Cell Tissue Res. 201, 409–422 (1979)

Cell and Tissue Research


# Studies on Fish Scale Formation and Resorption

## III. Fine Structure and Calcification of the Fibrillary Plates of the Scales in *Carassius auratus* (Cypriniformes: Cyprinidae)*

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**Summary.** Electron microscopic investigation of scales of the goldfish *Carassius auratus* revealed that the lamellae of fibrillary plates contain sheet-like structures composed of vertically oriented collagen fibers embedded in an organic matrix. The fibers (TC fibers) are smaller in diameter (35–45 nm) than those of the lamellae and the matrix is stained intensely with lead citrate.

The sheet-like structures as well as the lamellae are formed by fibroblasts located beneath the lamellae. The orientation of the collagen fibers of the sheets and the lamellae seems to be controlled by the orientation of the ridges and invaginations of the surface of the fibroblasts.

The fibrillary plate of *C. auratus* was found to be partially calcified. Calcification was initiated by the deposition of needle-like or flaky crystals of hydroxyapatite in the organic matrix of the sheet-like structure and proceeded into the TC fibers and the matrix region of the lamellae. The potassium pyroantimonate-osmium tetroxide method showed a heavy concentration of calcium in the osteoblasts, fibroblasts, and in the matrix regions of the fibrillary plate. Calcium-containing precipitates were also present in the "hole zone" of the collagen fibers in the lamellae, but the significance of this location in calcification remains to be elucidated.

**Key words:** Fish scale – Fine structure – Calcification – Fibrillary plate – *Carassius auratus*.

Scales of teleost fish are composed of two layers, the upper osseous layer and the lower fibrillary plate. The fibrillary plate is known to be composed of multiple layers of lamellae, each of which is filled with parallel collagen fibers and an organic

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0302-766X/79/0201/0409/$02.80

matrix (Neave, 1940; Oosten, 1957; Wallin, 1957; Brown and Wellings, 1969; Waterman, 1970; Yamada, 1971; Kobayashi et al., 1972; Maekawa and Yamada, 1972; Olson, 1976; Yamada and Watabe, 1979).

The fibrillary plate is partially calcified in some species, such as *Leuciscus rutilus* (Wallin, 1957), *Fundulus majalis* (Cooke, 1967), *Hippoglossoides elassdon* (Brown and Wellings, 1969), *Cyprinodon variegatus* (Olson, 1976) and *Fundulus heteroclitus* (Yamada and Watabe, 1979), and uncalcified in other species, e.g., *Salmo gairdnerii irideus* (Maekawa and Yamada, 1970), *Brachydanio rerio* (Waterman, 1970) and *Oncorhynchus keta* (Yamada, 1971). Yamada and Watabe (1979) reported that calcification of the plate in *Fundulus heteroclitus* proceeded by invasion of needle crystals of hydroxyapatite in the interfibrous spaces of collagen from the upper calcified osseous layer.

During the study of the fibrillary plate of the scale of the goldfish *Carassius auratus,* the present authors have found a new structure within the lamellae that seems to play a significant role in calcification. This paper describes the detailed morphology of this structure and calcium distribution, and discusses the process of calcification of the fibrillary plate.

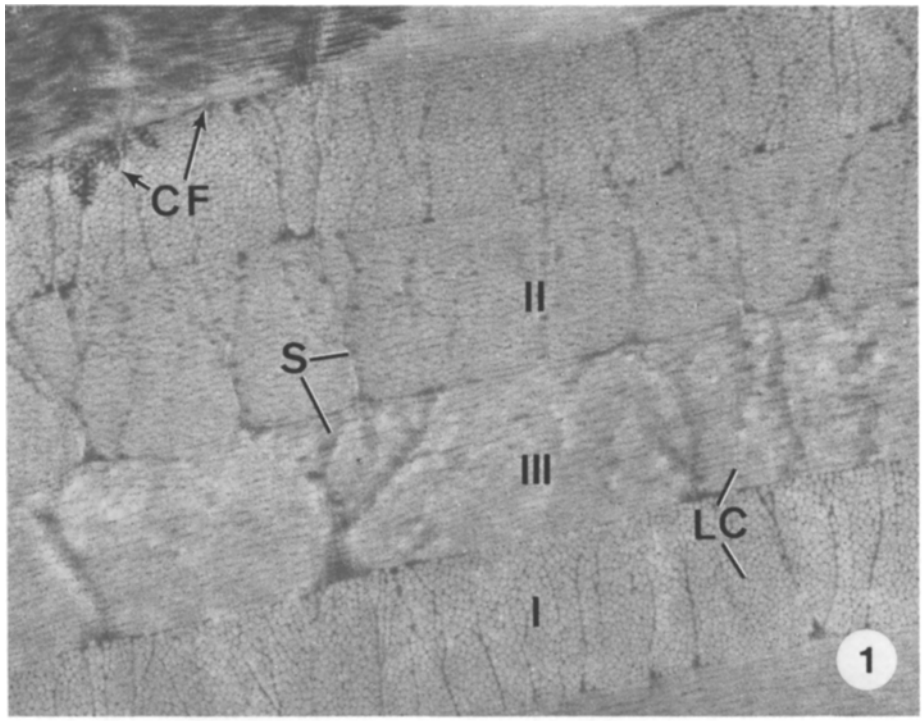
## Materials and Methods

Goldfish, *Carassius auratus,* weighing 2.0 to 4.8 g were obtained commercially and kept in a 10-gallon glass aquarium with dechlorinated tap water maintained at 19–21°C. They were fed on fish food pellets. From each fish, 5 to 10 scales were removed from the two rows on both sides of the anterior lateral line, usually at the left side of the body. A total of 60 to 70 scales were sampled from 10 fish. A regenerated scale which was formed after the removal of a scale was included in the study for observation of active fibroblasts. The scales were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for 2 h, postfixed with 1% osmium tetroxide in the same buffer for 1 h, dehydrated through an ethanol series, and embedded in Spurr resin (Spurr, 1969). Ultrathin sections were cut with a Sorvall Porter-Blum MT-2 ultramicrotome using a diamond knife. Sections were unstained, or stained with uranyl and lead citrate, and observed with JEOL 100-B or Zeiss EM-9S electron microscopes. Some sections were decalcified by floating on a drop of 0.13 M 2Na EDTA. For identification of the crystal type, electron diffraction analysis was performed on unstained sections.

For localization of calcium, scales were fixed in 1% osmium tetroxide containing 2% potassium pyroantimonate following the method of Simson and Spicer (1975). They were then dehydrated, embedded in Spurr medium and sectioned as described previously. Unstained sections were observed with a JEOLJSM-U3 scanning electron microscope equipped with a transmission detector, and elemental composition of the precipitates was analyzed with EDAX-EDIT energy dispersive x-ray microanalysis system.

## Results

The fibrillary plate of the goldfish is composed of stratified lamellae. Each lamella is 1 to 3 μm thick and made up of tightly packed collagen fibers (Figs. 1, 6), which will be called LC fibers (lamellar collagen) in the present report. The directions of orientation of the LC fibers within each lamella were identical. The directions of orientation of the LC fibers between any two adjacent lamellae differed, but were not aligned at right angles to each other. The LC fibers are embedded in an amorphous matrix and spaced at the intervals of 8 to 15 nm. In cross section, the LC



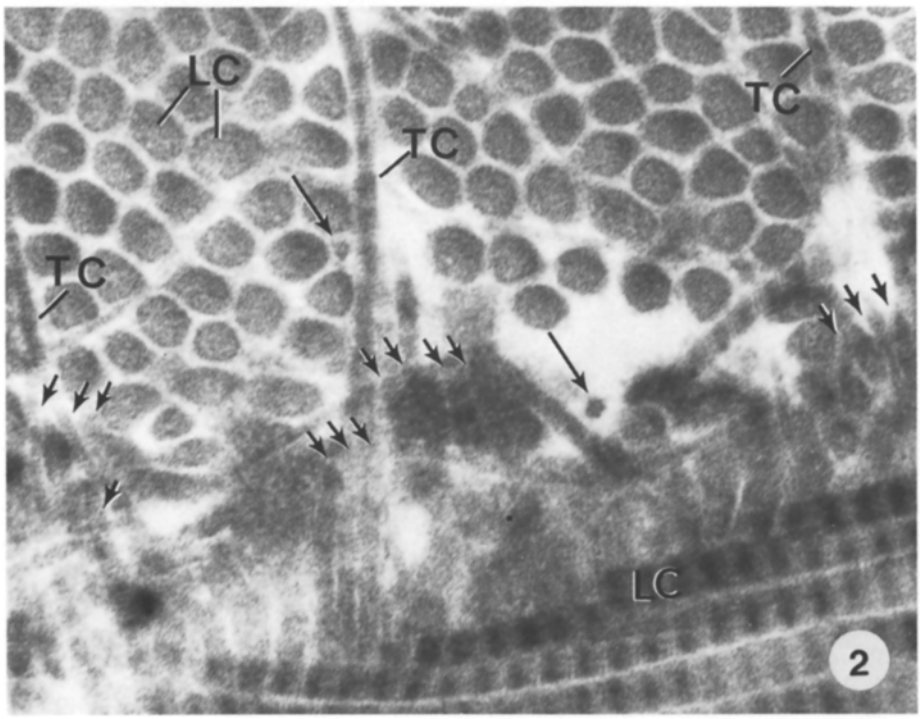




**Fig. 1.** Cross section of a fibrillary plate in *Carassius auratus*. The upper two lamellae are partially calcified. Sheet-like structures (*S*) appear as thin and distinct when *LC* fibers (*LC*) are cut transversely (*I*), but thick and obscure when fibers are cut obliquely (*II, III*). *CF* calcifying front. Unstained. ×10,400

**Fig. 2.** High-magnification electron micrograph of a boundary region between two lamellae. Several fibers (*short arrows*) diverge out from the TC fibers (*TC*) in a fan-shaped fashion at the base of lamellae. Thin fibers (*long arrows*) intermingle among LC fibers (*LC*). Uranyl-acetate and lead-citrate stain. ×80,500

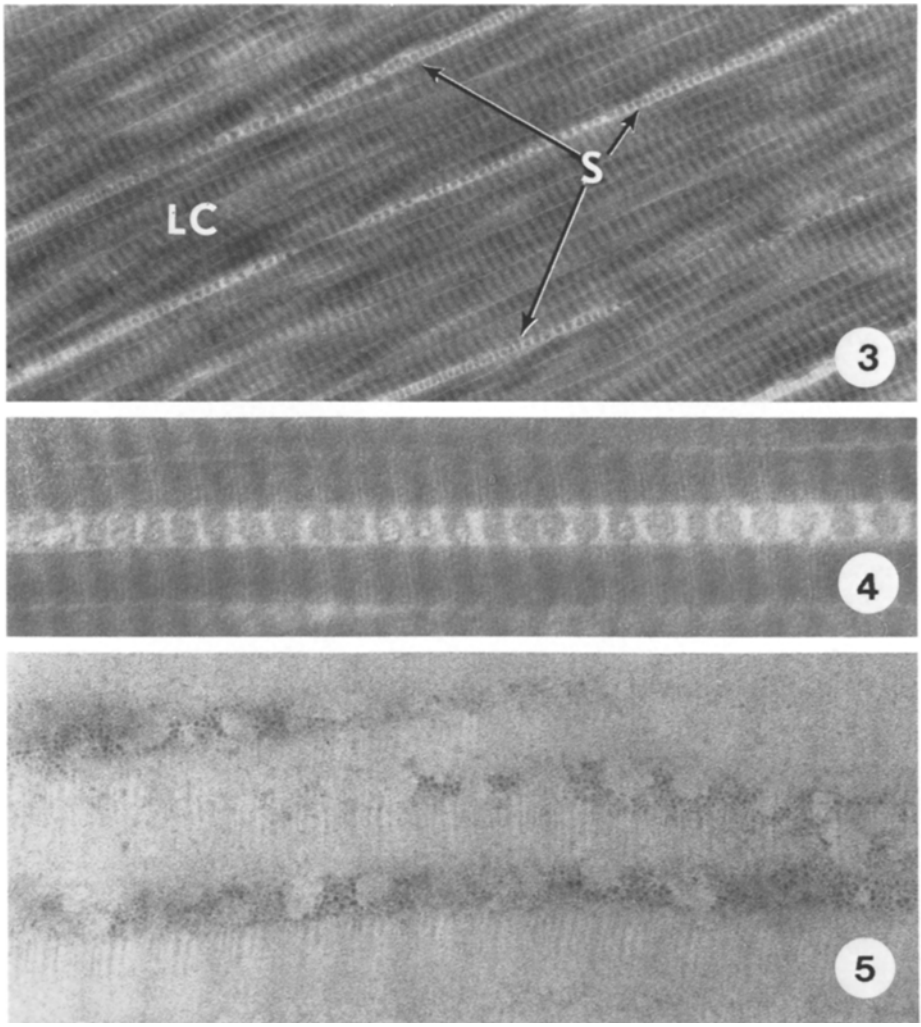



**Fig. 3.** Horizontal section of a lamella. Cross sections of sheet-like structures (*S*) are seen between the LC fibers (*LC*). Uranyl-acetate and lead-citrate stain. × 23,900

**Fig. 4.** Enlargement of a sheet-like structure as shown in Fig. 3. Uranyl-acetate and lead-citrate stain. × 93,000

**Fig. 5.** Sheet-like structure stained with lead citrate. The matrix is stained intensely and granular structures are evident. × 135,000

fibers exhibit polygonal outlines which measure 65 to 100 nm in diameter (Fig. 2). A few thinner fibers with a diameter of 20 to 40 nm are interspersed among the thick fibers (Fig. 2). When stained with uranyl acetate, longitudinal sections of LC fibers display distinct bands with a periodicity of 62.5 nm (Figs. 2–4, 7, 9).

The lamellae of the fibrillary plate were found to contain vertical sheet-like structures composed of thin collagen fibers and an organic matrix (Figs. 1–6, 8–10).

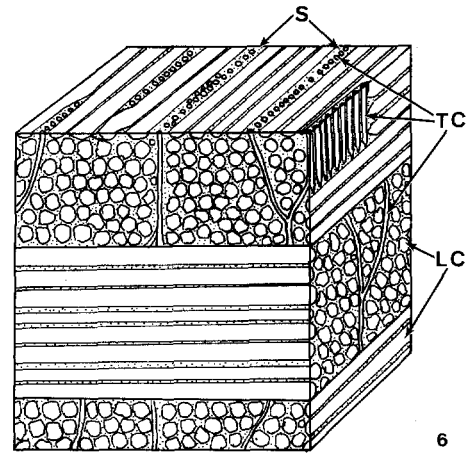



**Fig. 6.** Diagram of the lamellae. *S* sheet-like structure; LC *LC* fibers; *TC* TC fibers

The sheets are parallel to the direction of elongation of the LC fibers and spaced at intervals of 0.1 to 1 μm. The lateral length of the sheets varies from 0.3 μm to more than 10 μm. Collagen fibers, called TC fibers here, are 35–45 nm in diameter and exhibit an axial periodicity of about 62.5 nm (Fig. 2). The TC fibers contained in the sheets run in vertical directions and are mostly arranged in a single row (Figs. 3–6, 13), but sometimes several fibers can be grouped together. The lateral distance between each TC fiber is 7 to 10 nm when the fibers are relatively regularly arranged (Fig. 4), but the distance sometimes varies up to about 50 nm. In rare instances, a single TC fiber embedded in a matrix was present in a narrow space between the LC fibers.

The organic matrix of the sheets appeared to be stained more intensely with lead citrate than the matrix in the lamellae. Granular structures 4 nm in diameter are also evident (Fig. 5). No granular structures are observed in the matrix of the lamellae. Uranyl acetate appeared to decrease the contrast of this matrix (Fig. 4).

In sections where the LC fiber are cut transversely, many single TC fibers can be seen extending toward the basal portion of the lamella; also three or four fibers often converge to form a wide "trunk" (Fig. 1). A short distance above the base of the lamella, several fibers diverge out from both sides of each single fiber or each "trunk" in a fan-shaped fashion and form a triangular area at the base (Figs. 1, 2). Many of these fanned-out fibers are continuous with the TC fibers immediately below.

The lamella at the lowest level of the fibrillary plate is lined with a layer of fibroblasts (Figs. 7, 8, 10). The fibroblasts are usually squamous in shape, about 0.1 to 0.5 μm thick, and closely fitted together by interdigitation of the lateral plasma membranes. Occasional desmosomes were observed, but tight junctions could not be distinguished. Each cell contains a large single nucleus with one or two prominent nucleoli, a moderate number of mitochondria, granular endoplasmic reticulum, a Golgi complex in the supranuclear region, and occasional vesicles.

When collagen synthesis was very active, such as in the marginal region of the scale or in regenerating scales, the cytoplasm of the fibroblasts was filled with granular endoplasmic reticulum showing dilated cisternae (Fig. 7). The Golgi

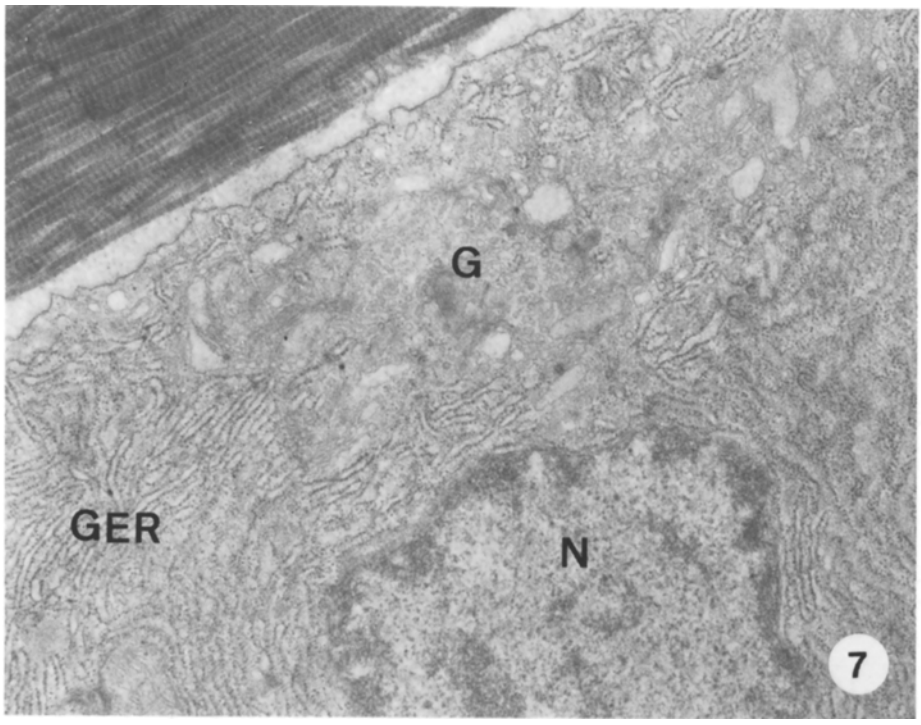



**Fig. 7.** Fibroblast (regenerating scale). The cytoplasm is filled with well-developed granular endoplasmic reticulum (*GER*) and Golgi complex (*G*). *N* nucleus. Uranyl acetate and lead citrate stain. ×43,000

complex was prominent, displaying vesicles with dense bodies and vacuoles. The cytoplasm was filled with fibrils about 5 nm thick running parallel to the plasma membrane and vesicles of about 20 to 130 nm in diameter. Some of the vesicles were fused with the membrane. The cell surfaces facing the fibrillary plate are characterized by many processes and invaginations (Figs. 8, 10), and one or two microtubules are present within each process (Fig. 9). Serial sections indicate that the processes and invaginations are outfoldings or infoldings of the cell surface running parallel to one another rather than cone-like protrusions or pits. The outer surface of the plasma membrane at each of these surface modifications is lined with fibrils, which rapidly increase in diameter with increasing distance from the cell surface and eventually become the TC fibers (Fig. 10). The remaining outer surfaces of the fibroblasts are lined with the LC fibers. The direction of elongation of these fibers is more or less parallel to the folds or to the invaginations.

### *Calcification of the Fibrillary Plate*

Except for the marginal region of the scale, a portion of the fibrillary plate in *C. auratus* was calcified (Figs. 1, 11–14). The degree of calcification differed in

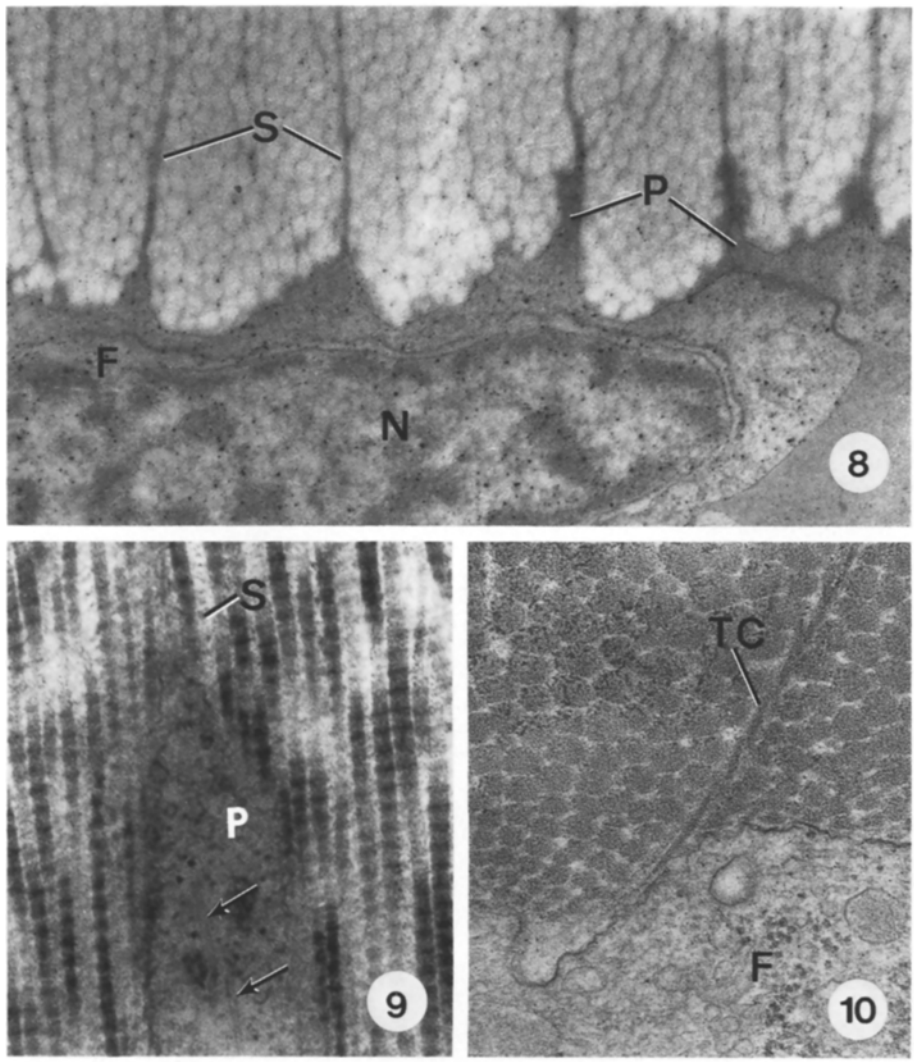



**Fig. 8.** Surfaces of fibroblasts lining the basal portion of the fibrillary plate. Many cell processes (*P*) are in continuation with sheet-like structures (*S*). *F* fibroblast; *N* nucleus. Lead-citrate stain. × 30,000

**Fig. 9.** Cell process (*P*) cut horizontally to the lamella. Two microtubules are present within the process (*arrows*). *S* sheet-like structure. Uranyl-acetate and lead-citrate stain. × 43,000

**Fig. 10.** Invagination of the cell surface of a fibroblast (*F*) showing the TC fiber (*TC*) formation. Regenerating scale. Uranyl-acetate and lead-citrate stain. × 55,600

individual animals and in different lamellar regions within a scale. For example, a fish weighing 2 g had 14 lamellae in the fibrillary plate, 7 or 8 of which were calcified close to the central portion of the scale, whereas only 1 or 2 out of 15 lamellae were calcified in another individual of similar weight. Regardless of the extent of the calcification, the uppermost lamella was always calcified in all 10 individuals observed.

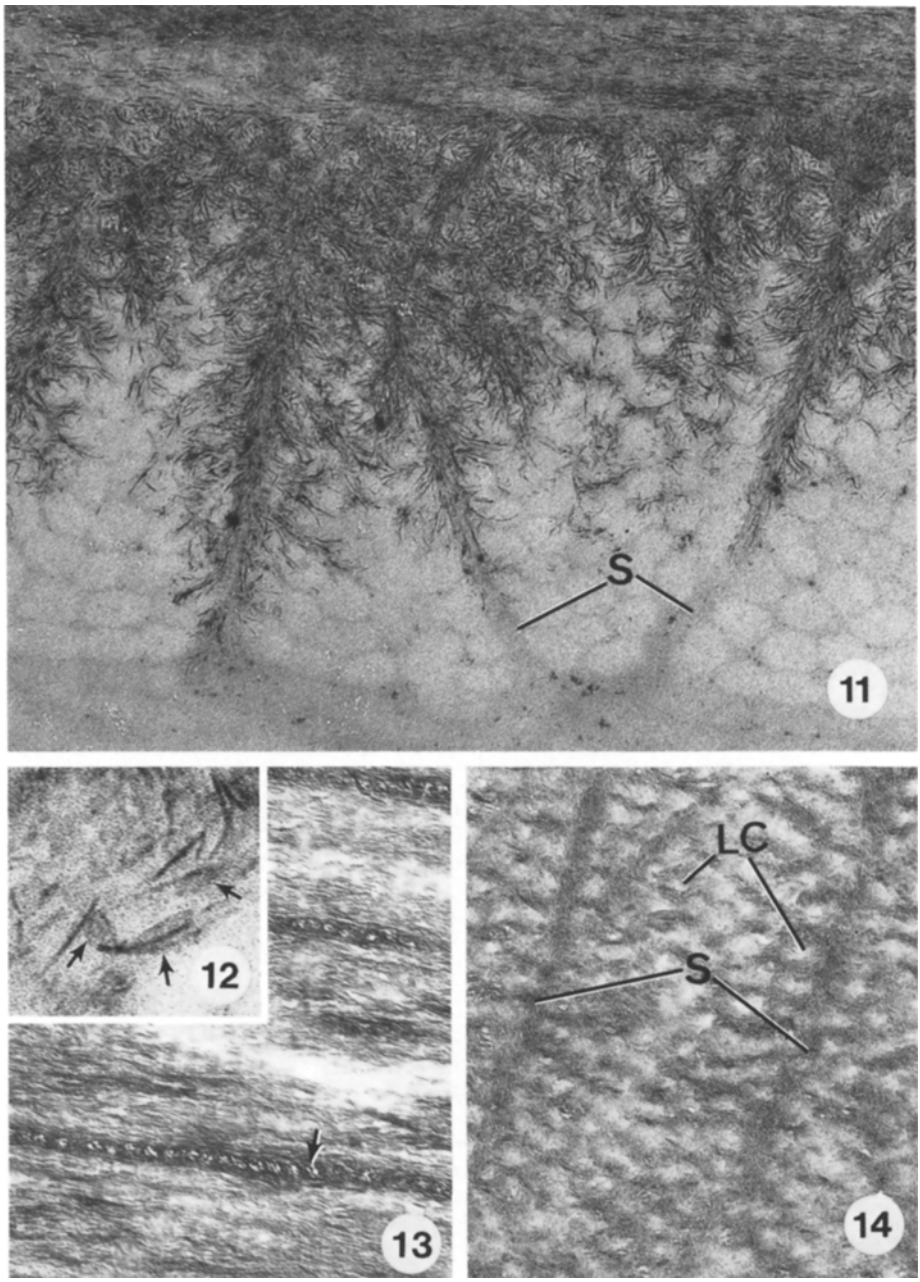



**Fig. 11.** Calcifying front of the fibrillary plate. Calcification proceeds downward along the sheet-like structures (*S*). Unstained. ×89,000

**Fig. 12.** High magnification of crystals. Both the needle crystals and the flaky crystals (*arrows*) are seen. Unstained. ×265,000

**Fig. 13.** Horizontal section of a fully calcified lamella. Some TC fibers remained uncalcified and are seen as circular, empty spaces (*arrow*). Unstained. ×45,000

**Fig. 14.** Cross section of a fully calcified lamella. Many LC fibers (*LC*) are calcified only at their surface. *S* sheet-like structure. Unstained. ×45,000

**Table 1.** Comparison of electron diffraction lines generated by the osseous layer, the calcified fibrillary plate of the scale of *Carassius auratus*, and hydroxyapatite

| Osseous layer (Å) | Calcified fibrillary plate (Å) | Hydroxyapatite[a] (Å) |
|---|---|---|
| 3.41 | 3.41 | 3.44 |
| 2.84 | 2.84 | 2.814 |
| 2.27 | 2.28 | 2.296 |
| 1.95 | 1.97 | 1.94 |
| | 1.86 | 1.871 |
| 1.75 | 1.74 | 1.754 |
| | 1.65 | 1.644 |
| | 1.55 | 1.542 |
| 1.48 | 1.49 | |
| | 1.34 | |
| 1.26 | 1.28 | |
| 1.16 | 1.17 | |
| | 1.13 | |
| | 1.05 | |
| | 0.95 | |
| | 0.88 | |
| | 0.84 | |

[a] From ASTM powder diffraction file

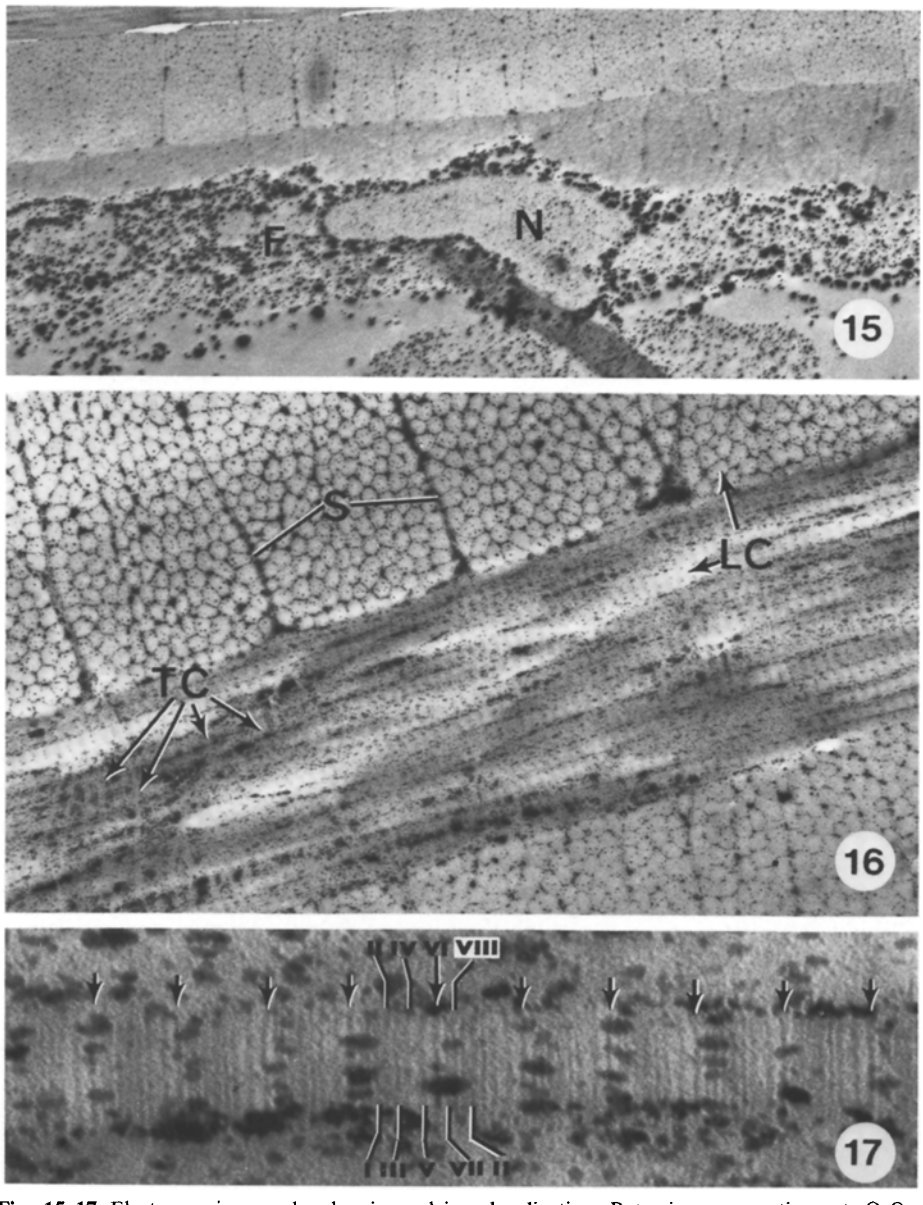
Calcification appeared to be initiated by the deposition of randomly oriented needle-like or flaky crystals in the matrix around the TC fibers; it proceeded downward along the fibers and branched out into the spaces between the LC fibers. Thus, in sections where the LC fibers were cut transversely, the calcified regions showed a configuration resembling inverted trees (Fig. 11). The needle crystals were about 60 nm long and 3 nm wide, and the flaky crystals were about 30 to 60 nm long and 15 nm wide (Fig. 12). Electron diffraction analysis of these crystals as well as of crystals in the osseous layer revealed that they consisted of hydroxyapatite (Table 1).

Although the crystals were deposited in the matrix, the TC fibers themselves did not calcify until a later stage of calcification and remained as circular, empty spaces in unstained sections cut horizontal to the lamellae (Fig. 13).

The crystal development in the matrix between the LC fibers appeared to take place almost simultaneously with that of the TC fibers, and the process was similar to that reported in *Fundulus heteroclitus* by Yamada and Watabe (1979); the crystal orientation was also random (Fig. 11).

Calcification of the LC fibers occurred subsequent to that in the matrix region and in TC fibers, and many LC fibers were calcified only at their surfaces even in heavily calcified lamellae (Fig. 14). The crystals in the LC fibers were usually oriented with their long axes parallel to the elongation of the fibers; however, the development and growth of the crystals did not seem to be structurally related to the 62.5 nm banding patterns (Fig. 13). In addition to the needle-like or flaky crystals, electron dense and amorphous areas were observed within fibers, forming regular bands spaced at a distance of 62.5 nm. These areas disappeared after staining with uranyl acetate or EDTA.

In both the sheet-like structures and in the lamellae, the degree of calcification was higher in the matrix region than in the collagen fibers.




**Figs. 15–17.** Electron micrographs showing calcium localization. Potassium pyroantimonate-$OsO_4$ fixation

**Fig. 15.** Heavy precipitations are seen in the cytoplasm of fibroblasts (*F*). Fine granules are also present in the nucleus (*N*). Unstained. × 7,600

**Fig. 16.** Uncalcified fibrillary plate. A high concentration of the precipitates is found within the matrix of the sheet-like structure (*S*). Fine granules less than 13 nm in diameter are located within the LC fibers (*LC*). *TC* TC fibers. Unstained. × 24,600

**Fig. 17.** High magnification of a horizontal section of a LC fiber. Eight intraperiodic bands are discernible (*I* to *VIII*). Granules are spindle-shaped and located between bands V and II. *Arrows*, band VI. Unstained. × 182,000

**Table 2.** Comparison of the distances between intra-period bands of the LC fibers in the scales of *Carassius auratus* with those of native collagen measured on Fig. 28 in Glimcher and Krane (1968).

| LC fibers in the scales of *Carassius auratus* | | | Native collagen | | |
|---|---|---|---|---|---|
| Band | Distance (nm) | %[b] | Band | Distance (nm) | %[b] |
| I –II | 7.8± 1.10[a] | 12.5 | $a_1$–$a_4$ | 7.9 | 11.3 |
| II –III | 7.4± 1.09 | 11.9 | $a_4$–$b_1$ | 9.5 | 13.5 |
| III –IV | 7.0± 1.21 | 11.2 | $b_1$–$b_2$ | 8.3 | 11.9 |
| IV –V | 10.9± 0.54 | 17.5 | $b_2$–$c_2$ | 13.9 | 19.8 |
| V –VI | 10.5± 1.12 | 16.9 | $c_2$–d | 10.7 | 15.3 |
| VI –VII | 7.4± 0.71 | 11.9 | d –$e_1$ | 7.9 | 11.3 |
| VII –VIII | 5.4± 0.54 | 8.7 | $e_1$–$e_2$ | 6.3 | 9.0 |
| VIII–I | 5.9± 0.52 | 9.5 | $e_2$–$a_1$ | 5.5 | 7.9 |
| Total distance (periodicity) | 62.3 nm | 100.1% | | 70 nm | 100.0% |

[a] Standard deviation from 12 measurements
[b] Percentage of the distance between each band to the periodicity

**Table 3.** Energy dispersive X-ray microanalysis of precipitates in the scale of *Carassius auratus*

| | Ca | P | S |
|---|---|---|---|
| Osteoblast-1 | 195[a](1) | 140 (0.33) | 82 (0.58) |
| Osteoblast-2 | 203 (1) | 60 (0.48) | 62 (0.41) |
| Fibroblast | 795 (1) | 181 (0.31) | 248 (0.42) |
| Fibrillary Plate-1 | 43 (1) | 40 (1.52) | 56 (1.77) |
| Fibrillary Plate-2 | 132 (1) | 170 (2.10) | 176 (1.82) |
| Fibrillary Plate-3 | 143 (1) | 141 (1.60) | 181 (1.72) |

[a] Peak intensity expressed by counts per 400 seconds. Each value of Ca was obtained after stripping Sb peak
( ) indicates the mol ratio estimated by EDAX-EDIT TEM program

### *Localization of Calcium in the Fibrillary Plate*

When the tissue was fixed with potassium pyroantimonate-osmium tetroxide, precipitation of a large number of spherical granules in the cytoplasm of osteoblasts and fibroblasts was observed (Fig. 15). The granules in the osteoblast were larger ($\sim$0.5 μm) in diameter than those in the fibroblasts (0.06 μm). The nuclei in both cell types contained finer granules. A high concentration of the precipitates was also seen throughout the uncalcified portions of the fibrillary plate and the matrix regions of the TC fibers (Fig. 16). The granules were not discernible in the heavily calcified areas due to the similar density of the crystals.

The granules within the LC fibers were less than 13 nm in diameter and their number was relatively small (Figs. 16, 17). The potassium pyroantimonate-osmium tetroxide fixation revealed eight intra-periodic bands in the longitudinal sections of the LC fibers (Fig. 17) (Table 2). These bands were numbered I through VIII.

Granules in these sections were spindle-shaped, less than 31 nm long, and located between band V and band II, but not between bands II and V.

Using x-ray mircoanalysis, the granules were shown to contain Ca, P, and S (Table 3). Os, K, Sb, and Cl were also present, but these elements were derived from the fixative and the embedding resin; thus, they are deleted from Table 3. As shown in Table 3, calcium content was found to be higher than P and S in the osteoblasts and fibroblasts, but was less in the fibrillary plate.

## Discussion

The present study reveals for the first time that the lamellae of the fibrillary plate of the goldfish *Carassius auratus* contain sheet-like structures composed of collagen (TC) fibers and organic matrix. The exact nature of this structure is unknown at present. However, the diameters of the TC fibers are smaller than the LC fibers; the organic matrix in the sheets stains more intensely with lead than in the lamellae and contains a granular structure. Therefore, these two types of collagen fibers and matrices are assumed to be structurally and chemically different.

Similar to the fibrillary plate reported previously (Brown and Wellings, 1969; Maekawa and Yamada, 1970; Yamada, 1971), the sheet was also found to be formed by fibroblasts. The outer surfaces of the cellular outfoldings and invaginations were lined with fibrils, which extended vertically following the slopes of these surface modifications and increased in diameter to eventually become the TC fibers. This indicates that the surfaces of the folds and invaginations are the sites of secretion of the fibrils of the TC fibers, and that the surfaces direct the orientation of the TC fibers.

Secretion of the LC fibers and their matrix presumably takes place at the surfaces between the outfoldings and invaginations. The ridges and valleys of these surface modifications are parallel with one another, and the areas of the secretion of LC fibers can be considered to be elongated parallel to these structures. Thus, the topography and orientation of the surface structure of the fibroblasts seem to determine the orientation of the TC and LC fibers.

The configuration observed at the base of each lamella, i.e., several TC fibers diverging out from a seemingly single fiber or from a wide "trunk" in a fan-like fashion may indicate that the surface folds of the fibroblasts gradually shift their orientation along the cell surface during the secretion and formation of the TC fibers. The shift takes place synchronously at the end of formation of each lamella. When the shift is completed, the grooves, now having a new direction of orientation, will accordingly direct the orientation of LC fibers of a new lamella being formed. The microtubules in the cytoplasm of the folds may control the rotation of these folds. It is not inconceivable that the fibroblasts themselves make a horizontal revolution to change the orientation of the TC fibers. However, this does not seem probable since the fibroblasts are tightly fitted together by interdigitating plasma membranes.

In contrast to the report by Maekawa and Yamada (1972), the present study shows that the fibrillary plate of the goldfish *Carassius auratus* is partially calcified. This discrepancy may be due to the differences between the individual animals. This raises the question whether all teleost scales possess calcifiable fibrillary plates. It

seems worthwhile to reexamine the species in which the fibrillary plate is reported to be uncalcified, e.g., the rainbow trout, *Salmo gairdnerii irideus* (Maekawa and Yamada, 1970), chum salmon, *Oncorhynchus keta* (Yamada, 1971), and zebrafish, *Brachydanio rerio* (Waterman, 1970).

This study clearly shows that the sheet-like structure is the major site of calcification in the goldfish fibrillary plate. Calcification is initiated in the organic matrix region of the TC fibers, proceeds into the TC fibers and almost simultaneously into the matrix region of the LC fibers. The LC fibers are the last to be calcified. Calcification of TC and LC fibers is not as heavy as that of the matrix regions.

In *Fundulus heteroclitus,* the sheet-like structure was not reported; however, the interfibrous spaces (the matrix region) were the first to calcify (Yamada and Watabe, 1979). Therefore, regardless of the possible differences in the overall structures or chemical characteristics, the organic matrices in different structures of the fibrillary plate of these fish apparently contain materials serving as nucleation sites for hydroxyapatite crystals. This is in agreement with Cameron (1972), who reported a similar role of the matrix in bone formation. Our results from the potassium pyroantimonate-osmium tetroxide method, which showed heavy calcium-containing precipitates in the matrix region, further substantiate the ability of the matrix to concentrate calcium ions.

The precipitates were also found in the LC fibers; specifically, each spindle-shaped granule spanned the spaces between the intra-period bands V and II. Simson and Spicer (1975) also reported similar precipitates occurring at intervals of 60 nm in collagen. By measuring the distance between the bands, $a_1$, $a_4$, $b_1$, $b_2$, $c_2$, d, $e_1$, and $e_2$ on the photograph of the native collagen cited by Glimcher and Krane (1968), we have found that our bands I through VIII correspond to their bands $a_1$, $a_4$, $b_1$, $b_2$, $c_2$, d, $e_1$, and $e_2$, respectively (Table 2), and the distances between V and II relative to the periodicity are similar to those between $c_2$ and $a_4$. The spaces $c_2$–$a_4$ are reported to be the "hole" zone of the collagen (Hodge and Petruska, 1963) and, accordingly, intra-collagen calcium in the goldfish is considered to be located in the holes. (The hole zone is actually $c_2$–$a_3$; however, $a_3$ was not clearly identified in their photograph and is very close to $a_4$. Since $a_4$ is located at the end of band group $a_1$, $a_2$, $a_3$, $a_4$, the distance $c_2$–$a_4$ was considered to be the hole zone here.) In spite of the precipitates that occurred in the hole zones, we were not able to observe crystal nuclei within these areas as reported by previous investigators (see Glimcher and Krane, 1968; Höhling et al., 1971). This does not necessarily imply that the hole zones are not the sites of nucleation of crystals in the LC fibers. Further studies may show that the calcification of the LC fibers is initiated in the holes.

The nature of the electron-dense areas in the fibers showing a periodicity of 62.5 nm is not known. The fact that these structures disappeared after uranyl acetate or EDTA treatment may indicate that they represent amorphous calcium phosphate or carbonate. The latter was found to be present in an appreciable amount in goldfish scales (Watabe and Weiss, unpublished). More data are needed to establish the identity of this structure.

Numerous calcium-containing precipitates were localized within the fibroblasts as well as in the osteoblasts. It seems most likely that the fibroblasts

secrete, in addition to collagen and matrix material, calcium ions to be utilized for calcification of the fibrillary plates. Olson (1976), in his study of the sheepshead minnow *Cyprinodon variegatus,* also suggested a similar function for fibroblasts.

*Acknowledgement.* The authors wish to thank Dana G. Dunkelberger, Tom Bargar and Andrea S. Blake for their excellent technical assistance and Carm Finneran for typing the manuscript. This study was supported by NSF grant PCM 77-01528 and PCM 77-27866, and a fund from the Electron Microscopy Center, University of South Carolina.

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Accepted June 23, 1979