UPTAKE OF S^{35}-METHIONINE IN THE EPIDERMIS OF CHICK EMBRYOS AND CHICKS

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By the method of radioautographs [1, 4, 8, 9] it has been shown that the injection of S^{35}-methionine into cells of the mammalian cutaneous epithelium before cornification results in selective uptake of S^{35}. Subsequently the cells containing the isotope became cornified, moved into the stratum corneum, and were shed. The movement of cells containing S^{35} is related to the physiological regeneration of the epithelium, and may give a measure of its rate.

To make a comparison with mammals and to establish the general features of cornification and of physiological regeneration, we made a study of changes in the distribution of S^{35} in birds. The work was carried out on chick embryos at the 17th day of incubation after they had been treated with an indicator [6, 10] which enabled the distribution of S^{35} during differentiation of the cornifying epithelium to be determined; observations were also made on 8-day-old chicks in which physiological regeneration was complete.

EXPERIMENTAL METHOD

For the work we took several portions of epithelium of various structures: the pad of the foot, the dorsal surface of the shank, and the skin of the head. Injections of methionine (1 μC/g body wt.) were given in the embryo through the eggshell into the yolk, while in chickens they were given into the axilla. Portions of skin were fixed in Bouin and in Carnoy, 2, 4, 24, 48, 72, and 78 hr after the injection, while in one embryo fixation was made after 112 hr. The fixed material was given the usual histological treatment, and embedded in paraffin. Radioautographs were made on photographic emulsion type R (made by the Scientific Research Institute of Motion Pictures). Trace autographs were obtained by an exposure of seven days and were stained in hemalum-eosin. The rate of uptake was determined by a count of tracks seen in one square of an ocular micrometer representing 40 μ^2. The mean value was determined from counts on 200 squares. Contrast autographs were obtained by exposure for 42 days.

The epidermis of the sole of a 17-day-old chick embryo is about 80 μ thick. On the outside can be seen a definitive stratum corneum 2-3 μ thick. In contrast autographs, two hours after the injection of S^{35} the emulsion was darker over the middle rows of cells (Fig. 1, a). After 24 hr, isolated labelled cells appeared in the stratum corneum. By the 20th day of incubation the thicknesses of the epidermis and of its cornified layer were 140 and 30-40 μ. Contrast autographs show the greatest uptake of S^{35} over the granular cells and over half the stratum corneum (Fig. 1, b). The cornified layer became completely filled with S^{35} 78 hr after the injection of methionine.

The thickness of the epidermis of the dorsal surface of the shank of 17-day-old embryos is 50-60 μ. Above the surface of the skin there project well-developed scales covered with a plate of stratum corneum 2 μ thick. Just as in the sole, 2 hr after the injection of methionine, radioactive sulphur becomes concentrated in the stratum spinosum, and 24 hr later a maximum rate of uptake is found in the granular layer. Before hatching, the stratum corneum, which has now increased in thickness, is completely filled with the radioactive marker (Fig. 2).

The epithelium of the skin of the head in 17-day-old embryos is covered with a thin layer of stratum corneum 15-16 μ thick. The rudiments of the feathers are well developed. In contrast autographs of the epithelium of the head it was difficult to see over which of the layers the emulsion was most darkened. In the region round a developing feather (where the epithelium surrounds the papilla, particularly in the ceratogenous zone of the quill) S^{35} uptake was greater than in the epidermis between the feathers. In trace autographs the distribution of tracks two hours after injection of the isotope, and at subsequent periods, resembled the picture found in the epidermis of the sole. Table 1

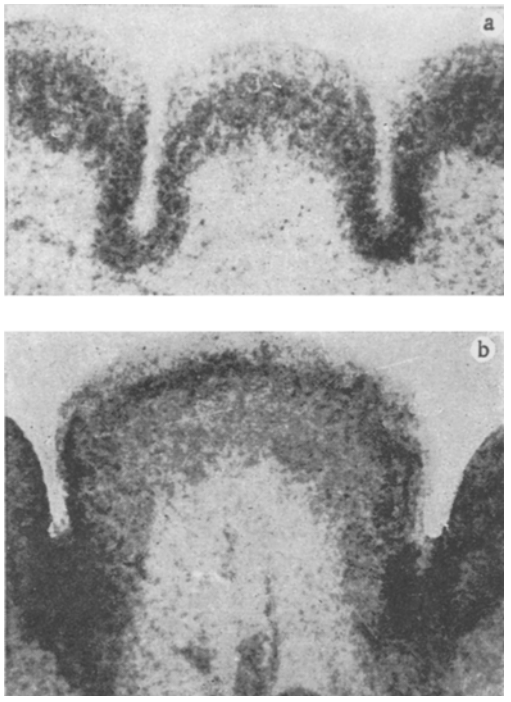

Fig. 1. Contrast autograph of the epidermis of the cell of the sole of an embryo. a) 2 hr after injection (17th day of development); b) 72 hr after the injection (20th day of development). Micrograph. Objective 20 ×, ocular 10 ×.

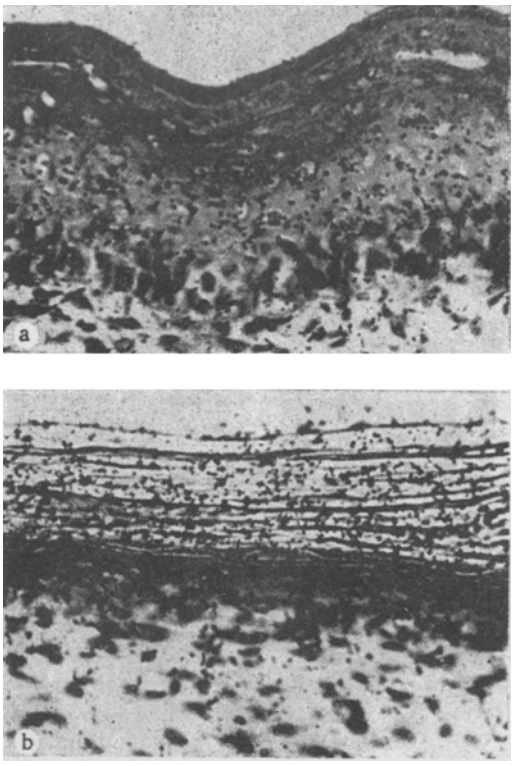

Fig. 2. Trace autograph of the epithelium of the shank of an embryo. a) 2 hr after injection (17th day of development); b) 78 hr after injection (21st day of development). Micrograph. Objective 40 ×, ocular 10 ×.

shows counts of tracks in the epidermis of different parts of embryo skin (exposure of the autographs seven days).

The epidermis of the sole of an 8-day-old chick differs from that of the adult in being thinner, and particularly in having a thinner cornified layer. The stratum germinativum is 70 μ thick, the stratum corneum is almost the same thickness. The thickness of the epithelial layer of the dorsum of the shank of a bird of the same age is 70-90 μ. Chickens fixed 2 hr after the injection of S^{35} show clearly that S^{35} is taken up most intensely into the stratum spinosum. The stratum corneum was divided into three portions for the purpose of counting. Because it consists of dead cells, the very small number of tracks which were found could be attributed to "flight" of electrons. A count of tracks from a chick after exposures to methionine of 24, 48, and 72 hr showed that in the stratum granulosum and stratum corneum there was a continuous increase of the number of tracks, while in the basal layer and stratum spinosum the number fell. Table 2 shows counts of tracks which reflect the movement of the cells containing radioactive marker from the deep layers into the superficial cornified layer of the sole and of the dorsum of the shank.

The epithelium of the skin of the head of the chick reaches a thickness of 17-18 μ in the regions between the feathers. The stratum corneum consists of fine scales. In the first hours after the injection of the isotope an intense uptake of S^{35} in the middle suprabasal layer is observed. In the stratum corneum there are practically no tracks, but 48 hr after the injection of methionine tracks are seen over the whole of the stratum corneum. With an exposure of 72 hr an even greater number are found in this layer.

If we compare the contrast autographs, and also counts of tracks in the epidermis of these selected portions in chicks and embryos it can be seen that S^{35}-methionine is taken up into the cutaneous epithelium of the embryo more intensely than into the epithelium of the chick. When we compare the rates of uptake by corresponding areas of epidermis from the different regions of a single embryo it is found that the greatest amount of S^{35} is taken up by the epithelium of the sole, then follows the epidermis of the dorsum of the shank, while least of all is found in the epithelium of the skin of the head.

TABLE 1. Number of Tracks in the Epidermis of Different Portions of Embryonic Skin

Layer of epithelium	Sole			Dorsum of shank			Skin of head		
	time after injection of S^{35}-methionine (in hr)								
	2	24	78	2	24	78	2	24	78
Basal	0,92	0,57	0,36	0,50	0,36	0,2	0,42	0,22	0,15
Stratum spinosum	1,38	1,01	0,73	1,27	0,60	0,36	0,56	0,43	0,40
Stratum granulosum	0,68	0,91	2,01	0,66	0,82	1,61	0,21	0,47	0,40
Stratum corneum	—	0,40	2,42	—	0,22	2,26	—	0,23	0,53

TABLE 2. Number of Tracks in the Epidermis of Different Parts of the Body of an 8-Day-Old Chick

Layer of epithelium	Sole				Dorsum of shank			
	time after injection of S^{35}-methionine (in hr)							
	2	24	48	72	2	24	48	72
Basal	0,71	0,56	0,38	0,21	0,34	0,28	0,19	0,12
Stratum spinosum	1,12	0,69	0,54	0,42	0,73	0,52	0,30	0,15
Stratum granulosum	0,51	0,95	1,28	0,62	0,37	0,54	0,72	0,46
Stratum corneum I	0,19	0,32	0,34	0,67	0,15	0,22	0,25	0,35
Stratum corneum II	0,04	0,05	0,08	0,22	0,10	0,10	0,09	0,07
Stratum corneum III	—	0,01	0,03	0,07	—	—	0,05	0,03

The quantitative analysis shows that the maximum uptake of S^{35} in the first few hours after the injection occurred in the stratum spinosum, while in the epithelium of the sole there was twice as much as in the epidermis of the skin of the head. In the stratum spinosum of the dorsum of the shank there were rather fewer tracks than in the sole. Thus in embryos killed two hours after the injection of radioactive sulphur there were on average 1.38 tracks, in the corresponding layer of the dorsum of the shank there were 1.27, and for the skin of the head the number was 0.56 track. In chicks given a 2-hour exposure to methionine, the number of tracks in the stratum spinosum of the sole was 1.12, of the epidermis of the side of the shank 0.73, and of the skin of the head 0.45.

In embryos fixed after 24 hours, a more intense uptake of S^{35} was found in the layers undergoing cornification, and a small number in the stratum corneum itself. The cornified layer was completely filled with tracks at the time of hatching (78 hours after the injection). The concentration of S^{35} found first in the granular and then in the cornified layer is related to the transference and cornification of cells from the deeper-lying layers of the epithelium. The greatest amount of S^{35} was found 72 hours after the injection of methionine in half of the cornified layer, and in the epidermis of chicks. From the results of mammalian experiments it is known that the rate of movement of cells which have taken up S^{35} differs in the different portions of the epithelium, and corresponds with the rate of physiological regeneration [2, 3, 5]. Comparison of the rate of movement of S^{35} in the different regions of the epidermis of chicks can, as we see, also be variable. The epithelium of the sole has a thick stratum corneum, and is renewed at a rate of 8.5 μ per 24 hr, while a change of the whole cornified layer 72 μ thick appears to require 8-9 days. The stratum corneum of the skin of the head is 7.5 μ thick and filled with tracks in 48 hr, therefore the amount of stratum corneum replaced per 24 hr is 3.7 μ, while in the epithelium of the dorsum of the shank it is 3.4 μ.

A comparison of the results obtained by radioautographs with those obtained previously [7] by a count of the mitotic activity of the different epithelial regions shows a good agreement for embryos and chicks. In the epidermis of the cell the uptake of S^{35} is maximum, and so is the rate of cornification and of physiological regeneration. At the same stages of development, the epithelium of the sole has the highest mitotic index. Mitotic activity of the dorsum of the shank is less than in the sole, and accordingly the uptake of S^{35} in the incubation and post-incubation periods is less intense than in the epithelium of the sole. The cutaneous epithelium of the head differs in showing a weak uptake of S^{35} in the embryonic and particularly in the post-embryonic period; the stratum corneum is replaced slowly, and the mitotic index is low.

Therefore the features of individual development of the different portions of epidermis correspond to differences in mitotic activity, rate of uptake of radioactive isotope, and unequal rate of physiological regeneration.

SUMMARY

The author studied changes of S^{35}-methionine distribution in various functional regions of the epidermis of 17-day-old chick embryos and 8-day-old chicks. In both, during the first hours after injection, maximum uptake of S^{35} occurred in the middle layers of the epidermis. Next the labelled cells gradually migrated into the stratum corneum. The rate of uptake of radioactive sulphur was not the same for all the regions, being higher the greater the rate of regeneration. In chicks the thickness of the stratum corneum of the epidermis regenerated per day was 8.5 μ, 3.7 μ, and 3.4 μ for the sole, head, and dorsum of the shank respectively; the differences were due to the different mitotic activities of these regions.

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