The mitotic pattern of the embryonic epidermis of chick during scale morphogenesis

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The epidermis of the chick embryo has been widely used for in vitro studies of many developmental problems (Matoltsy, 1960; Billingham & Silvers, 1963). The present attempt to determine the proliferation rate of chick embryonic epidermal cells was expected to provide a base for experimental studies, but a preliminary mitotic count revealed that the number of mitoses varied greatly in different areas. This suggested accumulation of mitoses in some restricted parts of the epidermis, and so a mapping experiment was carried out to determine the distribution of mitoses in this material. The characteristic mitotic pattern which was discovered is described and discussed.

Materials and Methods

About 300 White Leghorn embryos were used: 20 for study of the gross anatomy of scales, 200 for Colcemid treatment and 80 for [3H]thymidine treatment.

(a) Colcemid treatment

Embryos of 9, 10, 11, 12, 13, 15, 17 and 21 days incubation received a subcutaneous injection of Colcemid (CIBA, 1 mg/ml in distilled water) of about 1 μg/g body weight. Four hours after injection, embryos were sacrificed, the legs were cut off, fixed in Bouin’s fluid and embedded in paraffin. Transverse sections of the lower half of the shank were cut serially at 10 μ and stained with haematoxylin and eosin. These sections were then examined microscopically and selected according to the established criteria (Hooper, 1961). Finally, two to five examples of each stage were prepared and used in this study.

(b) [3H]thymidine treatment

Embryos of 10, 12 and 15 days incubation were given an intravenous injection of [3H]thymidine (sp. act. 5·0 ci/mmmole) of 1 μci/g body weight. They were

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sacrificed 2 h after injection. The legs were fixed in Bouin’s fluid, embedded in paraffin and cut serially at 5 µ. The sections were covered with NR-M 2 emulsion (Sakura, Tokyo), exposed for 30 days, developed and stained with haematoxylin and eosin through the emulsion.

(c) Mapping procedure

A microscope with a projection-screen attachment was used. The magnification of the picture on this screen was adjusted to be × 200; the contours of the basement membrane as well as those of some blood vessels and muscles were delineated on a tracing paper placed on the screen. The mitotic or the labelled cell figures were marked on this line as if projected on the basement membrane. A picture of each section of the selected region was thus prepared.

The mapping procedure itself was essentially the same as described by Marques-Pereira & Leblond (1965). All mitotic figures in a section were depicted as points on a straight line, and the resulting map made up of these lines gave a two-dimensional view of the distributional pattern of epidermal cell mitoses.

RESULTS

(a) Estimation of the map

The 9- and 11-day maps of Colcemid-treated embryos and a 10-day map of a thymidine-treated embryo are shown (Text-figs. 1-4). In these maps the regional difference in the distribution of mitoses can be observed distinctly. Two points should be noted on examination of the maps. (1) These maps do not show the relative mitotic rate of cells in each region but only mitotic density. The highest value of basal cell density is obtained from the site where the epidermal height was above average. This basal cell density is 25-30% higher here than in the region where it is at its lowest value. Even if these varying values are taken into consideration, the observed difference can be regarded as the result of differential mitotic activity of cells in each region in earlier stages. (2) Due to the scaly ridge formation the epidermis in transverse sections was cut tangentially at the distal boundaries of the scales and so the map showed a false increase of mitoses there. Hence, only a part of each scale was reproduced in the 13- and 15-day maps.

(b) Patterns observed on the maps

The map from a Colcemid-treated 9-day embryo showed a proximo-distally alternating pattern of mitotically active and inactive areas at the antero-lateral portion of the tarso-metatarsal region (Text-fig. 1). The size of both areas was approximately 150 × 300 µ. The wide medial portion showed no distinct regularity, though the mitotic activity seemed to be less in the region immediately medial to the active area (Text-fig. 2).

Patterns observed in the 10- and 11-day maps were essentially the same, but
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more complicated than those of the previous stage (Text-fig. 3); mitotically active and inactive areas were still present. The change consisted in: (1) the appearance of a new active area in the medial portion where the mitotic activity was generally low in the 9-day stage, and (2) the deformation of both mitotically active and inactive areas laterally.

Text-fig 1. Map of the antero-lateral portion of the left shank of a 9-day embryo. Colcemid-treated. From top to bottom, distal to proximal. Three active and four inactive areas can be observed.

The maps of thymidine-treated embryos showed similar patterns (Text-fig. 4), and numerically comparable results were obtained. Peridermal maps were also prepared, but no definite relation to the pattern revealed by the underlying basal cells could be detected.
From the 12th to 13th day an over-all increase in mitotic density was observed, which shows the onset of epidermal stratification. This can probably be attributed to the enhanced mitotic activity of epidermal cells, though not conclusively in the absence of a differential cell count.

Text-fig. 2. Map of the portion immediately medial to Text-fig. 1. Distribution of mitoses is almost completely random.

(c) Histological observation

The histological characteristics of the inactive area were clear. The epidermis is distinctly three-layered, and its thickness is almost constant at c. 20 μ. The basal cells are columnar, the nuclear position is higher and separate from the basement membrane (Plate 1, fig. A). A regular arrangement of nuclei is also observed, but this is partly due to scarcity of mitoses in these areas. Mitoses of
fibroblasts accumulate in the deeper dermis, but mitotic arrest cannot be observed so definitely as in the epidermis.

The active area is not uniform as is shown by the change in epidermal thickness. This is lowest at 10 μ near the proximal end of this area, and gradually increases distally to reach the highest value of 20 μ near the distal end where

passage to the inactive area occurs. At this transitional region dermal condensation is as thick as it is under the inactive area (Plate 1, fig. B). Hence, no exact boundary can be defined between these two areas, though the labelled cells are usually lower in height than the unlabelled ones within one transverse section (Plate 1, fig. D).

From the 12th to 14th day of incubation the basal cells gradually assume a high, columnar shape all over the shank. The characteristic feature of these stages is the existence of abundant mitoses in the upper layers of epidermis, which are found until the end of the 14th day of incubation.

Briefly, as development proceeds, the proliferation patterns of epidermal cells change in the following sequence: (1) towards random distribution of mitoses and a moderate mitotic activity, (2) depression of mitotic activity accompanied by histological change, (3) enhanced mitotic activity, probably reactive, in several places, (4) over-all increase in mitotic activity and formation of multi-layered epithelium.
Fig. A. Mitotically inactive area. Epidermal cells are tall and columnar.

Fig. B. Active area. In these two figures fibroblastic accumulation is conspicuous.

Fig. C. Indifferent portion, neither active nor inactive, as in Text-fig. 4. The epidermis is thin and fibroblastic accumulation is lacking. A, B and C are of a Colcemid-treated 9-day embryo.

Fig. D. Mitotically inactive area. [3H]thymidine-treated 9-day embryo.
DISCUSSION

The present study reveals a complex pattern of epidermal proliferation during scale morphogenesis. Because of the wide divergence of mitotic activity among cells, the shank epidermis in those stages described cannot be regarded as a proper material from which to derive numerical data for statistical analysis. This might explain the uncertainties of the quantitative results with regard to $[^3]$Hthymidine incorporation in vitro (Wessels, 1963). Wessels described a whole mount autoradiogram of shank epidermis in which scale tips failed to incorporate $[^3]$Hthymidine, but he made no further comment on this fact. Several in vitro experiments have also been performed with materials in the corresponding stages, but no authors have paid attention to the peculiar mitotic behaviour of these cells in situ (Wessels, 1961, 1963; Dodson, 1963, 1967; Mordoh & Lustig, 1966). This seems only natural, because the epidermis when peeled off from the dermis usually fails to develop the regional differences characteristic of skin in situ (original observation, but also see Wessels, 1961). This suggests the existence of some local control mechanism which exerts stimulatory or inhibitory effects on the proliferation of epidermal cells, though the peeling-off process itself may have some effect.

The transient mitotic arrest of epidermal cells in 9- and 10-day embryos is probably a similar phenomenon to those that have been observed in several cases of the early stages of feather or hair development (Wessels, 1965; Wessels & Rossener, 1965). In our material, however, it is constantly observed, while the mitotic arrest of dermal fibroblasts could not be as clearly demonstrated.

The existence of the mitotically active area is unusual, though no comparable data can be obtained at present in other materials. The mitotic density is often ten times or more greater than that of the indifferent portion such as the extreme lateral side on the 9-day map (Plate 1, C). Such a high mitotic activity seems to suggest the presence of some local stimulatory mechanism, but no signs of wave-like propagation of the mitotic stimulation could be discerned (Harding & Srinivasan, 1961). It would be of interest in this connexion to obtain data of the proliferation pattern of the epidermis of scaleless mutant (Sengel, 1964).

SUMMARY

1. Chicken embryos of 9–15 days of incubation were treated with Colcemid or $[^3]$Hthymidine. Serial sections of the shank were reconstructed in such a way that the distribution of epidermal mitoses could be observed two-dimensionally.

2. A regular, proximo-distally alternating pattern of mitotically active and inactive areas has been shown to exist in the case of 9-day embryos. Such areas persist in the 10- and 11-day stages, though the pattern becomes more irregular. Histological features of these areas are described.

3. The implications of the findings are discussed mainly in relation to the results of in vitro experiments of earlier investigators.
La répartition des mitoses de l'épiderme embryonnaire du Poulet pendant la morphogenèse des écailles

1. Les embryons de 9 à 15 jours d'incubation ont été traités à la colcemide ou à la thymidine H3. Des reconstructions de coupes sérées de la cuisse ont été faites de manière à ce que l'on puisse observer la distribution des mitoses épidermiques dans deux directions de l'espace.


3. La signification de ces résultats est discutée en relation avec les résultats antérieurs des expériences in vitro d'autres chercheurs.

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REFERENCES


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