinous scales, closely packed against each other; their borders could not be demonstrated by the usual staining methods.

In 5-day old rats the epithelial layer had become somewhat thinner. The proportions of the individual layers were changed (see Table 1). In addition, the number of rows of cells in each layer was also changed. In the stratum spinosum, for instance, one row of cells was usually found instead of two or three in the newborn rats,

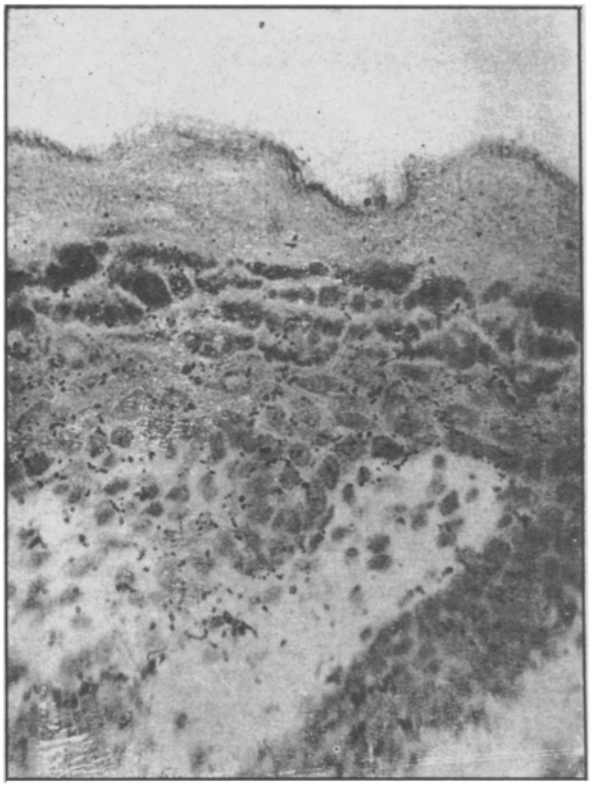

Fig. 1. Tracking autoradiograph of the epithelium of the dorsal skin of a newborn rat 2 hours after injection of methionine.
Staining by eosin-azure. Exposure of emulsion – 5 days. Magnification 420 ×.

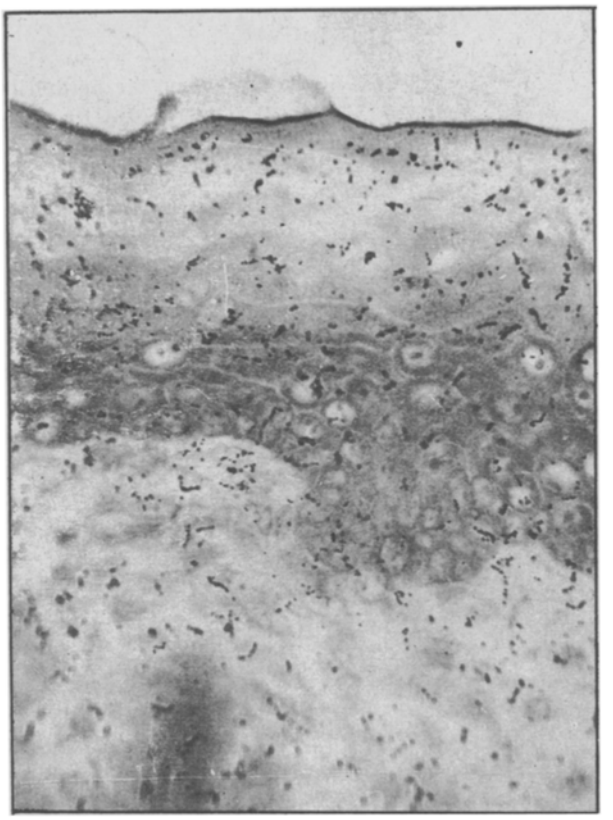

Fig. 2. Tracking autoradiograph of the epithelium of the dorsal skin 3 days after injection of methionine to a newborn rat.
Staining by eosin-azure. Exposure of emulsion – 5 days. Magnification 420 ×.

in the stratum granulosum – 2-3 rows, with complete disappearance of the cells with oxyphil granules, and the stratum corneum was slightly thickened. At the same time the skin contained only rudiments of hair, and their was no external covering of hair.

In the subsequent stages characteristic changes arose in the dorsal skin, associated with development of the hair. On the 10th day the total thickness of the epithelial layer was still further reduced, on account of thinning of all the strata of the epidermis except the corneum, which kept its original dimensions. Later, on the 20th and 30th days of life, in association with the development of a covering of hair the thickness of the epithelial integument continued to diminish, mainly on account of the stratum corneum (see Table 1).

Hence, during 20 days of postnatal development of the rat, the dorsal skin showed a gradual reduction in the thickness of its epithelial layers, on account of a decrease in the number of rows of cells in each stratum. In addition a change took place in the shape of the cells and they were reduced in size. The basophilia of the cytoplasm of the cells of the stratum basale was decreased. The nuclei were more lightly stained. Furthermore, because of the development of the covering of hair, the stratum corneum became thicker.

We studied the physiological regeneration of the epidermis at successive stages of development in newborn, 5- and 10-day old rats. In the newborn rats 2 hours after injection of radioactive methionine, serial autoradiographs showed the greatest intensity of inclusion of S^{35} by the cells of the stratum spinosum. A slightly less intense accumulation was found in the stratum basale and stratum granulosum (Fig. 1).

It must be pointed out that the radioactive sulfur content of the stratum granulosum of the epidermis of newborn rats was significantly higher than in adult animals. In the stratum corneum, radioactive sulfur was found only in areas bordering on the granular cells. After 24 hours and 2-3 days, the intensity of inclusion of S^{35} in the stratum germinativum showed no visible change. However, the S^{35} content of the stratum corneum was grossly

Fig. 3. Tracking autoradiograph of the epithelium of the dorsal skin of a 10-day old rat one hour after injection of methionine.
Staining by eosin-azure. Exposure of emulsion – 5 days. Magnification 420 ×.

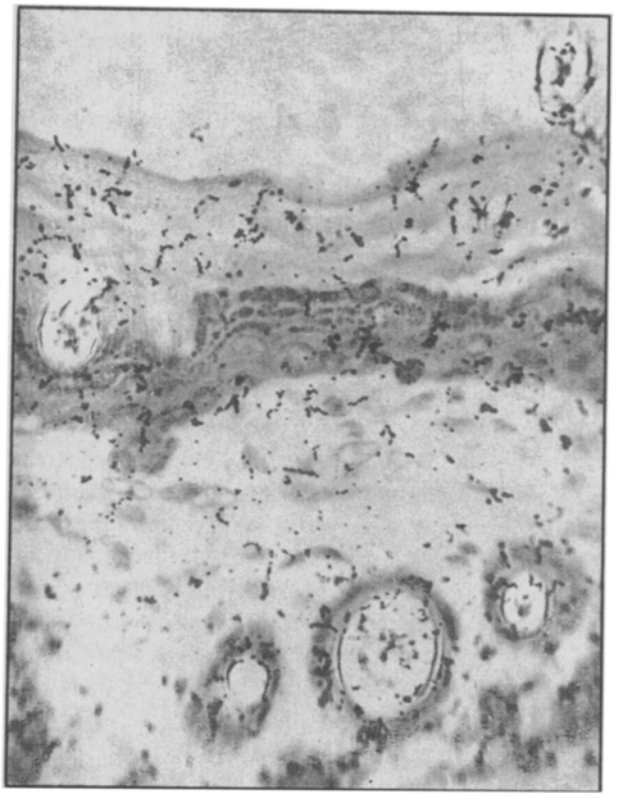

Fig. 4. Tracking autoradiograph of the epithelium of the dorsal skin 3 days after injection of methionine to a 10-day old rat.
Staining by eosin-azure. Exposure of emulsion – 5 days. Magnification 420 ×.

altered. In the animals killed 3 days after injection of the isotope, for instance, the stratum corneum as a whole was now filled with radioactive sulfur (Fig. 2, Table 2) on account of its replacement by cells of the strata spinosum and granulosum and the desquamation of old keratinous scales.

From the results obtained it might be considered that the whole of the stratum corneum was replaced in the course of 3 days. Its thickness in newborn rats was 23-25 μ, hence the rats of its replacement was about 8 μ in 24 hours.

In 5- and 10-day old rats the character of distribution of radioactive sulfur was the same as in the newborn animals. One hour after injection of radioactive methionine, these rats also showed selective accumulation of sulfur in the stratum spinosum of the epidermis (Fig. 3, Table 3), for its content in the strata basale and granulosum was smaller. In the stratum corneum, at the border with the stratum granulosum, there were isolated traces of radioactive sulfur. When 6, 12 and 24 hours had elapsed after the injection of methionine, an insignificant accumulation of S^{35} took place in all the strata. In 5-day old rats, 3 hours after injection of methionine, the S^{35} was transposed into the stratum corneum, filling it completely after 5 days (Table 3). The thickness of the stratum cornuem of the epidermis in our preparations was 22 μ. It took 5 days for it to be filled with S^{35} tracks; hence the average rate of replacement of the stratum corneum was 4.4 μ per day. In 10-day old rats the stratum corneum filled with radioactive sulfur in 3 days, with an average rate of 4.9 μ per day (see Table 3, Fig. 4).

Besides studying the replacement of the stratum corneum, on the same preparations and at the same time we studied the rate of growth of the hair. In the first few hours after injection of methionine, S^{35} accumulated intensively in the keratogenous zone of the developing hairs, whereas the overlying areas did not contain any radioactive label. Later, as the growth of the hair proceeded, the area labelled with radiosulfur moved along the length of the hair, which enabled the rate of its growth to be calculated. For instance, in the first 3 days

after birth the rate of growth of the dorsal hairs was on the average 150 μ per day. In the period from the 6th to the 8th day, i.e. at the moment of emergence of the hair on to the skin surface, the rate was more than doubled. On the 10th day the intensity of growth reached the charactertistic value for the adult animal (i.e. 500 μ per day).

In comparing the results of all these experiments it must be borne in mind that at different stages of postnatal development the thickness of the stratum germinativum and also of the stratum corneum are not the same. Correspondingly, the rate of replacement of the stratum corneum, by which we judge the intensity of physiological regeneration, is different. Physiological regeneration of the epidermis takes place most rapidly of all in newborn rats, and it gradually slows with age. This fall in the rate of physiological regeneration coincides with changes in the structure of the epithlium itself and with the development of a covering of hair. A converse relationship is observed in the development of the dorsal hair: this grows most slowly in young animals and three times quicker in adult animals.

SUMMARY

The authors studied the physiological regeneration of skin epidermis on the back and of the hair at the early stages of postnatal development (in newborns and 5-10-day old animals) by the autoradiographic method, by the movement of the cells with incorporated radiosulphur in the epithelial stratum and the shaft of the hair. Different velocity of physiological regeneration of the epithelium was established: in newborns it equals about 8 μ per 24 hours and in 5-10-day-old animals – 5 μ. Beginning from the 6th day of life the velocity of physiological regeneration of the epithelium drops, which corresponds to the decrease of the thickness of the horny layer and hair growth. On the contrary, an increase of the growth velocity is noted in the hair after birth. Thus, in the newborn rats the velocity of growth equals 150 μ per 24 hours, while in 10-day-old rats it averaged 500 μ.

The intensity of protein metabolism in various layers of the epithelial stratum was studied simultaneously.

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** See English translation.