

THE RATE OF PHYSIOLOGICAL REGENERATION OF AVIAN EPIDERMIS

(UDC 591.169.2:591.176]-082)

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Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 61, No. 3, pp. 96-99, March, 1966

Original article submitted November 18, 1964

The research work of a number of authors [1, 6, 7] had demonstrated a connection between the keratinization of mammalian skin epithelium, the incorporation of radioactive sulfur, and the subsequent translocation of the isotope in the epithelium. When methionine- S^{35} was injected, labelled cells were formed which, as keratinization advanced, completely filled up the horny layer. The rate of translocation of the cells corresponded with the rate of physiological regeneration and varied in different parts of the body [2, 3, 5].

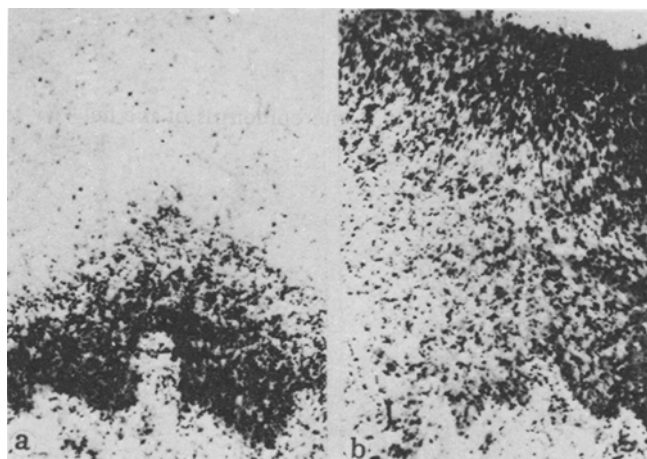
In order to study the rate of physiological regeneration of the epidermis in adult birds and to determine the mutual relationships in keratinization, we investigated the process of distribution of methionine- S^{35} injected into the hen.

EXPERIMENTAL METHODS

Pieces of epithelium, differing in structure and functional importance, served as experimental objects and were taken from the pad on the sole of the foot, the posterior side of the tarsometatarsus, and from the skin of the head. Radioactive methionine was injected under the skin in the axilla of the hen at a dose of 0.6 microcurie/g weight. The pieces of skin were fixed in Carnoy's fluid for two hours, 24 h, five or ten days after injection of the isotope, and then subjected to the usual histological treatment and embedding in paraffin wax. The uptake of methionine- S^{35} was studied by autoradiography. A photoemulsion type P, prepared by the Scientific Research Cine-photo Institute, was laid on the sections which were 7 μ thick. The tracked autographs were obtained from exposures of five to seven days and then stained with hemalum-eosin. A quantitative analysis of the uptake of radioactive sulfur was carried out by counting the number of tracks present in 200 squares of a squared microscope eyepiece and finding the average number of tracks per square (area of each square was 100 μ^2). Contrast autographs were obtained by exposing for 40 days.

EXPERIMENTAL RESULTS

The epithelium of the foot pad, which is continually subject to mechanical action, had a robust, horny layer 150-400 μ thick. The connective tissue grew into the epithelium in the form of papillae, the dimensions of which varied from 50-190 μ . The results of studying contrast autographs of the epidermis of the foot pad showed that, two hours after the injection of methionine- S^{35} , a most intensive blackening of the photoemulsion was observed in the prickly cell layer, less in the basal layer, and still less in the granular layer. There were almost no tracks in the horny layer. On the tracked autographs a similar picture of the distribution of S^{35} in the layers was seen. After one day the character of the distribution of radioactive sulfur had altered a little; in the basal layer there were fewer tracks than in the granular layer, the maximum number being found, as before, in the prickly cell layer. In tissues fixed five days after the injection of the isotope, radioactive sulfur was concentrated in the first half of the horny layer and after a further five days it was in the second half (see figure). However, in a few places in the skin of the foot pad, ten days after injection, the surface layer of keratinized cells (thickness up to 10 μ) was free from tracks. The table illustrates the process of translocation of labelled cells from the growing zone into the horny layer. For convenience in counting the tracks, this layer has been divided accordingly into three parts: - I) boundary layer with granules; II) middle layer; III) surface layer.



Tracked autograph of the epidermis of the foot pad of the bird (seven days exposure). a) Two hours after injection of methionine; b) ten days after. Microphotographs. Obj. $\times 20$, Oc. $\times 10$.

TABLE 1. Number of Tracks of S^{35} in the Epithelium of the Skin of the Foot Pad (Autographs exposed five days)

Period after injection of methionine- S^{35}	Layer					
	basal	prick-ly cell	gran-ular	horny		
				I	II	III
2 hours	1,70	3,61	1,38	0,30	—	—
24 »	0,96	2,12	1,40	0,61	0,02	—
5 days	0,61	0,84	1,72	1,95	1,02	—
10 »	0,56	0,76	1,78	1,90	2,63	4,10

TABLE 2. Number of Tracks of S^{35} in the Epidermis on the Back of the Tarsometatarsus (Autographs exposed five days)

Period after injection of methionine- S^{35}	Layer					
	basal	prick-ly cell	gran-ular	horny		
				I	II	III
2 hours	1,02	1,54	1,06	0,2	—	—
24 »	0,70	1,48	1,80	0,68	—	—
5 days	0,39	0,65	0,99	1,12	0,13	—
10 »	0,22	0,41	0,41	1,30	0,2	—

The thickness of the epithelial layer on the back of the tarsometatarsus attains a thickness of 220μ . The thickness of the horny layer of the scute is from 90 to 180μ , but on the outer side, under the edge, it was 73μ . Two hours after injection of methionine the maximum uptake took place in the prickly cells, as in the epidermis of the foot pad. In the basal and granular layers the amounts of radioactive sulfur was similar. The horny layer, consisting of dead cells, was free from tracks. A day after injection a very high level of S^{35} was recorded in the granular layer, and the intensity of uptake of the isotope in the first part of the horny layer increased. Five to ten days after injection of the isotope, the maximum accumulation of tracks was seen in the lower third of the horny layer. The results of the counts of the numbers of tracks in the epithelial layers of the tarsometatarsus are given in Table 2.

The epithelium of the skin of the head forms a layer about 24μ thick. The keratinization of the cells takes the form of elongated, exfoliating scales. The thickness of the horny layer is $12-15 \mu$. In contrast in autography of the skin of the head, excised two hours after injection of methionine, there was a gradual blackening of the emulsion over the epithelium in the region of the feather papillae. In the spaces between the feathers the maximum accumulation of radioactive sulfur was recorded in the first 24 h, and five days after injection it was observed in the middle layer of the epidermis. On counting the tracks in the autographs (exposure two hours) it was shown that in one square of the squared eyepiece there were 1.1 tracks in the prickly cell layer, 0.84 in the basal layer and 0.2 in the horny layer. After 24 h the number of tracks in the basal layer had decreased, but in the horny layer it had doubled. After ten days the number of tracks in all layers was less than that found in previous counts.

On comparing the intensity of uptake of radioactive sulfur into the epidermis of the selected pieces of skin of any one hen examined, it appeared that the epithelium of the foot pad incorporated more S^{35} than the epidermis of

either the posterior side of the tarsometatarsus or the skin of the head. On counting the tracks in the autographs two hours after injection of methionine, 0.84 tracks were found in the basal layer of the epithelium of the head, 1.02 in the corresponding layer of the epidermis of the tarsometatarsus and 1.70 in the foot pad. The maximum accumulation of S^{35} was recorded in the prickly cell layer, while in the epidermis of the head and in the scutes of the tarsometatarsus there were, respectively, one third and one half as many as in the foot pad.

On comparing the rate of translocation of the radioactive, labelled cells it may be seen that this also varied and corresponded with the mitotic activity of the cells [4]. The epidermis of the foot pad has a high degree of proliferation; the average rate of translocation amounted to 17-20 μ per day and, therefore, 10 $\frac{1}{2}$ days were required for replacing the horny layer which was 178-200 μ thick. In the epidermis of the head, which has a weak mitotic activity, the horny layer with a thickness of about 15 μ was renewed in two days at the rate of 7.6 μ per day. The rate of physiological regeneration in the epithelium of the tarsometatarsus varied in different parts. The horny layer of the outer scute, with a thickness of 90-110 μ , was filled with tracks at 2-3.4 μ per day. In the lateral scute, in which the horny layer was 115 μ thick, the rate of translocation of labelled cells was 6 μ per day. The whole horny layer in this site could be replaced, evidently, in 19 days, but the horny layer under the scute required 12 days for regeneration.

A comparison of the results of the present work with earlier investigations on the dynamics of the incorporation of methionine- S^{35} into the epidermis of embryos and young chicks showed that the character of the distribution of radioactive sulfur was similar in birds of different ages [5]. However, the intensity of uptake of isotope and also the rate of renewal of the horny layer varied in the different layers (in the epidermis of embryos it was considerably higher). So, with the same rate of translocation of labelled cells per day, the horny layer of the scutes on the tarsometatarsus of the hen was replaced two to three times slower than in the same, much thinner, layer of the epithelium of the chick.

Another relationship was recorded in the foot pad and the skin of the head; the rate of regeneration increased corresponding to the increase in thickness of the horny layer with age. It appeared that, with a higher rate of physiological regeneration per day in the foot pad of the hen, the whole horny layer was changed somewhat more slowly than in the chick. The horny layer of the epidermis of the head of the hen and chick was renewed in two days.

SUMMARY

For studying the rate of physiological regeneration in various parts of avian epidermis, differing in structure and function, methionine- S^{35} was injected into hens. The maximum incorporation of S^{35} was recorded in the prickly cell layer a few hours after injection and in the horny layer after several days. The rate of translocation of labelled cells in the horny layer was not the same in the different parts of the epidermis; the rate in the horny layer of the foot pad was 17-20 μ per day, in the skin of the head 7.6 μ and in the scutes of the tarsometatarsus 2-3.4 μ . The whole horny layer in the foot pad and in the tarsometatarsus of adult hens was renewed more slowly than that in young chicks.

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