The origin of the ectomesenchymal condensations which precede the development of the bony scleral ossicles in the eyes of embryonic chicks

By D. M. FYFE¹ and BRIAN K. HALL²

From the Department of Developmental Biology, University of Aberdeen, and Department of Biology, Dalhousie University, Halifax

SUMMARY

The origin of neural crest-derived ectomesenchymal condensations located subjacent to scleral papillae and the development of the more deeply situated scleral ossicles were investigated in scleral tissues explanted from the eyes of chick embryos at Hamburger & Hamilton (1952) stages 30–38 (6½–12 days of incubation). Explants were pulse labelled with [3H]thymidine for 4 h in vitro. Nuclear counts and % labelling indices were calculated for standardized areas within and between the condensations. At all stages exhibiting condensations, the % labelling indices were higher within the condensations than in tissues between condensations. % labelling declined with maturity, but the decline was greater between than within condensations. Regional differences in intensity of proliferation in the ectomesenchyme centred about the scleral papillae seemed to be the best explanation for the development of these condensations. The condensations disappeared concomitant with the complete degeneration of the adjacent papillae. A new distribution of labelled nuclei 70–100 μm deep in the tissue and beneath the original sites of the condensations preceded the appearance of ossicle primordia. The roles of the scleral papillae, the ectomesenchymal condensations and deeper primordia in the development of scleral ossicles are discussed.

INTRODUCTION

The sclera of the eyes of birds and reptiles contains a cup of hyaline cartilage and a ring of bony scleral ossicles (Walls, 1942). Scleral ossicles are the overlapping plates of membrane bone, encircling the cornea and originating in the embryo from neural crest-derived ectomesenchyme (Stewart & McCallion, 1975; Noden, 1978; Johnston et al., 1979). (The term 'ectomesenchyme' is used throughout this paper to denote mesenchyme derived from the ectodermal neural crest. Mesenchyme is used to denote tissue derived from mesoderm.)

The domestic fowl and most other birds have 14 scleral ossicles in each eye.

¹ Author's address: Department of Developmental Biology, Natural Philosophy Building, University of Aberdeen, Aberdeen, Scotland, AB9 2UE.
² Author's address: Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4JL.
Almost 4 days prior to the onset of ossification of prospective scleral ossicles, 14 papillae develop within the conjunctival epithelium of the eye surface in a ring around the cornea (see Fig. 1). Each of these scleral papillae directly overlies the future site of a scleral ossicle and consequently papillae are thought to have an inductive role in scleral ossicle formation (Coulombre, Coulombre & Mehta, 1962; Puchkov, 1964, 1965; Palmoski & Goetinck, 1970; Johnson, 1973; Hall, 1981a,b). The papillae arise in an orderly sequence starting at Hamburger & Hamilton (1952; H.H.) stage 30 (6½ days of incubation). They gradually change from simple placodes to filiform projections which proceed to degenerate and finally disappear just as the corresponding ossicles begin to form in the subjacent ectomesenchyme (see Fig. 2). Murray (1943) has divided papilla morphogenesis into six characteristic stages. His staging criteria (designated 'M. stages') were used throughout this study.

During papilla morphogenesis, localized areas of high cell density, known as cellular condensations, develop in the scleral ectomesenchyme subjacent to the papillae. Several studies of the cellular condensations involved in the initiation

![Fig. 1](image-url)  
Fig. 1. On the left of the figure is the head of an embryo at H.H. stage 32 (6½-8 days of incubation) when all the papillae have developed on the eye surface. As a convention for numbering papillae, papilla 1 is situated over the choroid fissure (c). The papilla 12 lies over the temporal long ciliary artery (t). Thus the numbering is clockwise in the left eye as shown here and counterclockwise in the right eye. The tissues used for autoradiographic labelling were removed from the eye in blocks measuring about 2 mm in their longest dimension. The blocks included the papilla 12, its two neighbouring papillae, the conjunctival epithelium (ce), the ectomesenchyme (e), the fibrous sclera (fs) and the pigmented epithelium (pe) as shown in the right hand part of the figure.
Fig. 2. The morphogenesis of scleral papillae and scleral ossicles including the six stages according to Murray (1943). M. stage 1 – The earliest appearance of a placode in the conjunctival epithelium (ce). There is no condensation of the ectomesenchyme beneath the papilla at this stage. M. stage 2 – The papilla is a thicker placode and there is the earliest appearance of a condensation in the ectomesenchyme immediately beneath the papilla (at c). M. stage 3 – The papilla enlarges with an ingrowing cone of epithelial cells which extends into the ectomesenchyme. The cone of the papilla has contact with the ectomesenchymal condensation (c). M. stage 4 – Folds of conjunctival epithelium surround the inward directed cone of the papilla. The condensation remains in contact with the papilla and close to the eye surface at c. M. stage 5 – The inward directed cone of the papilla has disappeared and the papilla now has a core of ectomesenchymal cells. The papilla is elevated above the surrounding conjunctiva. This is the last stage at which the ectomesenchymal condensation may be found adjacent to the papilla at c. M. stage 6 – The papilla has lost the core of ectomesenchymal cells and has degenerated to a thin filiform projection. There is no longer a condensation of ectomesenchymal cells beneath the papilla and in contact with it. The anlage of the scleral ossicle has become distinct deep in the sclera at A, 70–100 μm away from the eye surface. Late M. stage 6 – The papilla is in its final stage of degeneration and will shortly disappear. The Scleral Ossicle (so) is first evident as the membranes in the centre of the anlage begin to ossify deep in the sclera. Beyond M. stage 6 – The papilla has disappeared. The Scleral Ossicles grow laterally to overlap. The head skeleton. The head skeleton of an H.H. stage 39 embryo is shown with a ring of 14 scleral ossicles encircling the cornea in the sclera of the eye.
of chondrogenesis have been carried out (Hall, 1978; Ede, 1982; Solursh, 1982; Thorogood, 1982). However, the condensations preceding intramembranous osteogenesis have received less attention. Hale (1956) examined the distribution of mitotic figures in the perilimbic region of the sclera in the chick and attributed the origin of the condensations beneath each of the papillae to regional differences in mitotic activity in the ectomesenchyme. However, he studied only four embryos which were roughly staged at 7, 8, 9 and 10 days of incubation and thus omitted the preliminary stages of osteogenesis in the sclera at 11 and 12 days of incubation. Furthermore, he pooled data from unspecified ossicle primordia of the same specimen and hence did not take into account the fact that these primordia develop asynchronously. Thus a new investigation was required to extend Hale’s study.

In the present investigation, the ectomesenchymal condensations were studied through the six stages of papilla development (the M. stages), the embryos were accurately staged, and only one specified papilla was studied from each specimen to reduce anatomical variability. Pulse labelling of the scleral tissues with $[^3\text{H}]$thymidine was used to determine whether the formation of the ectomesenchymal condensations was characterised by regional variations in DNA synthesis and mitosis.

### MATERIALS AND METHODS

#### Incubation of eggs

Fertile eggs of the domestic fowl, *Gallus domesticus*, white Leghorn strain, from Cox Poultry Farm, Nova Scotia, were incubated in a forced-draft incubator without rotation at $37 \pm 0.5 ^\circ \text{C.}$ and $54 \pm 4 \% \text{ R.H.}$ for between 6½ and 12 days to obtain embryos at H.H. stages 30–38.

#### Dissection of tissues

The eggs were opened under sterile conditions and the embryos removed and placed in Tyrode’s solution containing phenol red (0.02 g/l). The anterior surfaces of the eyes were freed of investing tissues by dissecting away the eyelids and nictitating membranes. Small blocks of the sclera incorporating the papillae 11, 12 and 13 were removed from the left and right eyes as shown in Fig. 1. The anatomical variability was reduced by using only the papilla 12 for analysis.

#### Incorporation of isotope

It was necessary to expose the tissue to $[^3\text{H}]$thymidine for less than the duration of the S-phase of the cell cycle to minimize double labelling of cells, but long enough to ensure labelling of all cells undergoing DNA synthesis. A previous study has reported 4–6 h as the duration of the S-phase for several types of chick embryonic cells including stromal cells of the cornea (Nuttall, 1976); we
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therefore selected 4 h as a suitable period for isotopic labelling. The blocks of tissue were totally immersed in 1.5 ml of culture medium (the complex synthetic medium, BGJb supplemented with 15% horse serum, both from GIBCO, Grand Island, N.Y.) containing 10 \( \mu \text{Ci} \) (0.21 ml) of \( [\text{\textsuperscript{3}H}] \)thymidine (specific activity 64.7 Ci/mmol, New England Nuclear, Lachine Quebec, Canada) in 35x100 mm Falcon plastic Petri dishes. This 4 h pulse was followed by a rinse in Tyrode's solution and 10 min in cold BGJb with 15% horse serum, utilizing the presence of excess non-radioactive thymidine in BGJb to stop the incorporation of \( [\text{\textsuperscript{3}H}] \)thymidine.

Histology

The tissue explants were fixed immediately after the chase in 10% neutral buffered formalin. They were then dehydrated, cleared and embedded in 54°C m.p. paraffin. Serial sections were cut at 6 \( \mu \text{m} \) with the plane of section perpendicular to the front of the eye and in line with the three papillae. The explants were cleared of wax in xylene and rehydrated in a series of alcohols in preparation for autoradiographic processing.

Autoradiography

The slides were coated with Kodak NTB3 emulsion (diluted 1:1 with distilled water) with a roller in a darkroom, air dried for 30 min and then placed in a light-tight box containing silica gel for 7 days. The autoradiographs were developed for 2 min in Kodak D19 developer, fixed for 4 min in Kodak rapid fixer and rinsed for 20 min in running tap water. The sections were then stained with aqueous Harris haematoxylin (3-5 min) dehydrated, cleared and mounted.

Quantitative examination

In each specimen, only the section through the centre of the papilla 12 was used for the survey. The numbers of \( [\text{\textsuperscript{3}H}] \)thymidine labelled and unlabelled nuclei were counted in the sections within defined areas using an ocular reticule as a guide at 400\( \times \) magnification. The total area examined in each specimen measured 0.31 x 0.13 mm and included ectomesenchyme extending from the inner fibrous sclera to papilla 12 at the eye surface in one dimension and the tissue between papilla 12 and the two neighbouring papillae in the other dimension (see Fig. 3). Nuclei were identified as having incorporated isotope if they were overlain by three or more silver grains above background levels. An area occupied by 12-18 nuclei had a background of less than six silver grains (i.e. less than 0.5 grains/nucleus).

Abercrombie (1946) has drawn attention to the problem of nuclei counted in equivalent areas of tissue in sectioned material, particularly where the comparison involves non-equivalent nuclei in different tissues. In the present study, the size and shape of nuclei was fairly constant in different areas of the
ectomesenchyme, and in the tissue at all the stages examined. Furthermore, we sectioned some tissue explants at each of the M. stages of papilla development in a plane perpendicular to the plane of section employed for the autoradiographs and confirmed that changes in nuclear density were real phenomena and not simply due to the nuclei assuming new orientations.

The 6μm sections were too thick for all the nuclei to lie within the autoradiographic range for tritium beta particles (Modak, Lever, Therwath & Uppuluri, 1973). Thus the number of labelled nuclei counted in our sections represented an underestimate of the total number of radioactive nuclei in the section. However, since our aim was to compare and not quantify labelling indices in equivalent areas of ectomesenchyme it was not necessary to compensate for this constant underestimate.

Labelling indices were calculated for each specimen as follows:

\[
\frac{\text{Number of labelled nuclei}}{\text{Total number of labelled and unlabelled nuclei}} \times 100\%
\]

Paired statistical data were compared using the Student's 't' distribution.

RESULTS

Nuclear density in ectomesenchyme between and beneath papillae

The data in Table 1 and Fig. 3 show the density of ectomesenchymal nuclei counted within defined areas (6800 μm²) both beneath and between scleral papillae at all six of the M. stages of papilla development. Nuclear counts were the same in ectomesenchyme in equivalent areas beneath and between the M. stage-1 papillae. The specimens with M. stage-2 papillae were the youngest to show higher nuclear densities in ectomesenchyme beneath the papillae than between papillae. This change (significant at P<0.05) was entirely due to increased nuclear density beneath the papilla, for the nuclear density between papillae remained unchanged over that seen between M. stage-1 papillae. Nuclear density increased in ectomesenchyme both beneath papillae and between papillae through advancing stages, i.e. the M. stages 2–5 of the papillae (Table 1, Fig. 3). During this period, the increase in nuclear density was more marked in the condensations beneath the papillae than in the surrounding areas between papillae. Maximum nuclear densities were observed in specimens with the M. stage-5 papillae. Once the papillae reached M. stage 6, nuclear density in the two sample areas had declined below the levels found at M. stage 5 and were no longer significantly different from one another. Coincident with this decline in nuclear density, ectomesenchyme became more fibrous than at earlier stages (Fig. 12) as increased extracellular materials, new invasion of blood vessels, and large numbers of degeneration granules were evident in the tissue. When the ectomesenchymal condensations first appeared subjacent to M. stage-2 papillae, they were situated close to the eye surface and in contact with
the basal layers of the epithelium. Later in the H.H. stages-37 to -38 (11–12 days of incubation) embryos, when the papillae were either absent or in the final stages of degeneration, the earliest ossification of the membranes was observed. The ossifying membranes were not situated at the original site of the ectomesenchymal condensation but were deep in the sclera at a depth of 70–100 μm beneath the base of the papillae. A distinct anlage of the scleral ossicles, lying deep in the sclera was not evident until just before the membranes ossified. The surface condensations seemed to disappear after the papillae passed M. stage 5, at which time the specimens as yet had no discernable ossicle primordia (see Fig. 11).

**Percent labelling index in ectomesenchyme between and beneath papillae**

The data in Table 1 and Fig. 4 show the % labelling index of ectomesenchymal cells in the same areas in which nuclear density was counted. Specimens with M.
Table 1. Nuclear densities and labelling indices in ectomesenchyme beneath or between scleral papillae as a function of the stage of papillae and embryos

<table>
<thead>
<tr>
<th>H.H. stage of embryo</th>
<th>Stage of papilla</th>
<th>Nuclear density(^a)</th>
<th>% labelling index(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Beneath papilla(^c)</td>
<td>Between papillae(^c)</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>17.2 ± 0.71(6)</td>
<td>16.4 ± 0.38(6)</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>20.6 ± 1.05(6)*</td>
<td>17.2 ± 1.29(6)*</td>
</tr>
<tr>
<td>31–33</td>
<td>3</td>
<td>23.9 ± 0.61(7)*</td>
<td>21.2 ± 0.54(13)*</td>
</tr>
<tr>
<td>32–34</td>
<td>4</td>
<td>26.0 ± 0.81(11)*</td>
<td>22.7 ± 0.66(18)*</td>
</tr>
<tr>
<td>34–35</td>
<td>5</td>
<td>27.1 ± 1.20(8)*</td>
<td>23.5 ± 0.50(15)*</td>
</tr>
<tr>
<td>36</td>
<td>6</td>
<td>23.8 ± 1.20(6)</td>
<td>22.4 ± 0.62(10)</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± SEM (n) of nuclei/mm\(^2\) \times 1000

\(^b\) Mean % ± SEM (n)

\(^c\) as measured in areas of 6800μm\(^2\)

\(^d\) significantly different from same stage ectomesenchyme (P<0.05)

n = number of observations (1 observation/eye—beneath papilla; 1–2 observations/eye—between papillae)
Scleral condensations

Stage-1 papillae had identical % labelling indices (11%) in ectomesenchyme beneath and between papillae. Beneath papillae, % labelling index peaked at 13% in specimens with M. stage-2 papillae, flattened out at about 8% at M. stages 3–5 and fell dramatically to 3% at M. stage 6. Essentially the pattern is a relatively constant labelling index of around 9½% between M. stages 1–5 and a rapid decline to 3% at M. stage 6 for the ectomesenchyme just beneath the papillae. In contrast, ectomesenchyme exhibited labelling indices of 9–10% between papillae at M. stages 1 and 2 only (Table 1, Fig. 4), rapidly declining to 2% by stage 3 and remaining low thereafter. Labelling indices for tissues between papillae were lower than those for tissues beneath papillae at M. stages 3–5 (P < 0.05).

The distribution of labelled nuclei in relation to the position of the papillae

The sections used in the analysis above were subdivided into Zones 25 μm wide.

Fig. 4. The change in % labelling index in ectomesenchyme with advancing stages of maturity. The locations and dimensions of the sample areas are shown in the insert. % labelling index for areas beneath the papilla (○) is distinct from % labelling index for areas between papillae (●).
Table 2. Density of labelled ectomesenchymal cells as a function of distance on either side of the midline of scleral papilla number 12

<table>
<thead>
<tr>
<th>H.H. stage of papilla</th>
<th>M. stage</th>
<th>n</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1</td>
<td>6</td>
<td>20 ± 3</td>
<td>18 ± 2</td>
<td>24 ± 2</td>
<td>15 ± 3</td>
<td>20 ± 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>6</td>
<td>19 ± 5</td>
<td>24 ± 3</td>
<td>25 ± 2</td>
<td>22 ± 2</td>
<td>27 ± 4</td>
<td>29 ± 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31–33</td>
<td>3</td>
<td>7</td>
<td>7 ± 2</td>
<td>6 ± 3</td>
<td>8 ± 2</td>
<td>6 ± 2</td>
<td>12 ± 2</td>
<td>18 ± 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32–34</td>
<td>4</td>
<td>11</td>
<td>8 ± 2</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
<td>13 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
<td></td>
<td></td>
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<tr>
<td>34–35</td>
<td>5</td>
<td>8</td>
<td>7 ± 2</td>
<td>7 ± 3</td>
<td>11 ± 3</td>
<td>13 ± 3</td>
<td>19 ± 3</td>
<td>26 ± 5</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*M X ± SEM, where X is number of [3H]thymidine labelled cells/mm² × 100.*
Scleral condensations (numbered 1–12) and located within 150 μm on either side of the papilla 12. Data from specimens at all six M. stages are shown in Table 2 and in Figs 5 and 6.

Fig. 5. The distribution of labelled nuclei in ectomesenchyme lateral to the vertical line through the midpoint of the papilla (MP). The representative sections were subdivided into six areas on each side of MP as shown in the figure. The plots represent the density of labelled nuclei in these areas; M. stage 1 — ■, M. stage 2 — ○ and M. stage 3 — ◇. There is a peak in the number of labelled nuclei at MP for the specimens with M. stage-3 papillae but the younger specimens having M. stage-1 papillae have a random distribution of labelled nuclei about the papilla.
Fig. 6. The distribution of labelled nuclei in ectomesenchyme lateral to the vertical line through the midpoint of the papilla (MP). The six sampled areas on each side of MP are shown in the figure. The data for specimens having M. stage-4 (●) and M. stage-5 (□) papillae have been plotted. Both classes of specimen have a peak in the number of labelled nuclei in areas beneath the papilla (at MP) and small numbers of labelled nuclei in lateral areas.

\[ ^{3}H \]thymidine-labelled nuclei were uniformly distributed in the ectomesenchyme in the sections with M. stage-1 papillae. By M. stage 2, the zones of ectomesenchyme directly beneath the papilla had a significantly higher concentration of labelled nuclei than the zones 100 µm or more away from the central area beneath the papilla. This peak in the distribution of labelled nuclei beneath the papillae became more pronounced in specimens with papillae at M. stages
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3–5 (Figs 5 and 6). It may be seen from Fig. 4 that this peak reflects an increase in % labelling index immediately beneath the midpoint of the papilla and a decline in % labelling index away from the papilla with increasingly older specimens up to the final degenerative stages of the papilla at M. stage 6.

Until M. stage 5, an ectomesenchymal condensation was seen adjacent to the conjunctival epithelium and immediately beneath each papilla (Figs 8–10). When the papillae were in their final degenerative stages, these condensations seemed to disappear as their previous locations had lost their high % condensations. (Fig. 11). An area with numerous labelled nuclei appeared deep in the

Fig. 7. This scleral papilla in the youngest stage of development (M. stage 1) is a placode surrounded by unthickened conjunctival epithelium (ce). No condensation of ectomesenchyme was found at this stage and the labelled nuclei were randomly distributed. p, papilla; fs, fibrous sclera; pe, pigmented epithelium, e, ectomesenchyme. The donor embryo was at H.H. stage 30. Bar in 7 & 8 = 50 μm.

Fig. 8. An M. stage-2 papilla (p) explanted from a H.H. stage-31 embryo. The thickening of the papilla has caused it to extend into the subjacent ectomesenchyme (e). Numerous labelled nuclei can be seen scattered in the ectomesenchyme (e).
ectomesenchyme beneath the site of the superficial condensation but now at the future location of the scleral ossicle. The new pattern of labelling appeared just before the anlage of the ossicle became cytologically distinct for the first time (see Fig. 12). The more mature anlage had its peripheral nuclei heavily labelled but its central region was almost devoid of labelling in a condition which lasted
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Fig. 11. An M. stage-6 papilla in an explant from a H.H. stage-35 embryo. The ectomesenchymal condensation which was seen just beneath the papilla at previous stages (see Fig. 10) has disappeared. In younger stages, the labelled nuclei were numerous in the condensation close to the eye surface. Here the labelled nuclei have an accumulation deep in the ectomesenchyme at the presumptive site of the scleral ossicle (at a). Bar in 11 & 12 = 50 μm.

Fig. 12. The anlage of the scleral ossicle (a) in the H.H. stage-37 embryo. Labelled nuclei were concentrated around the periphery of the anlage. There is an absence of labelled nuclei in the preosteogenic centre of the anlage. The papilla has degenerated and disappeared by this stage. There are no longer numerous labelled nuclei in the former site of the ectomesenchymal condensation (c).

for 12–24 h until ossification commenced in the centre of the anlage. While a zone of proliferation surrounded the outer limits of the anlage, there was clearly a decline in proliferative activity between the papilla and the anlage in the previous location of the ectomesenchymal condensation.

OUR DATA RELATING TO THE ORIGIN OF THE SUPERFICIAL CONDENSATIONS WHICH LIE CLOSE TO THE CONJUNCTIVAL EPITHELIUM, AND BELOW EACH SCLERAL PAPILLA, ARE CONSISTENT WITH LOCALIZED CELL PROLIFERATION AS THE MECHANISM FOR THEIR FORMATION, IN AGREEMENT WITH HALE'S CONCLUSIONS (1956). OUR RESULTS ARE NOT CONSISTENT WITH THE POSSIBILITY THAT THE CONDENSATIONS ArISE PRIMARILY AS A RESULT OF CENTRIPEDAL MIGRATION OF CELLS. IF ECTOMESENCHYMAL CELLS WERE MIGRATING FROM BETWEEN TO BELOW PAPILLAE TO INCREASE CONDENSATION SIZE OR CELL PACKING BY PERIPHERAL ADDITION, ONE WOULD EXPECT TO SEE NUCLEAR DENSITIES DECLINE BETWEEN PAPILLAE CONCOMITANT WITH THE INCREASE BELOW PAPILLAE. SUCH A DECREASE WAS NOT OBSERVED (TABLE 1). THE POSSIBILITY THAT CELLS LEAVING THE AREA BETWEEN PAPILLAE TO TAKE PART IN CENTRIPEDAL MIGRATION MIGHT BE REPLACED BY PROLIFERATION OF ECTOMESENCHYMAL CELLS BETWEEN PAPILLAE CAN BE RULED OUT BY THE OBSERVATION THAT LEVELS OF PROLIFERATION BETWEEN PAPILLAE PROBABLY DECLINED ACCORDING TO THE LEVELS OF % LABELLING AFTER M. STAGE 3.

AS THE CONDENSATIONS ADJACENT TO THE PAPILLAE DISAPPEARED AT THE END OF PAPILLA DEVELOPMENT, % LABELLING DECLINED IN THE ECTOMESENCHYMEX EXPECT IN THE AREA OF THE OSSICLE PRIMORDIA, DEEP IN THE SCLERA. THE DISTRIBUTION OF LABELED NUCLEI AROUND THE PERIPHERY OF THE OSSICLES AND LACK OF LABEL IN THE CENTRE OF OSSICLES FOR A PERIOD OF 12–24 H INDICATED THAT PROLIFERATION AND DNA SYNTHESIS WAS TEMPORARILY CONFINED TO THE PERIPHERY. THIS PATTERN WHICH PRECEDED OSSIFICATION PARALLELS THE DECLINE IN CENTRAL PROLIFERATION IN THE DERMAL CONDENSATIONS WHICH PRECEDE FEATHER DEVELOPMENT IN THE 20–30 H PHASE BEFORE FEATHER GROWTH.
Scleral condensations proceeds (Wessells, 1965). In the two systems, a focal halt in DNA synthesis is a prelude to differentiation.

Ossification of the membranes that form the scleral ossicles occurs in beds situated 70–100 μm away from the surface of the eye and beneath the original location of the superficial condensations of ectomesenchymal cells which had contact with the papillae. It has been suggested that cells migrate away from the superficial condensations to form the deeper primordia where the ossicles actually form. Another possibility is that the condensation may get buried by new generations of less-tightly packed cells which then place it deeper in the sclera. We have yet to demonstrate the relationship between the scleral papillae, the subjacent ectomesenchymal condensations and the development of the scleral ossicles deep in the sclera. Since the sclera is readily accessible for in ovo and in vitro experiments, the papilla–ossicle system will provide a useful model to understand how membrane bones and other tissues differentiate where they do.

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