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Histological Analysis of the Development of Scleral Ossicles in Chick Embryo

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Abstract

The morphogenesis of conjunctival papillae, the early development of scleral ossicles, and the condensation of mesenchymal cells located subjacent to the papillae were investigated in tissues from the eye walls of chick embryos at HAMBURGER and HAMILTON (H.H.) stages 28 to 38. Early papillae were found as flat thickenings of the conjunctival epithelium at H.H. stage 30. By H.H. stage 32, the central region of each thickening formed a slight bulge above the general level of the conjunctiva and projected steeply downward into the mesenchyme where a slight cellular condensation beneath each papilla was noted. The papillae then began to degenerate from the interior of the downward projection. Simultaneously, mesenchymal condensation started to migrate downward and flatten out, and collagen fibers appeared. These fibers extended from the basal surface of the papilla among mesenchymal cells. Scleral ossicle primordia could be seen at H.H. stage 35. The cells of the flattened mesenchymal condensation started to transform into osteoblasts and the collagen fibrils of future scleral ossicles appeared among them. Later during H.H. stages 37 to 38, when the papillae were either absent or in the final stage of degeneration, the earliest calcification of scleral ossicles was apparent. Quantitative examination of mesenchymal condensation showed cellular density to be significantly higher beneath the papillae than in tissues between papillae in H.H. stage 31 to 35 embryos. The condensation disappeared with the degeneration of papillae. The roles of conjunctival papillae, mesenchymal condensation and primordia in the development of scleral ossicles are discussed.

Introduction

The eyes of all birds contain a ring of overlapping plates of membrane bones known as scleral ossicles encircling the margin of the cornea⁵⁾. The primordium of each scleral ossicle arises as a condensation of mesenchymal cells of neural crest origin subjacent to transient thickening of conjunctival epithelium designated as conjunctival papilla^{12,14,17)}. In chick embryos, 14 papillae arise in an orderly sequence starting at HAMBURGER and HAMILTON¹⁰⁾ (H.H.) stage 30 (6.5 days of incubation) in each eye directly overlying future sites of the 14 scleral ossicles. Each of these papillae is thus thought to have an

inductive role in scleral ossicle formation^{4,8,11,15,16)}. The first papillae appear over the ciliary arteries and later papillae form about these until the ring of papillae is complete at H.H. stage 33 (8 days of incubation)^{1,4)}. Each papilla undergoes complex morphogenesis which MURRAY¹³⁾ has divided into six stages. During papilla morphogenesis, localized areas of high cell density, cellular condensations, develop in the scleral mesenchyme subjacent to the papillae. HALE⁷⁾ examined the distribution of mitotic figures in the perilimbic region of the sclera in only four chick embryos roughly staged at 7, 8, 9 and 10 days of incubation and attributed the origin of the condensations beneath each of the papillae to regional differences in mitotic activity in the mesenchyme. Mesenchymal condensation preceding intramembranous osteogenesis has been given only little attention.

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This study was conducted to clarify the development and fate of papillae, the early development of scleral ossicles, and the mesenchymal condensation at various stages of papilla development.

Materials and Methods

Fertile eggs of White Leghorn chicken were incubated in a forced-draft incubator at 37.6°C and 55% relative humidity from 5.5 to 12 days to obtain embryos at H.H. stages 28 to 38.

As a check on the stages of papilla development described by MURRAY¹³⁾, blocks of the eye wall including the papillae and underlying mesenchyme were fixed in 10% neutral buffered formalin. They were then dehydrated in a graded series of ethanols, cleared and embedded in paraffin. Serial sections were cut at 6 μ m with the plane of the section perpendicular to the front of the eye and in line with the three papillae. The sections were mounted on glass slides, and stained with hematoxylin and eosin or with Azan stain.

For quantitative examination, only the section through the center of the papilla number 12, overlying the temporal long ciliary artery, was used in each specimen. Papilla number 12 was chosen to reduce variation in the tissue used, since each papilla and ossicle develop on their own time scales⁴⁾. The number of mesenchymal cells was counted in sections within specified areas using an ocular reticle as a guide at $\times 400$ magnification. The area examined in each specimen included the mesenchyme extending from the basal surface of papilla number 12 to the inner fibrous sclera in one dimension and the tissue between papilla number 12 and two neighboring papillae in the other dimension.

Results

Histological observation

H.H. stages 28 to 29

No papillae were found in embryos at these stages.

H.H. stage 30

Early stage in the development of papillae was evident. The earliest papilla was a flat thickening of the epidermis in which periderm cells appeared unchanged while the basal layer was two or three nuclei deep, the whole being about twice the thickness of the unaltered epidermis (Fig. 1).

H.H. stages 31 to 32

Differentiation between central and peripheral regions of each thickening was apparent. The central region began to bulge downward into the mesenchyme, and upward above the general level of the conjunctiva (Fig. 2). The thickness of each papilla was greatest at the center. During these stages, mesenchyme underlying papillae began to show definite condensation, the extent being greatest near the downward projection of the central portion of the papilla, with gradual thinning noted in all directions. In more advanced papillae, the downward projection had increased in size to give rise to a large conical structure (Fig. 3).

H.H. stages 33 to 34

Mesenchymal condensation beneath papillae was more conspicuous than in H.H. stage 31 to 32 embryos (Fig. 4). The whole condensation had essentially the rough form of a thick disc, but later, it appeared as a thick column extending from the papilla to the boundary with fibrous sclera. The principal observation in these stages was the presence of fine collagen fibers which stained with aniline blue. The fibers extended from the basal surface of the papilla into the area of mesenchymal condensation. An important change in more advanced specimens was regression of the downward projection, accompanied by further increase in the elevation of the papilla above the general surface of the conjunctival epithelium.

H.H. stage 35

Following the disappearance of the downward projection, the surviving portion of the papilla stood higher above the general surface of the conjunctival epithelium than at earlier stages (Fig. 5). The papilla became more elongated and narrow. Large vacuoles and basophil granules in the cells continued to show

their degeneration. Numerous collagen fibers ran from the basal surface of the papilla down through the mesenchyme, spreading out among the cells. The mesenchymal condensation began to spread outward, flattening itself above the fibrous sclera. In the flattened part of the condensation, very delicate blue-staining collagen fibrils could be seen between mesenchymal cells. These were the beginnings of collagen fibrils of future scleral ossicles.

H.H. stage 36

The final regression of papillae began at this stage. Each papilla had a filiform structure (Fig. 6). The descending collagen strand itself emerged from the base of the papilla usually as a single thick fiber running downward, fraying out into smaller fibers and fibrils, which continued to diverge and branch with approach to the bone anlage.

H.H. stage 37

Only three or four papillae were present. A descending collagen strand could clearly be seen in a few cases, but could not be traced to the bone anlage formed in the sclera at a depth of 70–100 μm from the conjunctival surface (Fig. 7). In other cases, scraps of collagen were embedded in the mesenchyme at the base of the papillae. Ossicle formation had advanced, but it was apparently still uncalcified. The

cells of developing osteoid took on the features of osteoblasts, with increased quantity of their cytoplasm and irregularly rounded or polygonal in shape.

H.H. stage 38

The papillae had completely disappeared. The bones at this stage showed increased thickness and surface area, and were situated between two layers of periosteum (Fig. 8). Neighboring bones overlapped with no periosteum between them. Each bone, which was calcified, appeared to be a simple plate. The outer and inner surfaces were thickly covered with osteoblasts. A few osteocytes could be seen in the bone matrix.

Cell density in mesenchyme beneath or between conjunctival papillae

The data in Table 1 show the density of mesenchymal cells counted within specified areas beneath or between conjunctival papillae in H.H. stage 28 to 38 embryos. Cell density was the same in mesenchyme in equivalent areas beneath and between papillae in H.H. stage 28 to 30 embryos. The specimens from H.H. stage 31 to 32 embryos were the first to show higher cell density in mesenchyme beneath the papillae than between papillae. This was due entirely to increased cell density beneath the papilla, in that

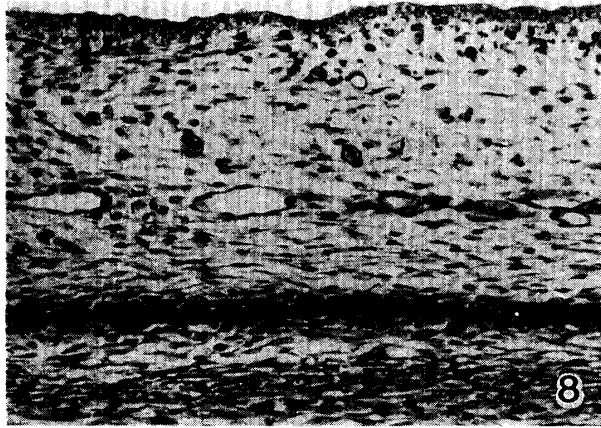
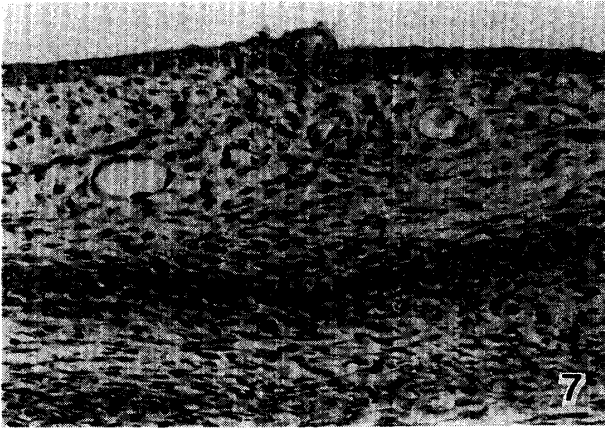
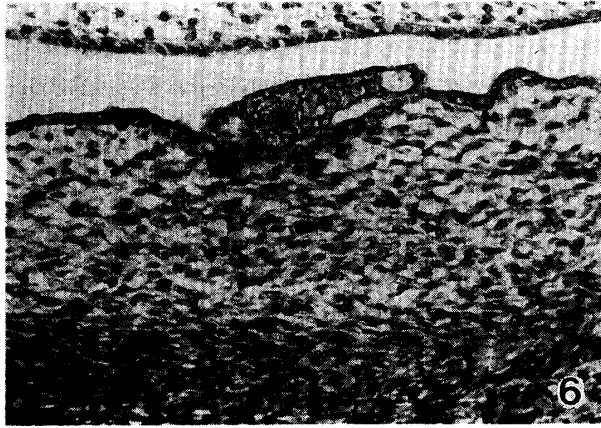
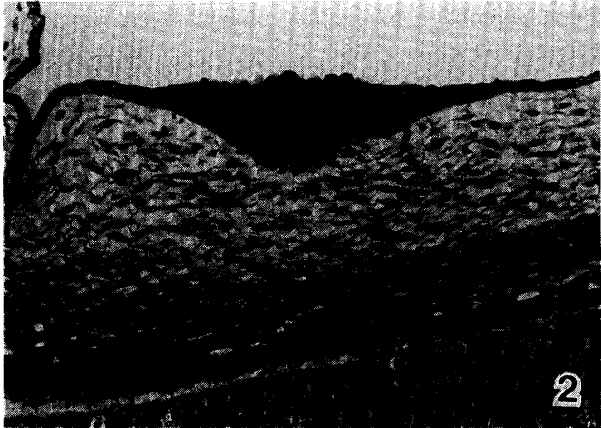
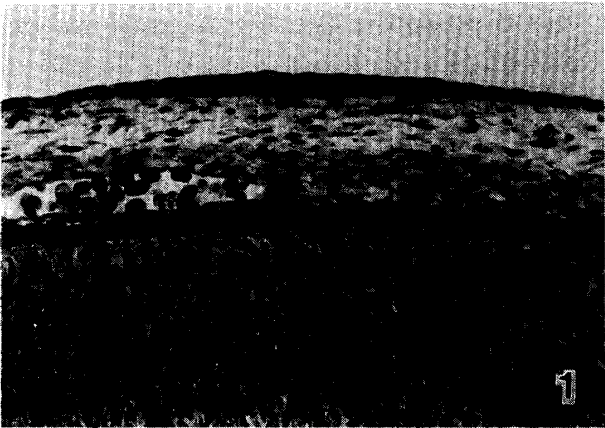
Table 1. Cell density in mesenchyme beneath or between conjunctival papillae as a function of embryo stage

H.H. stage of embryo	No. of embryos	Cell density ^a	
		Beneath papilla	Between papillae
28–29	7	16.2 \pm 0.7	15.6 \pm 0.8
30	8	17.1 \pm 0.9	16.8 \pm 1.0
31–32	10	21.8 \pm 0.6 *	18.1 \pm 0.7
33–34	12	24.8 \pm 0.7 *	21.9 \pm 0.6
35	10	27.3 \pm 0.9 *	23.6 \pm 0.6
36	6	23.5 \pm 1.2	22.2 \pm 0.7
37	9	18.7 \pm 1.3	17.6 \pm 1.0
38	7	15.8 \pm 0.8	15.4 \pm 0.7

Results are means \pm SEM.

^aExpressed as the number of mesenchymal cells per 1000 μm^2 .

*Values are significantly different from those between papillae at the same stage ($P < 0.01$).



cell density between papillae remained essentially unchanged compared to that seen between papillae in H.H. stage 30 embryos. With embryo stage advancement, cell density increased in mesenchyme beneath and between papillae. In H.H. stage 31 to 35 embryos, increase in cell density was more marked in condensation beneath papillae than between papillae. Maximal cell density was observed in specimens from H.H. stage 35 embryos. When the embryos reached H.H. stage 36, cell density in both areas had decreased below that observed at H.H. stage 35. Later, mesenchymal cell density beneath and between papillae was not significantly different from each other.

Discussion

Examination of conjunctival papillae in this study confirmed the adequacy of MURRAY's staging (M stages 1-6)¹³⁾ and demonstrated the morphogenesis and growth of papillae. The elongation of papillae, followed by

basal constriction, cell degeneration, and ultimate loss of the entire structure, is a very elaborate means of removing a structure that has fulfilled its morphogenetic role. PUCHKOV¹⁶⁾ has proposed that cell migration is vitally essential to papilla formation. Certainly, the surface area occupied by the base of a papilla decreases rapidly at earlier stages. At later stages, loss of the downward projection and elongation of papilla contribute to the morphogenesis and growth of papillae. The precise role of a papilla in the initiation of scleral ossicles remains obscure. MURRAY¹³⁾ observed fibers of material which he called "descending collagen strands" originating at the basal surface of the papilla as early as H.H. stage 31. In his study of various developmental stages of papillae, he noted fibers to descend internally from the papilla, through the column of condensed cells orientated vertically among the fibers, to the anlage of the scleral ossicle where they fanned out to become continuous with newly formed osteoids. Collagen strands extending from papillae to

Explanation of Figures

Photomicrographs in all figures are taken at $\times 200$. Figures 1, 3 and 5-8 show photomicrographs of sections stained with hematoxylin and eosin, and figures 2 and 4 with Azan stain.

- Fig. 1. A conjunctival papilla from an H.H. stage 30 embryo. This papilla in the earliest stage of development is a placode surrounded by unthickened conjunctival epithelium. No condensation of mesenchyme can be seen at this stage.
- Fig. 2. A conjunctival papilla from an H.H. stage 31 embryo. The papilla shows early formation of the downward projection. There is diffuse condensation in the mesenchyme beneath the papilla.
- Fig. 3. A conjunctival papilla from an H.H. stage 32 embryo. The papilla has a cone-shaped downward projection into mesenchyme and exhibits an upward projection on the conjunctival surface. Mesenchymal condensation in the tissue beneath the papilla is evident.
- Fig. 4. A conjunctival papilla from an H.H. stage 34 embryo. The downward projection of the papilla has maximally developed. Condensation of mesenchymal cells is densest close to the downward projection of the papilla. Slender collagen fibers from the downward projection run among mesenchymal cells.
- Fig. 5. A conjunctival papilla from an H.H. stage 35 embryo. The downward projection of the papilla has disappeared. There is extensive degeneration in the central mass of cells in the papilla, with the formation of vacuoles.
- Fig. 6. A conjunctival papilla from an H.H. stage 36 embryo. The papilla has degenerated to a filiform projection. There is no longer condensation of mesenchymal cells beneath the papilla.
- Fig. 7. An anlage of the scleral ossicle from an H.H. stage 37 embryo. A platelike condensation of cells presages the development of membrane bone deep in the mesenchyme beneath the thin filiform papilla.
- Fig. 8. A scleral ossicle from an H.H. stage 38 embryo. Developing bone shows increased thickness and is covered with numerous osteoblasts on both surfaces.

underlying mesenchyme may possibly be essential to the initiation of ossicle formation. Although experimental evidence is currently lacking, a plausible mechanism may be derived from available morphological evidence. Since collagen strands are specifically localized beneath papillae and absent from mesenchyme between papillae, and growth of these strands continues inward from the epithelium, papillae may direct in some manner the polymerization of collagen. At later stages, when collagen strands extend from papillae to the mesenchyme, mesenchymal cells are oriented parallel to the axes of strands along their entire length. If collagen strands beneath papillae direct mesenchymal migration to the pre-ossicular plate, papillae may at least be responsible for localizing the bone anlagen. Factors responsible for ossification in these regions are unknown, but would not arise in papillae, since these structures are separated considerably, and papillae are already undergoing disruption at the time of pre-ossicular plate formation.

The present data show that mesenchyme has a uniform cell density beneath and between papillae in H.H. stage 28 to 30 embryos. Mesenchymal condensation could be detected in fibrous sclera subjacent to papillae at H.H. stages 31 to 32. In histological sections from H.H. stage 33 to 34 embryos, this condensation appeared as a thick column of tightly packed, predominantly vertically orientated cells extending from the basal surface of the papilla to fibrous sclera. At H.H. stage 35, a column of vertically orientated cells extending internally from the papilla to the fibrous sclera could still be seen. Within this column, a flat condensation just above and parallel to the fibrous sclera, the anlage of the scleral ossicle, was seen. Osteogenesis commenced at H.H. stage 37 just as the papillae went through their final degenerative stage and as the column of vertically orientated cells between the papilla and the ossicular bed disappeared. HALL⁹⁾ suggested that the development of skeletal derivatives of condensations depends on a threshold number of

mesenchymal cells accumulated in the condensation. The role of collagen fibers in pre-ossicular condensation formation may either be to promote mitosis or migration so that a sufficient number of cells for osteogenesis accumulates. Various studies demonstrate regional differences in mitosis associated with the development of mesenchymal condensation beneath papillae. Condensing mesenchyme, corresponding to the area of collagen fiber accumulation beneath papillae, has a significantly higher mitotic index than mesenchyme overlain by unthickened epithelium^{6,7,19)}. The mitotic index peaks in mesenchyme in contact with the basal surface of a papilla when collagen fibers appear most prominent in the same area. FYFE and HALL⁶⁾ showed that there was a halo of dividing cells about the newly formed plate of osteoid while proliferation ceased in the bone anlagen. Experimental evidence for migration as a mechanism for the accumulation of cells in condensation in ossicular beds is lacking. Stromal fibroblasts have a predominantly horizontal orientation between papillae, while mesenchymal cells directly beneath each papilla are orientated vertically at H.H. stages 35 and 36^{13,18,19)}. This may be evidence of internally directed mesenchymal cell migration along or between descending collagen strands. Additional evidence for internal migration may be the dispersal of necrotic debris from the basal surface of a degenerating papilla to the depth of the ossicular bed along the length of descending collagen strands^{13,18)}. Particle movement may possibly be due to displacement by migrating cells¹⁸⁾. Thus, tissue beneath papilla may promote cell migration in a way essentially the same as that of neural crest cell migration^{2,3)}.

In this study, collagen fibers were noticeably concentrated beneath papillae at H.H. stages 33 to 35 when mesenchymal condensation could clearly be seen in contact with the basal surface of a papilla. Collagen fiber localization was most distinct when mesenchymal condensation was maximal. Papillae of H.H. stage 35 to 36 embryos had descending collagen strands extending beneath papillae to the

ossicular bed. When the ossicular bed was undergoing differentiation into membrane bone, with the appearance of osteoblasts in developing bone, collagen strands disappeared and accumulated delicate collagen fibrils could still be seen beneath papillae. Thus, change in the distribution of collagen fibers corresponds very closely with the time of pre-ossicular condensation. MURRAY¹³⁾, in examining histological sections of eyes between 7 to 12 days of incubation, noted fine aniline blue-positive fibrils confined to the subpapillary region as descending collagen strands. The strands extending from the basal surface of a papilla into the mesenchyme were vertical and stained more intensely with aniline blue than the predominantly horizontal fibers in surrounding stroma. COULOMBRE *et al.*⁴⁾ traced fibers in the same area, which stained positively with PAS stain. VAN DE KAMP¹⁸⁾ found numerous banded fibrils with an interval periodicity of 60 μm in channels open to the mesenchyme along the basal surface of papillae. Based on the restricted distribution of aniline blue-positive collagen fibers beneath papillae, these fibers may have a role in determining the location of scleral ossicles.

References

- 1) AMBROSI, G., M. E. COMOSSO and L. RONCALI : *Boll. Soc. Ital. Biol. Sper.*, **49**, 135-140, 1972.
- 2) BRONNER-FRASER, M. E. : *Develop. Biol.*, **91**, 50-63, 1982.
- 3) BRONNER-FRASER, M. E. : *J. Cell Biol.*, **98**, 1947-1960, 1984.
- 4) COULOMBRE, A. J., J. L. COULOMBRE and H. MEHTA : *Develop. Biol.*, **5**, 382-401, 1962.
- 5) COULOMBRE, A. L. and J. L. COULOMBRE : *Develop. Biol.*, **33**, 257-267, 1973.
- 6) FYFE, D. M. and B. K. HALL : *J. Embryol. Exp. Morph.*, **73**, 69-86, 1983.
- 7) HALE, L. J. : *Quart. J. Microscop. Sci.*, **97**, 333-353, 1956.
- 8) HALL, B. K. : *J. Embryol. Exp. Morph.*, **66**, 175-190, 1981.
- 9) HALL, B. K. : In *Factors and Mechanisms Influencing Bone Growth*, ed. by A. D. DIXON and B. G. SARNAT, pp.205-215, Alan R. Liss Inc., New York, 1982.
- 10) HAMBURGER, V. and H. L. HAMILTON : *J. Morph.*, **88**, 49-92, 1951.
- 11) JOHNSON, L. G. : *Develop. Biol.*, **30**, 223-227, 1973.
- 12) JOHNSTON, M. C., D. M. NODEN, R. D. HAZELTON, J. L. COULOMBRE and A. J. COULOMBRE : *Exp. Eye Res.*, **29**, 27-45, 1979.
- 13) MURRAY, P. D. F. : *J. Anat.*, **77**, 225-240, 1943.
- 14) NODEN, D. M. : *Develop. Biol.*, **67**, 296-312, 1978.
- 15) PALMOSKI, M. J. and P. F. GOETINCK : *J. Exp. Zool.*, **174**, 157-164, 1970.
- 16) PUCHKOV, V. : *Arkh. Anat. Gistol. Embryol.*, **46**, 16-24, 1964.
- 17) STEWARD, P. A. and D. J. MCCALLION : *Develop. Biol.*, **46**, 383-389, 1975.
- 18) VAN DE KAMP, M. : *J. Exp. Zool.*, **169**, 447-462, 1968.
- 19) VAN DE KAMP, M. and S. R. HILFER : *J. Embryol. Exp. Morph.*, **88**, 25-37, 1985.

ニワトリの胚子における強膜骨の発生に関する組織学的解析

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要 約

ニワトリの強膜骨の形成過程を明確にするため、HAMBURGER and HAMILTON (H.H.) stage 28 より 38 までの胚子の眼球壁について、各時期の組織変化を観察するとともに強膜における間葉細胞の分布状態を検索した。その結果、強膜骨の骨組織形成に先行して、H. H. stage 30 ごろより、瞳孔を輪状に取り囲んで14個の結膜上皮の部分的増殖が認められた。すなわち、それらの上皮細胞は急速に増数して細胞塊を形成し、上皮表面から乳頭状に突出した。このような結膜乳頭の基底部下方の強膜内には間葉細胞が密集し、それらの細胞の間に多数の膠原線維の伸長が観察された。その後、乳頭の内部から細胞の退行変性が進み、乳頭は次第に縮小し、やがて消失した。このような結膜上皮における組織変化にほぼ並行して、各乳頭の位置に当る上皮下の強膜内で間葉細胞が索状に配列し、H.H. stage 37 にはそれらの細胞から分化した骨芽細胞の間に類骨組織が出現した。類骨組織へのカルシウム沈着はH.H. stage 38 において明瞭となり、各骨組織は拡大して両端が互いに重なり合った。また各時期の間葉細胞の分布状態について計測した結果、H.H. stage 31 より 35 までの胚子において結膜乳頭下の強膜で細胞密度が著しく増加した。しかし、その計測値は乳頭の退行とともに減少し、H.H. stage 38 には乳頭下の強膜と乳頭間のそれとの間で差がほとんど認められなくなった。