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Author(s)	YAMADA, Jurô
Citation	北海道大學水産學部研究彙報, 7(3), 202-207
Issue Date	1956-11
Doc URL	http://hdl.handle.net/2115/22965
Type	bulletin (article)
File Information	7(3)_P202-207.pdf

ON THE MECHANISM OF THE APPEARANCE OF THE SCALE STRUCTURE

VI. Some Observations Associating with the Absorption of Scale in the Goldfish

Jurô YAMADA
Faculty of Fisheries, Hokkaido University

Foreword

In order to take materials for the histochemical study of alkaline phosphatase, goldfishes were reared in the laboratory for four months as reported in the previous paper. During that period, the writer obtained various stages of regenerated and absorbed scales. It was noticed regarding these scales that the calcified portion differs in area individually with a certain regularity. The phenomenon will be dealt with in this paper. Other observations on the formation of radii, which are the radial grooves on the bony layer of the scale, and on the manner of absorption of the scale under mal-condition for the fish will also be described. These phenomena will be discussed on the common basis of being associated with the absorption of scale.

Material and Method

The scales were taken from the goldfishes which were being reared in four groups. At the beginning, certain scales were removed from each fish to obtain the regenerated scale. Group "WF" means the fishes under the condition of feeding in warm (about at 20°C) water; in this manner, each "WS", "CF", and "CS" means the condition of, in turn, starving in warm water, feeding in cold (about at 5°C) water, and starving in cold water. The numerals followed these initials represent the number of days in rearing. For instance, WF-50 means the fish reared at 20°C for 50 days under food supply.

For the detection of the depositing calcium salts of the scale *in toto*, the writer tried with advantage the procedure of the latter half of Gomori's calcium-cobalt method for alkaline phosphatase. A fresh scale was rinsed in 2% solution of cobalt chloride for 5 minutes, then, the deposit of calcium phosphate or carbonate was changed to cobalt phosphate or carbonate. After thorough washing in distilled water, the scale was transferred into the dilute solution of yellow ammonium sulphide, about 2 minutes; thus the site of calcium salts was demonstrated as black precipitate of cobalt sulphide.

Results and Consideration

The normal growing scale treated with cobalt sulphide method shows a deposit of calcium salts covering almost the whole surface excepting the outermost margin. The uncalcifying margin in which one or two circuli are usually counted is somewhat narrower in the anterior area than in the posterior. Just within the edge of the uncalcifying margin, an intensely stained band borders the central calcified area, and along either side of each radius is an area stained also in dark black, though the radius itself is never stained (Fig. 1). This is supposed to be an evidence that the just calcified margin as well as the area along the radii has a larger deposit of calcium salts than other calcified area.

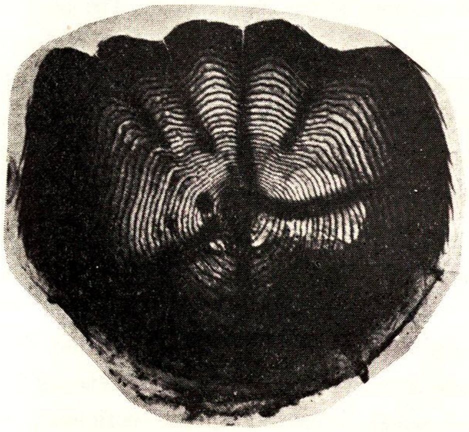

Fig. 1. Typical ontogenetic scale removed at the outset of rearing. Treated with cobalt sulphide method. Marginal and radial areas are intensely stained. × 30

In WF and WS, the regenerated scale appears in a few days. It attains almost an equal size and a similar shape with the removed ontogenetic scale within about two weeks. The feature of the regenerated scale of goldfish is characterized by a large central space showing abnormal grooved network and a marginal area in which a few concentric ridges are arranged in considerably wide intervals. The regenerated scales in WF and WS show no difference in scale structure at early stages.

The calcified central areas of these scales are stained uniformly in dark black, and the

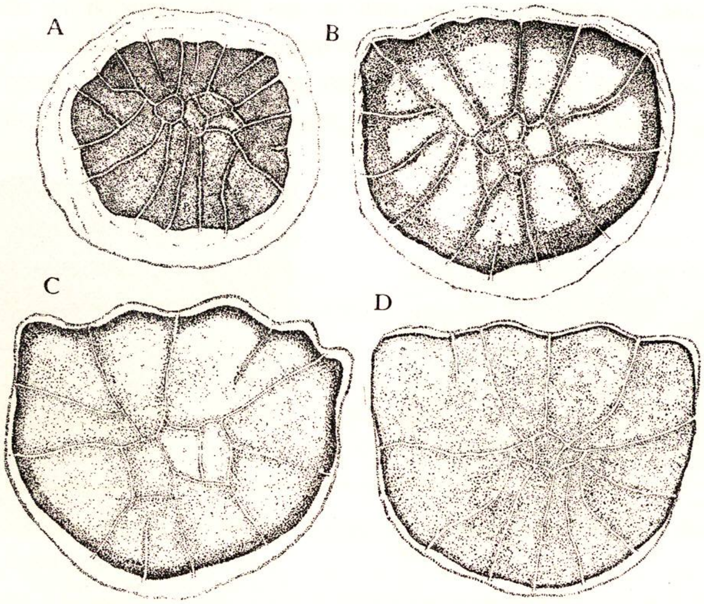

Fig. 2. Semi-diagrammatic drawings showing the order of decalcification in four stages of regenerated scale. Scales were obtained respectively from: A. WF-26; B. WF-50; C. WF-78; and D. WS-50

stainless marginal areas are relatively wider than those of ontogenetic ones, while that of the scale taken from WF-50 shows pale stained portions among the radii. Finally, it comes to take a similar appearance to the ontogenetic one with respect to the calcified area, showing a dark marginal border and narrow regions along each radius. The process is shown in Fig. 2. It is faster in WS than in WF. In WS-50, most scales of both the ontogenetic and regenerated type come to be absorbed at the "shoulder". The intensely stained marginal and radial areas are reduced in the absorbed scale, and at last the areas take on the same appearance as the other pale stained area. In the fishes of CF and CS, in them no regenerated scale appeared, the ontogenetic scales are absorbed with difficulty and their mode in staining shows the preservation of calcium salts in marginal and radial areas as compared to those of WF and WS.

In interpreting the above facts, it is obvious that a part of once deposited calcium salts dissolves out from the bony layer of the scale under even normal condition, and the dissolution is accelerated in warm circumstances probably because of the active metabolism of the fish. If the absorption of scale is caused by the deficiency of calcium salts, it may take place after the dissolution of the soluble salts over the whole surface under the condition of some physiological change. That the area along the radius usually retains much deposit of the calcium salts is probably due to the difficulty of dissolution of the salts in this area for some reasons. However, it is questioned here, whether the staining of radial or marginal area is due to the artefact in the treatment of cobalt solution since the area corresponds to the absent portion of bony or fibrous layer. As control, the scale with bony layer being hurt in linear groove with a fine needle or the scale with a portion of it cut off was treated in the same manner. The vicinity of the artificial groove or of the incision showed no peculiarity in staining.

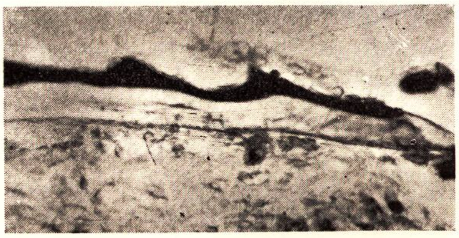

Fig. 3. Section of the absorbed scale margin. Treated with cobalt sulphide method. Uncalcified fibrillary plate is extended beyond the black stained bony layer. × 480

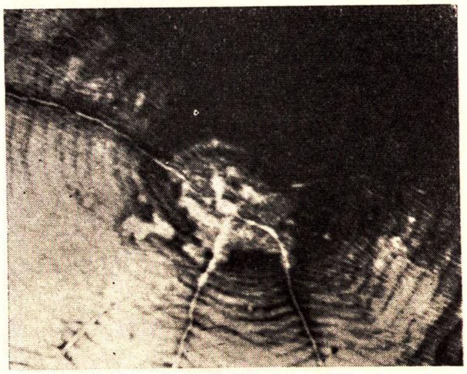

Fig. 4. A portion of the ontogenetic scale showing the absorption of the focal bony layer and exposed fibrillary plate. × 60

The scale grows at the periphery in the laying down of bony material and the fibrillary plate is formed behind this outermost margin. But in the absorbed scale, the corresponding section of absorbed edge represents the inverse relation (Fig. 3). Moreover, a crack being parallel with the absorbed margin was often observed in the flat mount of the ontogenetic scale. These observations indicate that the absorption of the scale occurs at first on the bony layer and consequently the unsupported fibrillary plate, which is left behind freely, is destroyed by mechanical rubbing action. The absorption of the bony material takes place also on the central focal area (Fig. 4).

Then, it is evident that the absorption of scale, which originally means the partial destruction of scale, involves three processes in a broad sense, that is, the dissolution of calcium salts, the erosion of bone matrix, and the mechanical destruction of fibrillary plate. The dissolution of calcium salts may be caused by the local humoral factor, because the alkaline phosphatase, which is responsible for the deposition of solid calcium phosphate, works at high pH level such as 9.0 or above, and at that level calcium phosphate is easily deposited. However, the raising of pH level *in vivo* at the site where the laying down of bony material is taking place may be probable, but usually, the humoral pH does not indicate such higher level. Accordingly, a part of the precipitate should be dissolved again as soon as the lowering of the pH level occurs. Concerning the erosion of bone matrix, the osteoclastic absorption should be kept in mind; this will be discussed below together the consideration of the manner of formation of radius.

The general ontogenetic scale of the goldfish shows a few radii cutting the intersecting circuli. This is a matter of course, for the circuli are the concentric linear elevations of bony layer while the radii are the radial grooves lacking the bony material. But it was frequently observed in the regenerated scale that certain circulus is not cut by the radius but continues across it (Fig. 5). The radius, excepting the absorbed portion, never reaches to the outermost margin, fading out in the uncalcifying bony substance. Therefore, it is quite likely that the radius elongates in the absorption of once formed bony substance. The regenerated scale grows so rapidly until it becomes restricted by the wall of scale pocket, that the above phenomenon might be caused by the elongation of radius unproportionate to the accelerated laying down of bony material. As shown in Fig. 5, a part of peripheral osteoblasts enter into the groove, and if

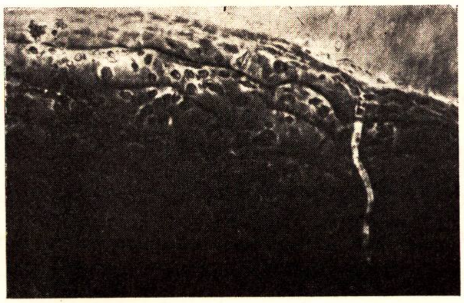

Fig. 5. Phase-contrast photomicrograph of a portion of the regenerated scale. Outermost circulus continues across the radius. Mallory's triple stain. × 160

these cells are associated with the elongation of radius, they should certainly have an osteolytic property.

It is understood that the elongation of radius as well as the absorption of scale are based on a similar phenomenon, that is, the erosion of bone matrix. Osteoclasts are found in general bone tissue especially where the absorption is taking place. The nature of their function or the stimuli which cause the cells to form, still remain as unsolved questions. They are regarded as arising from fusion of relatively immobile connective tissue cells or of mobile cells such as macrophage. The transformation of cells is said to be reversible from one to the other. In the present study, it can be assumed that the cells found in the radius may be the osteoclasts derived from the fusion of peripheral osteoblasts. Such transformation of cells is very suggestive also in the absorption of bony layer of scale.

Ichikawa (1953) reported similar results on the disappearance of calcium salts before the destruction of the scale of the carp under starvation. He observed on the surface of these scales the remarkably thinner calcified layer compared with that of normal scale and considered that the calcium salts were absorbed from the fibrillary plate, and that thereafter mechanical breaking would take place on the softened portion by decalcification. However, the calcium salts deposit by no means on the fibrillary plate without the calcium oxalate crystals demonstrated by Kato (1953), but are confined to bone matrix. The thinning of bony layer is probably due either to the dissolution of calcium salts from the intimate layer of bone or to the actual thinning of bony layer according to the decalcification. At the initial stage of bone destruction, the marginal area under absorption yet retains much deposit of calcium salts (Fig. 2D). This is a counter-evidence to the conception of the mechanical destruction of bone effected against the softened portion.

The writer is greatly indebted to Prof. S. Saitô who has critically reviewed the manuscript.

This work has been carried out partly with funds from a Grant in Aid for Miscellaneous Scientific Research from the Ministry of Education.

Summary

1) The ontogenetic, regenerated, and absorbed scales of goldfish obtained in the rearing experiment were stained by the cobalt sulphide method, and the phenomena associated with the absorption of scale were observed.

2) The absorption of scale can be interpreted in a broad sense as involving three processes, which are the dissolution of depositing calcium salts, the erosion of bone matrix, and the mechanical destruction of fibrillary plate.

3) A part of once-deposited calcium salts dissolves out from the bony layer even

under normal circumstances. This tends to accelerate as a result of active metabolism accompanied with insufficient nutrition. As a cause of the dissolution of calcium salts, the effect of lowering of humoral pH level is considered. Thereafter, the erosion of bony layer begins on the marginal area, especially on the portion of the "shoulder". It takes place also on the focal area.

4) The radius is elongated also by the partial erosion of bony material. The absorption of scale as well as the elongation of radii seems to be caused by the osteolytic property of certain cells derived in the transformation of osteoblasts.

5) The remaining free edge of fibrillary plate may be destroyed by the mechanical rubbing action.

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