THE DYNAMICS OF S^{35}- METHIONINE INCLUSION IN THE CUTANEOUS EPITHELIUM OF THE FROG Rana temporaria L.

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Compariosn of the dynamics of S^{35}-Methionine inclusion in noncornifying [6] and cornifying epithelium [3,4, 5,7,8,10] revealed essential differences. In the morphologically similar areas of epithelium of the axolotl there was no direct relationship between cornification and the intensity of inclusion, and there was also no turn over in the tag after 5 days.

The presence of beginning stages of cornification was shown in the salamander, as compared with mammals [9]. In this case, the appearance of a middle layer (Str. intermedium) in the cytoplasm of the cells was noted; this layer consisted of keratin granules, markedly different histochemically from the keratohyalin granules in the granular layer of the mammalian epidermis.

Using S^{35}-methionine, it was of essential interest to study the process of cornification in representatives of amphibia, in which it occurs sufficiently intensely, and undergoes seasonal fluctuations. In this relation, a convenient subject is the epithelium from the leg of male frogs, which undergoes morphological changes connected with the appearance of tubercles in the leg calluses prior to the period of reproduction, and their absence after it. For a comparison, we studied the epithelium of the back, where cornification is less intense, and also the corresponding areas of epithelium from females.

Four series of experiments were performed at varying times of the year, using 80 adult male and female frogs.

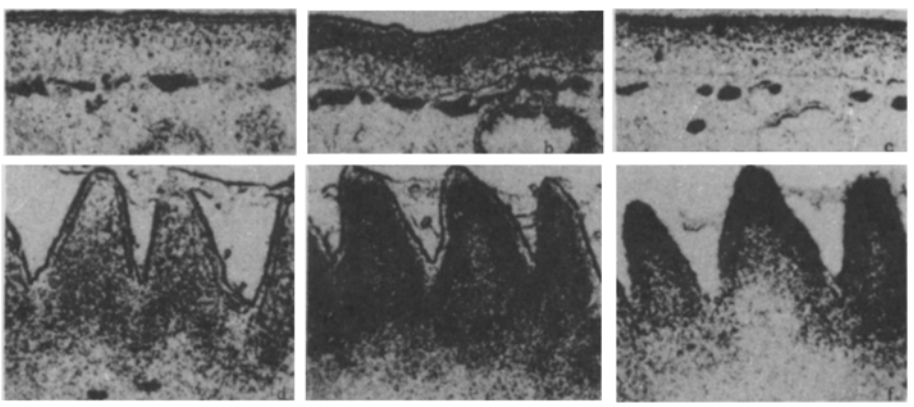

Fig. 1. Contrast autographs (first February series). Exposure of 25 days. a, b, c—epithelium of the back; d, e, f—epithelium of the leg callus; a, d—at 2 hours after administration of S^{35}-methionine; b, e—at 48 hours; c, f—at 72 hours after administration of S^{35}-methionine. Magnification: obj. 15x, ocul. 10x.

The S^{35}-methionine solution was injected intraperitoneally, using 1–0.5 microcuries/gram. The material was fixed after varying intervals (from 2 hours to 5 days) following the injection of the indicator, using Carnoy's Bouin's solution, and was then imbedded in paraffin. Tracing and contrast autographs were obtained on Type R fluid emulsion, released by the Scientific-Research Cinema-Photo Institute. Further treatment of the preparations was carried out according to the method described in a series of works [1–2].

In the winter (experiment performed in February, 1959), at a temperature of 10–12 deg, the epithelium of the leg callus from the males appeared as a heavy layer, thicker than other areas by approximately 3 times. It was unique and complex in structure; the epithelium formed tapered tubercles, consisting of 10–12 rows of cells, and the connective tissue, correspondingly, formed connective tissue papillae. The width of the layer at the sites of tubercles was 203 micra, while between them it was 103.3 micra. The cells of the basal and 1–2 rows of the suprabasal layer had a prismatic form, and were somewhat larger than those in the epithelium of the back; the upper rows of cells had a polygonal form, with round nuclei and oxyphilic cytoplasm. The layer was topped by two cornified disks, consisting of one row of cells in each; the height of the first disk was 3.6 micra, and of the second, 3.3 micra.

The epithelial layer from the back of the same frogs was considerably thinner (63.5 micra), formed by 4–5 rows of cells, the uppermost of which was cornified.

Analysis of the contrast autographs made from the epithelium of the back showed that, starting with the first hours after injection of the indicator, selective accumulation of the S^{35}-methionine was observed in the upper rows of cells of the spiny layer (Fig. 1,a), along with comparatively weak accumulation of it in the basal and suprabasal cells and the cornified disk. These differences were retained up to 48 hours, but the intensity of the accumulation rose considerably (Fig. 1,b).

After 72 hours, an apprecialbe decrease occurred in S^{35}-methionine accumulation, which was apparent from the generalized, weak darkening of the emulsion over the entire layer with the exception of the superficial cornified layer and the 1–2 rows of cells lying beneath it (Fig. 1,c).

Studying the contrast autographs of the epithelium from the leg calluses showed that at 2 hours after administration of S^{35}–methionine the tag was observed as most prevalent in the suprabasal row cells of the spiny layer (Fig. 1,d); the basal and upper rows of the spiny layer incorporated the preparation less intensively. At 5 hours after the S^{35}-methionine administration, anincrease in the intensity of inclusion occurred. After 24 hours, we observed uniform distribution of the tag from the suprabasal to the superficial rows, and after 48 hours (maximum accumulation), the presence of S^{35}-methionine was noted in the uppermost rows of the spiny layer and the first cornified disk (Fig. 1,e). After 72 hours, a generalized decrease occurred in the number of incorporated radioactive sulfur-atoms, but many remained in both cornified disks (Fig. 1 f).

The results of counting the tracks on the tracing autographs from the epithelium of the spine and leg after various intervals following administration of the S^{35}-methionine (2,6,24,48, and 72 hours) are presented graphically in Fig. 2.

These data coincide with the data obtained on the contrast autographs for both areas. Even in the first hours after the S^{35}–methionine administration we observed selective accumulation of the tags in the upper rows of the spiny layer from the back epithelium, and in the suprabasal cells of the epithelium from the leg callus.

The unique distribution of the S^{35}-methionine in the epithelium of the leg callus is apparently related to the high intensity of the processes of cornification that occur here. Up to 48 hours, an increase in the intensity of radioactive sulfur atom incorporation occurs in both areas, while in the epithelium of the leg, up to this time, the larger portion of the S^{35}-methionine is shifted from the suprabasal layer to the upper rows of the spiny layer. The intensity of inclusion in the epithelium of the leg callus (at all intervals of the investigation) was approximately 2 times higher than in the epithelium of the back (see Fig. 2).

The decrease in the amount of S^{35}-methionine within the cornified disks after 72 hours, as compared with the content of tag in the spiny cells after 48 hours, is evidence that a part of the radioactive sulfur stoms incorporated into these cells come back out, the remaining part entering into the composition of the keratin and moving with the cornifying cells.

A repeat series of experiments was performed in February, 1960, under the same conditions.

The data from the second series of experiments differed somewhat from the data of the first series. In the winter frogs, within the tubercle-containing epithelium of the leg callus, the cornified layer appeared as a single cornified disk, 2.5–3 micra in thickness.

Selective accumulation of the tag by the cells of the spiny layer was also demonstrated at 48 hours after its administration, and its predominance in the epithelium of the leg callus by a factor of approximately 2 as compared with the epithelium of the back. In addition, we noted (beginning with 2 hours after injection of the indicator) predominant accumulation of S^{35}-methionine in the upper rows of the spiny layer from the back and the leg callous. The observed relationships were retained up to 120 hours, with gradual decrease of the accumulation and without marked transference of the tag to the cornified layer. This discrepancy is apparently explained by the fact that, in the experiment, we used frogs in which the formation of a new cornified layer did not occur during the investigation intervals, and thus, we did not observe such active movement of the cells from the spiny layer as in the first series of experiments.

Repeat experiments were performed again at the end of December, 1960 and the beginning of January, 1961, at 20–22 deg; the epithelium of the leg callus was noted to be in the process of forming the tubercles, and there was a single cornified disk in the cornified layer. We observed distinct transfer of the S^{35}-methionine from the

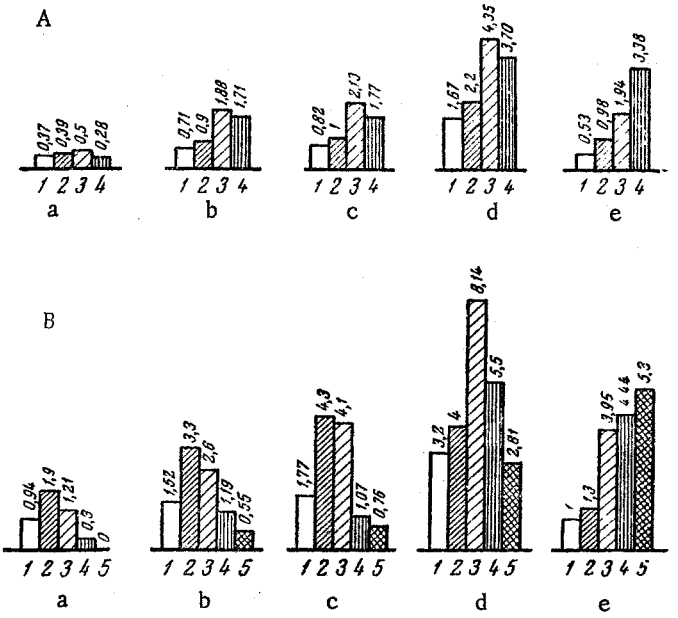

Fig. 2. Diagram of the count made on the S^{35}-methionine tracks in the autographs (first February series). Exposure of 3 days. A–epithelium from the back of the frog; B–epithelium from the leg callus; 1–basal layers; 2–suprabasal layer. 3–upper spiny layer; 4 for A–superficial layer; 4 for B–the first cornified disk; 5–second cornified disk; a–at 2 hours after administration of S^{35}-methionine; b–after 6 hours; c–after 24 hours; d–after 48 hours; e–at 72 hours after administration of S^{35}-methionine.

spiny layer to the cornified layer at 72 hours after administration of the indicator.

At the end of the period of reproduction, the leg callus of the males became smaller, and its surface became smooth; 6–7 cell rows formed the epithelial layer. At the apex of the connective tissue papillae, the thickness of the layer was equal to 64.2 micra; in the areas between them–101.8 micra. The cornified layer was 6.3 micra in height, and formed by one row of cells.

The epithelium of the back was also considerably thinner (37.5 micra), and the size of the cells, smaller. The epithelium taken from the back of females did not essentially differ in structure from the epithelium of the

males, but it was somewhat thicker (44 micra).

At this time, the epithelium from the leg of females was thickened in places (up to 88 micra), and was somewhat similar in its structure to the epithelium from the leg calluses of the males, but differed by the absence of connective tissue papillae and by the smaller number of glands.

Compariosn of the character of S^{35}-methionine inclusion in the epithelium of the males in July (experiment performed at 17–18 deg) and February showed that the intensity of inclusion at varying intervals after the administration was 1½ times greater in the epithelium of the back than in the epithelium of the leg callus (Fig. 3b, e). At 2 hours after the administration of S^{35}-methionine, a certain predominance of the tag in the two lower rows of the spiny layer was noted in the epithelium of the back (Fig. 3a), and after 6 hours (maximum inclusion), and in all the subsequent intervals (24, 48, 72 hours), this predominance was in the upper rows of the spiny layer, with gradual reduction of the inclusion. Thus, in the 72-hour autograph we noted a reduction in the S^{35}-methionine, but, as before, the most intense inclusion was observed in the upper rows of the spiny layer (Fig. 3c).

Beginning with the first hours, inclusion of the S^{35}-methionine in the epithelium of the leg callus was characterized by selective accumulation in 3–4 rows of the upper spiny cells; the basal and suprabasal layers incorporated it less intensively (see Fig. 3d, e). An analogous type of S^{35}-methionine distribution was also preserved in all the subsequent intervals (24, 48, 72 hours), but by 72 hours, the amount of radioactive tag had decreased

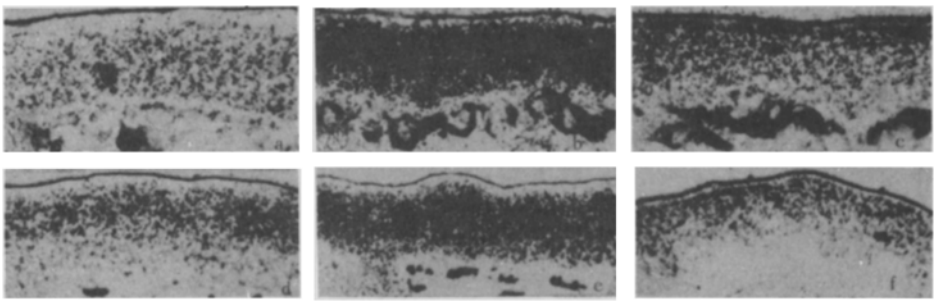

Fig. 3. Contrast autographs (July series). Exposure of 25 days. a, b, c–epithelium of the back (magnification: obj. 20 x, ocul. 15 x); d, e, f–epithelium of the leg callus (magnification: obj. 10 x, ocul. 15 x); a, d–at 2 hours after S^{35}-methionine administration; b, e–after 6 hours; c, f–at 72 hours after S^{35}-methionine administration.

considerably (Fig. 3f), without any noticeable shift of the material. The cornified layer of both the back epithelium and the epithelium of the leg callus appeared in the autographs in the form of a light band with weak S^{35}-methionine inclusion, which increased insignificantly up to 72 hours and is apparently explained by "dispersion of tracks".

In comparing the nature of the S^{35}-methionine distribution in the epithleium of the back and the legs of males and females, we did not discover any essential differences, with the exception of the different rates of the metabolic processes. Thus, in February the maximum inclusion in the females occurred after 24 hours, while in the males–after 48 hours. In July, on the other hand, the maximum inclusion in the females occurred 1–2 days later than in the males, which apparently is related to the varying functional state of the organism.

Thus, studying the process of cornification in the cutaneous epithelium of the frog, using S^{35}-methionine, made it possible to demonstrate that the rate and intensity of inclusion into the epithelium of the frog depends on both external (temperature) and internal (hormonal, etc.) factors.

Comparing the dynamics of S^{35}-methionine inclusion in frogs of the first February series with the dynamics of inclusion into the intensively cornifying epithelium of mammals, one notes selective accumulation of the tag, in both cases, within the cells of the spiny layer, preparing for cornification, and its subsequent shift into the cornified layer.

As has already been noted [6], the shift of the tag into the composition of the cornfied layer, characteristic of mammals, is not observed in the cornifying epithelium of the axolotl, and the inclusion intensity in this epithelium is lower than in the non-cornifying portion.

In comparing the dynamics of S^{35}-methionine inclusion in summer frogs with the inclusion into the epithelium of axolotls, one notes the same relationship as in the axolotls: the intensely cornifying epithelium of the leg callous in the frogs shows weaker inclusion than the weakly cornifying epithelium of the back. Here, as in the epithelium of the axolotls, movement of the tag is not observed.

Thus, the results of the winter and summer series of experiments fail to coincide to a definite degree. On the one hand, the data from the February series of experiments indicate the presence of a direct relationship between the process of cornification and S^{35}-methionine inclusion; on the other hand, in the summer frogs this relationship is not observed. This contradiction may be explained by the fact that cornification does not occur uninterruptedly, but periods of keratin synthesis and cornification of the superficial cells alternate with periods when these processes appear to be markedly inhibited in the layer, or completely absent. In addition, in the different portions of the cutaneous integument, the periods of cornification and the latent state of the layer may not coincide. Based on this point of view, one can explain the results of the experiments with the July frogs as characterizing S^{35}-methionine inclusion in the epithelial layer at a time when it was in the latent state.

The results of the experiments in the second February series, where we did not observe movement of the tag into the composition of the cornified layer, appears to confirm the presence of such periodicity for these processes in frogs.

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