Alkaline phosphatase in the sweat gland of the cattle embryos

By

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Preface

In the previous paper, Yasuda et al (1957) have described on the developmental change of the sweat gland of the cattle embryo, aging from fourth to the eighth fetal month. They reported that the primordia of the sweat glands of the cattle embryos almost appear in the same fetal stage and take the same developmental course as those of the human embryos. They also published the histochemical research on the Gl. cerminosa of the cattle embryo (Yasuda et al, 1959). This study concerned chiefly the activity of the alkaline phosphatase in the primordia of the sweat glands in several regions of the bodies.

Materials and Methods

The most difficult thing that perplexed us was to get fresh materials following the fetal months, covering the whole course of this investigation. The National Institute of Animal Health in Tokyo was kind enough to help us to obtain fresh materials from the Shibaura Slaughter House. The small pieces of the skin specimens from several parts of the bodies were fixed with chilled absolute acetone, four hours after the embryos were excised from the abdomens of the mothers. The specimens were then embedded in paraffin, cut in serial sections of 5μ in thickness and stained with Gomori's revised method for alkaline phosphatase (1952). The eight embryos were used in this study, aging from the beginning of the third fetal month to the end of the eighth fetal
month. The six parts examined were forehead, breast, abdomen, hypogastrium, back and sole. The detailed definition for each body region and the standard points for the judgement of the fetal month were the same as revealed in the previous paper (Yasuda et al., 1957).

**Observations**

Before describing the results in this study, the authors divided the primordia into seven stages, according to the feature obtained from the specimens stained by hematoxylin-eosin staining method.

1. hill-like primordia
2. mammellary primordia
3. globular primordia
4. clavate shaped primordia
5. hook-like primordia
6. hook-like primordia with a cleft
7. the primordia provided with glandular lumen

Though we could not be informed about the kind of the mother cattles, they might have been a Japanese species with brown hair. The fetal month of each specimen is judged by the same standard as mentioned in the previous paper. The course and the condition of the development of the sweat gland primordia are common to all the regions examined, as the authors already reported (Yasuda et al. 1957). But there is a significant difference in the progress of the development. Regarding these differences here, we referred to the previous paper and do not repeat.

In the embryo which is 11.5 cm in body length (middle of the third fetal month), both epidermal cells and the cells of the hair base give feeble activity, while the cells in the connective tissue present intense activity. In this fetal month the primordia of the sweat glands occurred in the sole, back and breast are presumably the most primitive form of the primordia. A group of cells in the hair base demonstrate strong reaction, at an obtuse angle where the hair-rudiment and the epidermis cross. No sign of protrusion of the cell group of the hair base toward the subcutaneous connective tissue is recognized at this stage, when observed the specimens stained with hematoxylin and eosin. The cells of the prospective anlagen of the sweat gland give stronger activity than the surrounding cells of the hair-rudiments. The sweat gland primordia can be easily
found in the hair-rudiments, as the prospective sweat gland cells demonstrate remarkable activity, though the sweat gland primordia do not grow into the corium yet. The most intense activity is recognized in the nuclear and cellular membranes of the sweat gland primordia and the lesser reaction in the cytoplasm and karyoplasm. The connective tissue cells have strong activity in the surroundings of the hair-rudiments, especially near the apical region of them.

The primordia in the next developmental stage revealed themselves at the abdomen and the sole of the same specimen as mentioned above. The hill-like protrusion of a cell group into the corium is recognized in the hair-rudiments at the adjacent part to the epidermis, in the specimens stained with hematoxylin and eosin. The cells of these hill-like primordia react more intensely to the alkaline phosphatase than the cells of the hair-rudiments. The nuclei of the sweat primordial cells show a larger concentration of the enzyme than their cytoplasm which has far stronger reaction than the cytoplasm of the hair-rudiment cells. The connective tissue cells, which lie in a line around both hair-rudiments and hill-like primordia of the sweat gland, contain a greater amount of alkaline phosphatase than the other cells in the connective tissue.

In the mammellary-form primordia, the cells with strong activity increase in number and give rise to the cell groups consist of seven to ten cells. Moreover, these strongly reactive cells are in general located at the center of the primordia, showing opposite figure to the weakly reactive cells in the peripheral part of the primordia. In the cells of the strongly reactive group, the nuclei contain greater amount of enzyme than the cytoplasm, resembling the profiles of the cells in the previous hill-like primordia. As mentioned above, the cytoplasm have so weak reaction that the border line between each cell is not distinctly demonstrated.

In the globular-form primordia, only few cells, numbering three or four, give remarkable enzymatic reaction, unlike the case in the previous developmental stage of the sweat gland. In these cells described above, both nuclei and cytoplasm show intensely reactive alkaline phosphatase. With the gradual approach to the peripheral cell group in the primordia, the reaction of the primordial cells decreases. First of all, the gradual decrease of the activity in the cytoplasm is recognized, in proportion as the distance from the central cell group increases. Next, the fall of the activity in the nuclei follows by degrees. The cytoplasm gives hardly any reaction
at the peripheral extremity of the primordia. The peripherally located primordial cells have as weak an activity as the cells of the hair rudiments. A remarkable activity is recognized in the elliptic-shaped cells which arrange themselves around both the hair rudiment and the sweat gland primordia.

The primordia in the next stage is a little longer than the globular-form and the transitional form primordia from globular shaped to clavate shaped primordia. The apical ampulla is not clearly differentiated from the cell cord, on the morphological point of view. The primordial cells in the elongated cell cord are weakly reactive to alkaline phosphatase, except the nuclear membrane that have a moderate activity. Some cells in the apical ampulla show a larger concentration of the enzyme than the other cells in the same place.

Occasionally, two or three cells with an appreciable amount of enzyme are encountered scattering in the cell cord. These cells appear not only at this stage, but in the previous stages, too, and also in the hair rudiments. These cells are presumably the pigment cells. The connective tissue cells which surround both hair rudiments and sweat gland primordia react more intensely than the cells of either hair or sweat gland primordia.

In the clavate shaped primordia, the cells in the center of the apical ampulla are rich in alkaline phosphatase, in both nuclei and cytoplasm. The enzyme occurs weakly in the cell cords, showing the gradual decrease of its activity in accordance with the move from the ampulla to the cell cord. In the further developed clavate-shaped primordia, both the number of the cells which are strongly reactive to the enzyme and the intensity of the reaction in each cell increase, when compared with those in the preliminary stages. The cells in the elongated cell cord, which contain feeble reactive enzyme at the previous stages, contain moderate amount of enzyme. In occasional primordia, the activity of the cell cord is as strong as that of the apical ampulla.

In the further developed clavate-shaped primordia, the apical ampulla is easily distinguished from the cell cord, as the former gives intense reaction and the latter feeble activity. The cells in the central region of the apical ampulla contain an appreciable amount of alkaline phosphatase, both in nuclei and cytoplasm. The karyoplasm is reactive in the granular pattern and the nuclear membrane is also strongly reactive. The cytoplasm as well as the karyoplasm has strong a activity. The cells in the peripheral region of the apical
ampulla contain a smaller amount of enzyme than those in the central region, especially the trace of enzyme in the cytoplasm. The connective tissue cells which surround both apical ampulla and cell cord show a larger concentration of the enzyme.

In the hook-like primordia, both cytoplasm and nuclei of the cells in the apical ampulla have intense activity. At the neck of the hook-like bending, namely the transitional part from the apical ampulla to the cell cord, the cells demonstrate weaker reaction than those in the ampulla but have a somewhat larger amount of enzyme than those in the cell cord. The cell cord is lined by two layers of cells which may be the prospective cells of the excretory duct. The linear structure with feeble reaction is recognized along the central longitudinal axis of the cell cord. This figure is suggestive of the formation of the secretory lumen in the cell cord. A layer of cells which arrange themselves along the greater curvature of the hook-like primordia exhibits intense reaction. These cells must be the prospective or young myoepithelial cells in the adult sweat gland, and are never clearly encountered along the smaller curvature. The alkaline phosphatase reaction occurs so strongly in the apical ampulla that it is difficult to differentiate the nuclei from the cytoplasm, by Gomori's method. The cleft appears at the central part of the apical ampulla at this stage. The cells with strong reaction are noticed at both sides of the sweat gland primordia which is in the stage of "hook-like primordia with lumen" after skipping over the stage of "hook-like primordia without lumen".

In the next stage, the activity of the cells in the outer region of the apical ampulla becomes weaker, in disregard of the intense activity in the cells of the outermost layer. The cells in the central part of the apical ampulla still maintain strong activity. Occasionally the cells in the central part of the primordia have an inclination to loose enzymatic activity, break down and then disappear.

In the primordia which are provided with secretory lumen, the prospective myoepithelial cells which arrange themselves around the sweat gland primordia have a strong concentration of enzyme, while the prospective glandular cells contain a small amount of enzyme, much less than in their nuclei. Narrow strips of apical cytoplasm of the luminal cells present intense activity, and become narrower in accordance with the developmental course in the perfection of lumen to indicate lineal appearance. The cell group with feeble reaction
is recognized in the lumen in the earlier stages of the development, but they soon disappear to form a perfect lumen.

**Discussion**

There have been several reports on the activity of the phosphatase in the apocrine sweat glands, published by Montagna (1956), Montagna, Chase and Lobitz (1953) and Bunting, Wislocki and Dempsey (1948). According to these descriptions mentioned above, this enzyme occurs diffusely in the cytoplasm of the luminal cells, in greater amount in the cuboidal cells than the flattened cells, and densely in the apical cytoplasm of the columnar luminal cells. The enzyme is found also in the basal cytoplasm and in the myoepithelial cells, but is hardly recognizable in the myoepithelial cells of the dilated secretory tubules. But these findings are noticed in the adult human sweat glands and somewhat different from the pictures obtained in the cattle embryos. In the cattle embryos, the most primitive primordia of the sweat gland are noticeable within the hair-rudiment by the alkaline phosphatase reaction at the beginning of the third foetal month, when the germination of sweat gland primordia from the hair-rudiment has not yet observed in the specimens stained with hematoxylin and eosin (Fig. A-1, A-2, B).

The authors have reported in the previous paper (Yasuda et al, 1957) that the first primordia of sweat gland are recognizable at the forehead, sole and abdomen in the middle of the fourth foetal month, when the sections stained by hematoxylin and eosin can be observed. Nevertheless, presumptive primordial cell groups are noticed by the staining method of the alkaline phosphatase in the hair-rudiments of an embryo that is 11.5 cm in body length (middle of the third foetal month), as illustrated in Fig. A-1, A-2 and Fig. B. In the foregoing papers written by Iwata (1927), Mogi (1938), Kagami (1939), Kan (1941) and Morise (1954), the first appearance of the sweat gland primordia is judged by the first protrusion of the cell groups from the hair-rudiments at an obtuse angle where the hair-rudiments and the epidermis are crossing each other. But, from the findings obtained in this study, it is evident that the occurrence of the enzymatic activity is ahead of the appearance of the morphological changes in the awaited sweat gland primordia within the hair-rudiment.
It must be considered that the variation of the staining methods gives the influence to the problem whether the cell group with strong enzymatic activity or the solid cell group protruded from the hair-rudiment is the true primordia of the sweat gland. If the spherical protrusion of the cell group were called sweat gland primordia, the cell group with enzymatic activity in the hair-rudiment must be named the “prospective primordia of the sweat gland”. This must be the same case as Roche (1950)'s report, in which he said that the prospective osteoblast of the chick embryo presents intense activity of the alkaline phosphatase just before the calcification of bones, proceeding to be recognized morphologically. As Roche (1950) has reported, the alkaline phosphatase plays an important role in the protein formation in the development of the living creature. This enzyme is said to appear in the course of cellular differentiation, as regards the development of the chick embryo. This phenomenon is similar to the appearance of the activity in the “prospective primordia of the sweat gland” in this study. Besides, this enzyme occurs in the recovery of the wound, from a burnt, in the bone fracture, in the formation of the proteinous secretory substances and in the increase of cell number stimulated by sex hormone or other materials. From the facts mentioned above, it is evident that there is intimate relationship between protein formation in a body and occurrence or increase of the activity of the alkaline phosphatase. Moreover, it is presumed that the alkaline phosphatase might participate directly or indirectly in the metabolism of nucleoprotein and nucleic acid. Considering Roche's assumption and also from the fact that the nucleic acid can react as one of the substrate material for alkaline phosphatase, the activity of the alkaline phosphatase in the sweat gland primordia of the cattle embryo is thought to increase, because of the rapid turn-over of the phosphate in the nucleic acid in the primordial cells. As a matter of fact, the alkaline phosphatase in this early developmental stage can be named “phosphatase for differentiation”, in contrast to the enzyme which concerns the secretory function in the adult sweat gland.

Then the prospective primordia of the sweat gland gives gradually rise to the rudiment which is definitely recognizable as a solid spherical protrusion from the hair-base (Fig. c, d, e). Though the hill-like primordia with intense enzymatic activity is suggestive of vigorous differentiation, the activity tends to concentrate at the
central part of the primordia, as the developmental stage proceeds from mammellary (Fig. D) to globular-form primordia (Fig. E, F). This figure indicates that the cells in the central parts undergo intense differential action to send many differentiated cells towards the outer side, and at the same time, to become larger in their own size.

In accordance with the morphological change of the sweat gland primordia, the undifferentiated mesodermal cells which surround the primordia are stimulated to have strong reaction to the alkaline phosphatase. The fact that the mesodermal cells which are far from the sweat gland primordia are also markedly reactive to the enzyme is presumed to be due to the differential activity of the mesodermal cells themselves.

In the clavate shaped buds (Fig. J), the enzymatic activity occurs not only in the cells of the central part but also in the whole cells of the apical ampulla, notwithstanding the appearance of the activity limited in the central part of the apical ampulla in the foregoing two stages.

The activity in the apical ampulla seems to increase, prior to the morphological change from the apical ampulla to the hook-like primordia with a cleft, as the fore-step to the formation of the secretory lumen and to accomplish the secretory epithelium. It must be only within the range of the imagination whether the cells with strong activity in the apical ampulla of the clavate shaped primordia originate from the cells with strong reaction at the center of the globular primordia, or the former regain the enzymatic activity after the latter once loose their activity to become the latter.

The reaction of the cell cords gets weaker in proportion to the development of the primordia, though the reaction strongly occurred in the earlier stage of the clavate shaped primordia. In the cell cord, two layers of cells arrange themselves on both sides of the narrow linear cleft which is centrally located. It is conjectured that the prospective excretory duct would be accomplished morphologically during the developmental course from the clavate bud to the hook-like primordia. The authors (1957) noticed the underdeveloped lumen in the prospective excretory duct in the eighth foetal month, while observing the specimens stained by hematoxylin and eosin. But by employing the histochemical method for alkaline phosphatase, the histochemical figures reveal the prospective lumen as a linear structure in the earlier stage than the eighth
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As illustrated in Fig. K, the apical ampulla of the hook-like primordia presents intense activity, without regard of the weak activity in the cell cord. It is worthy of note that one layer of cells which line up in a line along the greater curvature of the hook-like primordia have large concentration of alkaline phosphatase. These cells are presumed to be the myoepithelial cells and thought to have strong activity, as they are in a more intense state of functional activity in correspondence to the remarkable development of the cells in the greater curvature of the hook-like primordia. Then these cells appear at both sides of the sweat gland primordia and keep on to demonstrate intense activity until the end of the accomplishment of the lumen. Supposing the cells mentioned above give rise to the myoepithelial cells, the myoepithelial cells are distinguished histochemically from the glandular cells in the middle or at the end of the sixth foetal month.

With the embryonal stage develops, the cells in the apical ampulla and show a gradual decrease of activity, from the outer to the inner layer, except the outer most cells which are considered to be the undifferentiated myoepithelial cells (Fig. L-1). First of all, the cells in the outer third layer lose their activity, though the cells in the central region still maintain intense reaction. As demonstrated in Fig. L-2, the central cells fall into necrosis to disappear, after they lose their activity of the alkaline phosphatase. Then the glandular lumen is formed, being surrounded with a layer of glandular cells with weak activity.

In regard to the formation of the glandular lumen, Kölliker (1850), Mogi (1938) and Kan (1941) insisted that the lumen was built up after the formation of a narrow cleft at the center of the cell group in the apical ampulla, the accumulation of the mucous substance in the cleft and the disappearance of the cells near the cleft. The authors (1957) reported that the nuclei of the cells at the central region of the apical ampulla are weakly stained with hematoxylin and probably develop to be necrosis. Anyway, the progressive changes of the formation of the lumen go parallel to the regressive figures of the cells in the central part of the apical ampulla.

Fig. L-2 and L-3 are indicative of the strong activity of the luminal cytoplasm, in a bounded structure.

In these pictures, the lumen is not provided with the adult forms and filled with some cells with weak activity. This belt shaped
prolif are suggestive of the presence of the secretory function, because these figures correspond with the site of apical hyaline border in the adult luminal cells which undergo secretory action and, as Kölliker stated, the enlargement of the cleft may be due to the accumulation of the secreted substances. On the other hand, this activity may be due to the function of the absorption of the necrotic substances in the undifferentiated lumen. The fact that this belt-shaped activity in the luminal cytoplasm is encountered in the primordia at the end of the seventh and of the eighth foetal month suggests the existence of the secretory function in the primordial gland.

Nakagawa (1957) noticed the primitive secretory function without the preliminary secretory preparation in the glandular cell body, in the axillary apocrine sweat gland of a sixth month human embryo. From the facts mentioned above, it is conjectured that the phosphatase activity in the luminal cytoplasm is concerned with the secretory function of the glandular cells and must be named "phosphatase for secretory function", in comparison with the so-called "phosphatase for differentiation" which is encountered in the earlier stages of the development. So far as the phosphatase activity is concerned, the differential course in the earlier developmental stages was altered into the secretory course in the seventh or eighth foetal month.

Regarding the relationship between the secretory function and the activity of the alkaline phosphatase, Buntig, Wislocki and Dempsey (1948) stated that the phosphatase might intermediate in sending some substances from blood to sweat or in regulating the transmission of something from blood to sweat. But the function of the alkaline phosphatase will become definite after the metabolism of many things in the glandular cell is clearly demonstrated.

Conclusion

The changes of the activity of the alkaline phosphatase in the sweat gland primordia were studied on six parts of the eight cattle embryos, aging from the third to the eighth foetal month. The main results obtained were as follows:

1) The cell group which was strongly reactive to the alkaline phosphatase reaction revealed itself in the hair-rudiments of the
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forehead, breast and sole of a cattle embryo in the middle of the third foetal month. This cell group must be called the prospective primordia of the sweat gland. In this developmental stage, there were no sign of the protrusion of the cell group from the hair-rudiments, in the specimens with hematoxylin and eosin. It was evident that the first appearance of the primordial sweat gland in the histological specimen (middle of the fourth foetal month) is a little later than that examined by in the histochemical routine (middle of the third foetal month).

2) In the hill-like or mammerallary primordia, the primordial cell group of the sweat gland which protruded from the hair-rudiment contained an appreciable amount of alkaline phosphatase.

3) In the globular primordia, the primordial cells situated at the center of the primorida showed larger concentration of the enzyme, while the cells at the peripheral part of the primordia showed only traces of the enzyme.

4) In the earlier stage of the clavate-shaped primordia, both the apical ampulla and the cell cord which connects the primordia with the hair-rudiment are distinctly reactive to the enzyme. In progress of the developmental stage, the cells in the apical ampulla maintained intense activity, on the other hand, the cells in the cell cord gradually loose their activity.

5) In the hook-like primordia, the apical ampulla presented strong reaction, but the cell cords contained only a trace of enzyme. The cells, which arranged themselves along the greater curvature of the sweat gland primordia and were presumed to be the undifferentiated myoepithelial cells, were rich in alkaline phosphatase activity.

6) In the preliminary stage to the formation of the glandular lumen, the cells in the outer two or three layers in the apical ampulla gradually lost their activity, though the cells in the central part were still strongly reactive. Then the centrally located cells showed the decrease of enzyme and fell into necrosis to form the cleft or the primitive glandular lumen.

7) In the stage of the formation of the glandular lumen, the luminal cytoplasm of the prospective glandular cells presented the belt-shaped strong reaction to the alkaline phosphatase. The nuclei of the primitive glandular cells demonstrate weak activity. The undifferentiated myoepithelial cells contained appreciable amount of enzyme.
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References


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Explanation of Figures

Figs. A-M Alkaline Phosphatase staining.
Figs. a-m Hematoxylin-Eosin staining.
Fig. A-1 Forehead: Beginning of third fetal month.
Fig. A-2 Back: Middle of third fetal month.
Fig. B Back: Middle of fourth fetal month.
Fig. C Breast: Middle of fifth fetal month.
Fig. D Back: Middle of fifth fetal month.
Fig. E Abdomen: Middle of fifth fetal month.
Fig. F Abdomen: Middle of fifth fetal month.
Fig. G Sole: Middle of sixth fetal month.
Fig. H Abdomen: Beginning of seventh fetal month.
Fig. I Back: Middle of sixth fetal month.
Fig. J Hypogastrium: Middle of sixth fetal month.
Fig. K Sole: Middle of sixth fetal month.
Fig. L-1 Hypogastrium: Middle of sixth fetal month.
Fig. L-2 Breast: Beginning of seventh fetal month.
Fig. L-3 Forehead: End of seventh fetal month.
Fig. M Back: End of seventh fetal month.
Fig. a Forehead: Beginning of third fetal month.
Fig. b Back: Middle of fourth fetal month.
Fig. c Breast: Beginning of fifth fetal month.
Fig. d Back: Middle of fifth fetal month.
Fig. e Abdomen: Beginning of fifth fetal month.
Fig. f Abdomen: Middle of fifth fetal month.
Fig. g Sole: Middle of sixth fetal month.
Fig. h Sole: Beginning of seventh fetal month.
Fig. i Back: Middle of sixth fetal month.
Fig. j Hypogastrium: Middle of sixth fetal month.
Fig. k Sole: Middle of sixth fetal month.
Fig. l Forehead: End of seventh fetal month.
Fig. m Back: End of seventh fetal month.
Plate IV

K. Yasuda, K. Kobayashi & O. Saeki