ON THE MECHANISM OF THE APPEARANCE OF THE SCALE STRUCTURE

V. The Possible Role of Alkaline Phosphatase in the Formation of the Teleost Scale

Jurô YAMADA
Faculty of Fisheries, Hokkaido University

INTRODUCTION

The teleost scale which is one of the hard tissues developing in the mesodermal connective tissue under the epithelium is distinguishable from the other integumental structures such as spine, teeth, and placoid scale of the selachians etc. of ectodermal origin. This suggests that the process of scale formation corresponds with that of the membrane bone which occurs directly in the connective tissue without passing through the processes of chondrogenesis and secondary ossification after its destruction. A teleost scale consists of two portions, an upper bony layer and a lower fibrous layer. The former increases in area with growth but not in thickness and calcium salts deposit on it, while the latter, the so-called fibrillary plate, consists of several thin fibrous laminae which are not calcified. The histogenesis of two portions of the scale still remains as an undecided problem. The concentric ridges which are the most available structures for age determination are homogeneous with the bony layer and confined to it.

Neave (1936, 1940) suggested in his histological studies of the scale of the trout and the goldfish, that the stages in the laying down of the bony layer are typical of the formation of membrane bone in general. On the other hand, Robison and his collaborators (1923, 1924, 1933, 1934) offered an explanation on the mechanism of ossification from the evidence that the increase of the amount of alkaline phosphatase and glycogen is recognizable in the hypertrophic cartilage cells, that is, alkaline phosphatase is concerned in the process of ossification by effecting on phosphoric esters in the blood, thus bringing about a local increase in the concentration of inorganic phosphate ions, so that the product of the concentrations of phosphate and calcium ions becomes greater than the solubility product of calcium phosphate, which is thereupon deposited in the solid state. Later on, it was supposed that hexosemonophosphoric esters liberated in the process of glycolysis become the substrate for phosphatases. Although the opinion stated by Robison and others yet contains some unsolved problems, it is undoubtedly true that the alkaline phosphatases play an important part in the process of ossification.

During the developmental stages as well as the growth of fish scale, also, it is interesting to ascertain whether or no alkaline phosphatase exists in associated tissues. If that is so, the histochemical tests for tissue sections will offer effective pictures to determine the remaining problem concerning the histogenesis of both the bony and the fibrous layers of the scale.

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MATERIAL AND METHOD

For the observation on the developmental process of the scale, 33 young of the rainbow trout, *Salmo irideus* GIBBONS, ranging from 2.1 to 4.2 cm in body-length, were used. They were from half a month to three months old after hatching in the rearing pond of our Faculty. Besides, an about nine-months old fish, 15.5 cm in body-length, reared in the same pond, was used to observe the tissue surrounding the normal growing scale.

Early in winter, when the season causes the growth of fishes to cease, 24 of the goldfish, *Carassius auratus* (LINNÉ), were brought from the nursery. They were about half a year old, ranging from 3.5 to 4.5 cm in body-length; they were then reared continuously in the laboratory during the period from December to the following April. The fishes were divided into four groups; each was kept in a cylindrical glass tank containing five litres of water. The water temperature of two of the groups was controlled at 20 to 22°C and the other two at 4 to 7°C during the experimental period. One of the two groups respectively was fed on earthworms and grain foods; the other was kept under the starved condition. Therefore, fishes in rearing experiment consisted of four groups: fed in comparatively warmer water (WF), starved in warmer water (WS), fed in comparatively colder water (CF), and starved in colder water (CS). Previously, from the fishes in each group 15 scales were flayed off from the left body-side in 3 rows, putting the lateral line between them, just below the dorsal fin. Then the regenerated scales in the operated area and normal ontogenetic scales from the just opposite body-side were examined at intervals.

As fixatives, Bouin's and Helly's fluids for the histological preparations, cold 80% alcohol or acetone for the histochemical detection of alkaline phosphatase, and Gendre's modification of Allen-Bouin's fluid for that of glycogen, were respectively employed. The fixed materials were sectioned into 8μ by the paraffin-embedding method. In the histochemical test for alkaline phosphatase, Gomori's calcium-cobalt method, soduim-β-glycerophosphate buffered pH at 9.0 as the substrate, was employed. For the demonstration of glycogen, Bauer's cromic acid-Schiff reaction and Best's ammonia carmine reagent were used on the collodionized tissue sections of developing scale. The usual histological stainings such as Ehrlich's or Delafield's haematoxylin, Mallory's triple connective tissue stain, and Heidenhain's Azan stain were also employed.

Apart from the histochemical observation, the measurement of hydrolytic power of alkaline phosphatase in the kidneys of the fishes being under experimental conditions was once performed *in vitro* to find out about any possible relationship between the enzymic activities of the kidney and the connective tissue.

OBSERVATIONS

I. Developmental process of the scale in the rainbow trout

The skin is composed simply of two layers, a stratified epithelium having many scattered mucous cells and a subjacent comparatively thinner connective tissue layer of corium, until the scales begin to develop. Beneath the connective tissue is a pigment cell layer which divides the skin from the muscular tissue. No alkaline phosphatase is demonstrated in this stage.

When the fish grows to about 3 cm in body-length, the corium seems to differentiate into two layers: the spindle-shaped fibroblasts are seen sporadically in the upper loose fibrous layer, while the under layer consists of conspicuously compact fibrous bundles amongst which the cellular elements are rarely encountered. The fibres of the upper loose layer mostly take the blue color of anilinblue dye but the under compact fibres are stained red by the application of Heidenhain's Azan.

The fibroblasts in the loose connective tissue layer, now, begin to aggregate at regular intervals to form the "cutis papillae" or the "scale papillae"; at this stage the enzyme is first detected in the fibroblasts as black stained cells (Fig. 1). The cells transformed into the papilla are larger in size and more rounded in shape than the original fibroblasts, showing an intense activity of phosphatase. The network of blue-stained fine fibrils connecting the osteoblasts with each other can be seen in the section to which Mallory's triple stain was applied.

The cells of the scale papilla arrange themselves into two layers, and a thin origin of scale appears among them (Fig. 2). This is the origin of the upper bony layer and is similar to osteoid or preosseous tissue of the bone. It is observed, as the growth of scale advances, that the osteoblasts in scale papilla receive reinforcement of cells from the surrounding connective tissue, not from the underlying general corium, because no fibroblast possessing phosphatase activity is seen in the latter (Fig. 3). The osteoblasts adhering closely to their own products get lean and are flattened gradually; they begin to degenerate from the central region of the scale. The degeneration takes place sooner on the upper side, so the cells of the lower side remain until comparatively later (Figs. 4 and 5). The naked region resulting from the disappearance of the contacted cells calcifies simultaneously, stained in black also by Gomori's technique (for the technique shows the enzymic site as a deposition of calcium phosphate). From this fact, it is understood, as in the case of the calcification of bone, that the osteoblasts bring forth the matrix of bony layer first, and the secondary deposition of calcium

salts is effected on it by their phosphatase activities.

The remaining cells of the lower side also disappear finally, so the lower surface of the scale becomes in direct contact with the wall of connective tissue, and only around the periphery do the active osteoblasts always surround the growing scale. Thus the scale is enclosed by fibrous connective tissue named the "scale pocket". It is necessary to consider the functional difference between the upper and lower cells of the scale, although the cells of both sides are originally the same. Before the lower cells degenerate, above them uncalcifying lower layer of scale appears beneath the calcified layer. Accordingly, it is obvious that the intimate lamina of the under uncalcifying layer is the product of the remained lower cells. A discussion will be presented below how to interpret the thickening of the fibrillary plate after the disappearance of these cells. At any rate, osteoblasts of lower side remain for a while after the laying down of bony material, and break down embedding themselves in their own products (Fig. 6). The behavior of these cells resembles that of the "osteocyte" which commonly lives on in the bone matrix in general ossification.

Circulus as a linear ridge of upper bony layer is formed at the peripheral region and also calcifies (Fig.4). In tissue section, it occurs among two or three osteoblasts so as just to fill their intercellular space with bony material; the tip of the circulus is always towards the scale focus. Inner osteblast is slightly larger than outer one, but the difference in activity of alkaline phosphatase is not indicated.

The scale enlarges according to the growth of the fish; the upper bony layer grows in area by the action of marginal osteoblasts while the lower fibrillary plate increasingly thickens by the addition of lamina from the under wall of the scale pocket (Fig. 7). In the skin of nine-months-old fish, 15.5 cm in body-length, the posterior region of scale in imbricated arrangement penetrates into the epithelium, and alkaline phosphatase is demonstrated in marginal osteoblasts and fibroblasts in the connective tissue wall around the scale, being especially prominent in the former (Fig. 8). There can be seen a thin fibrous lamina on the under wall of scale pocket which is in contact with the fibrillary plate. This is considered as the most freshly formed fibrous lamina of connecitve tissue which may be taken into fibrillary plate by and by. The lamina has no cellular element and just beneath is a row of small nuclei of connective tissue cells, stained also in black, which may have fibrogenic properties.

On the histochemical tests for glycogen, polysaccharides in general, both the methods presented negative results except in the mucous cells in epithelium.

II. Scale regeneration in the goldfish

When a scale is removed from the body surface, an accompanying portion of epithelium and connective tissue is also broken off. The scales were regenerated in a few days in the warmer groups, while in the colder ones they did not appear even four

months later in spite of the rapid recovering of epithelium as in the warmer groups. The result seems to be not influenced by feeding or starving.

Because of the smallness of the tanks neither the growth of the body nor that of the scale was observed in each group during experimental period. The alkaline phosphatase was not represented in the osteoblasts or the fibroblasts surrounding the ontogenetic scale contrary to the active enzyme in the epithelial cells so differing in those of rainbow trout.

Twenty-six days after operation, in a fish of the colder water starved group (CS-26), the operated area is covered with regenerated epithelial cells under which the lost connective tissue is repaired already; the enzyme is detectable only in the epithelial cells which are unresponsible for the scale formation (Figs. 9 and 10). Similarly, in CF-78 and CS-78, the scale pocket is filled with multiplied fibroblasts, but in them no alkaline phosphatase is proved (Fig. 11). As it was thought that the alkaline phosphatase should be synthesized in the epithelial cells regenerated freshly, then, the unoperated skin of just opposite body-side was examined in the same way. The tests for the normal epithelium resulted the same as for the regenerated; the operated and unoperated regions of the skin of WF-50, WS-50, WS-83, WF-85, and WS-90 respectively offered the same pictures, the osteoblasts and fibroblasts around normal ontogenetic and regenerated scale are not stained despite the intense black of upper epithelial cells (Figs. 12 and 13). These facts seem to show that the deficiency of phosphatase under these conditions probably restrained the growth of the scale.

Eighty-six days after operation, the water temperature of CF-tank was raised to 20°C gradually, then, the fish regenerated the scales in 12 days. Both the fibroblasts in connective tissue and osteoblasts adhering to newly formed scale gave clearly positive reaction for phosphatase (Figs. 14 and 15).

The regenerating process does not indicate any essential difference with the developmental process, but it takes place in almost the whole region where scales were previously situated. Osteoblasts dispose themselves in two layers and between them the upper bony layer is formed —— likewise is the process in the rainbow trout —— and also the lower cells remaining in a layer after the disappearance of the upper one are buried away finally in their own products (Fig. 14).

On the regenerated scale, the cellular elements are often seen to be rather more numerous than on the ontogenetic one because of its rapid growing (Fig. 16). Inner attached cells are clearly visible along each ridge especially of the inner region of the scale, while the wide-spread cell-masses of osteoblasts can be seen near the marginal ends of the ridges; between them there are the degenerating osteoblasts under transformation to the inner attached remaining cells. As the capillaries running above and below the scale are destroyed by the scale removal operation, blood corpuscles are often seen

in company with the scale. It would seem that the irregularity of their appearance may have introduced the confusion in the interpretation concerning the cellular contributions to the scale, regarding them as the cells which associate with the scale formation.

Generally, a kidney is known to be abundant in alkaline phosphatase in addition to the osseous tissue. As the appearance of regenerated scale was assumed to be influenced by water temperature, it was questioned whether any correlation exists with phosphatase activity of kidney and connective tissue under various conditions. Accordingly, an attempt was made *in vitro* to measure the enzymic activity of kidney obtained from the fish of 50 days after operation in each group. Water-extracted enzyme was added in the substrate containing known amount of phosphoric ester and after incubation of 18 hours at 36°C, liberated inorganic phosphate was measured by means of photoelectrocolorimeter. The result is given in Table 1.

Table 1. Alkaline phosphatase activity of the kidneys of the goldfishes reared for 50 days under various conditions. Eighteen hours incubation at 36°C in the substrate containing sodium-β-glycerophosphate in veronal buffer adjusted pH at 9.0. Colorimetric measurement was performed by Youngburg & Youngburg's method.

Experimental condition	Fish		Org.-P in substrate (mg)	Inorg.-P liberated per 10mg of kidney, less than control (mg)	Org.-P hydrolysed (%)
	Body-length (cm)	Body-weight (g)			
WF	4.45	2.50	0.054	0.036	66.1
WS	4.00	1.65	〃	0.032	58.8
CF	4.00	2.80	〃	0.019	34.9
CS	4.50	3.60	〃	0.005	9.2

Perhaps owing to the active metabolism of the fishes in warmer groups, their fatness is conspicuously lower than that of those in the colder one. The enzymic activity of the kidney is larger in the order of WF, WS, CF, and CS. The difficulty of sacle regeneration in the fish at lower temperature is supposed to be caused by the intracellular synthetic weakness of alkaline phosphatase due to the inactivation of internal metabolism.

DISCUSSION

The alkaline phosphatase is synthesized in associated cells whenever a scale origin is established or a scale is regenerated, but it is not demonstrated at the time when scale growth is retained. This confirms the idea that the enzyme is indispensable for the laying down or growing of a scale as in the case of bone. The deposition of calcium phosphate as a principal bone salt is a result undoubtedly of the action of alkaline phosphatase, but the manner of production of bone matrix, which may be albuminoid such as ossein, is yet uncertain, though it is well accepted that the enzyme

associates in the formation of intercellular fibrils because of its general occurrence in fibrogenic cells. At any rate, bone matrix is formed primarily by the osteoblasts containing active alkaline phosphatase and calcium salts deposit secondarily on the matrix.

The substrate for alkaline phosphatase *in vivo* is said to be probably hexose-monophosphoric esters formed in phosphorolysis of glycogen (Harris, 1932). Jackson (1955) reported the existence of polysaccharide and protein components in the cytoplasmic granules of the fibrogenic cells of the fowl embryo and suggested that the substances may have fibrogenic properties and may synthesize the collagen fibrils. In the present study, polysaccharides were not proved in osteoblasts of rainbow trout at the scale developing stage. That would seem to be due to the fact that substance is actually absent or that there is only a little present in this case, or that there was some deficiency in the technique of examination. However, in the section on which Mallory's triple stain was employed, blue stained network of fine fibrils connecting the osteoblasts of papilla was seen. These fibrils, judging from their affinity with aniline blue dye, may be supposed to be identical with the osteogenic collagenous fibrils which commonly appear as the precursor of bone.

The cells enclosing the just-formed material show equally positive reaction for Gomori's technique (Fig. 2); they begin to degenerate in the upper layer before they do in the lower (Fig. 5). This is more remarkable in the regenerating stage in the goldfish (Fig. 14). As the upper layer of scale calcifies, it stains in black also by Gomori's technique; between the calcified layer and lower remaining osteoblasts an uncalcifying layer can be distinguished. Hitherto, this layer has been considered as the intimate layer of fibrillary plate, so that, concerning the cellular contributions of the bony and the fibrous layers several different opinions have been advanced. One of them is that the fibrillary plate is laid down by the lower layer cells of the papilla while the bony material is the product of upper elements (Ussow, 1897; Paget, 1920). Another opinion is the supposition of the cell migration around the margin of scale from upper to lower (Creaser, 1926), or from lower to upper side of scale (Setna, 1934). It is said that the same cells produce different materials according to the local movement.

These opinions can not explain the fact of the thickening of fibrillary plate all over the surface which is now in contact directly with the under wall of scale pocket after the disappearance of these lower cells. Setna's view is based on the observation that the fibrillary plate precedes the bony layer, but the actual fact is really the opposite.

Furthermore, there is an opinion which seems to be most likely, viz., that the original cells of the papilla contributes merely to the formation of upper bony layer and it grows only at the periphery while the fibrillary plate is formed as succesively larger laminae being added from the under wall of scale pocket after the degeneration of

interposed osteoblasts (Klaatsch, 1890, 1894; Neave, 1936, 1940; Yamada and Saito, 1953). However, the appearance of the uncalcified layer before the degeneration of the interposed osteoblasts is contradictory to this opinion, if this layer is truly the intimate lamina of the fibrillary plate.

The writer considers the reason of this contradiction as existing in the misunderstanding which makes this layer a part of the fibrillary plate. According to his opinion, the intimate layer of the fibrillary plate is probably not the true fibrous lamina but is uncalcifying bone in which the lower cells are buried. As Neave pointed out, the behavior of lower osteoblasts resembles that of the osteocytes which are normally alive in the bone matrix in general osteogenic process. In the scale, they degenerate forming the uncalcifying layer which is indistinguishable from the fibrous laminae being added later from the underlying connective tissue. At the periphery of the scale only the bony material is laid down, neverthless, inner calcified layer is often less thick than the outermost yet uncalcifying bone. This is a further evidence showing that whole bone matrix does not always receive the deposition of calcium salts.

On the under wall of the scale pocket being in contact with the fibrillary plate, newly formed fibrous lamina beneath which a row of small connective tissue cells, which also show enzymic activity, may be seen. In considering the fibrogenic property of these cells, the existence of alkaline phosphatase is acceptable as a matter of course; notwithstanding, the calcium salts do not deposit on the fibrillary plate. This is probably due to the difference in the physiological state of the two sides of the scale.

The occurrence of concentric ridges has much importance in view of the availability of these structures for the determination of the age of the individuals. There have been approximately three different opinions about above subject. Many investigators noticed a row of cells attaching closely to the inner side of each ridge and supposed that the ridge may be secreted by these cells.

Nusbaum (1907) considered that some of the upper cells may have osteolytic property and that they may absorb the bony material to form the ridge. Also, Neave (1936) suggested the possibility of the osteoclastic absorption of these inner cells and described that the ridges appear to be formed as much by a hollowing out behind as by a building up in front, but he changed his opinion in a later paper (1940). Setna (1934) stated that the ridge is formed in the damming up of scale margin by the restrictive action of scale pocket. Neave's later opinion allows the restrictive action of scale growth itself but not of scale pocket. Setna's view should be denied since the ridges are not always parallel with scale margin or they appear in the regenerated scale before the margin reaches to the pocket wall. Suzuki (1952) reported the gradual increase in the number of ridges being formed inwards on the regenerated scale of goldfish. If his observation is correct, Neave's later opinion should have also to be damaged. As

mentioned above, the setcion of scale in process of the ridge formation shows slightly larger cells closely attached to the inner side of it. Their nuclei are also larger than those of other osteoblasts but the reaction for alkaline phosphatase is the same as that of the others. It must be noticed here that the artefact owing to the diffusion of the precipitates during the incubation in the substrate is unavoidable in the treatment by Gomori's procedure. Hence, the cytochemical specificity of the inner attached cells along the ridge is somewhat uncertain whenever the present method is employed.

The osteoclast is usually a multinucleated giant cell which is said to be derived from the fusion of connective tissue cell or macrophage or even from any cell under histiocytic state (Hancox, 1949). The occurrence of osteoclasts from certain osteoblasts under some stimulation is likely to happen. The present writer can not refer to these inner large cells with regard to their osteolytic property, but the possibility of their possession of such property should be further investigated.

Kato (1953) reported the linear precipitates of calcium carbonate on the inner side of the ridge and of calcium phosphate on the outer side. It is not unreasonable to suppose that the cells in question may have other enzyme which liberate carbonate ions.

The epithelium of the goldfish shows invariable activity of alkaline phosphatase contrary to that of the rainbow trout, so it is supposed that physiological difference of the epithlial cells exists between the two species.

The circumstances, which make the internal metabolism of fish inactive, introduce the idea of the decrease of the alkaline phosphatase activity of the kidney, and probably also of connective tissue. The active metabolism accompanied with sufficient nutrition may bring forth the growth of the scale as well as the body, but in the case with insufficient nutrition it may result in the absorption of scale. However, the regeneration of the scale is controlled chiefly by the former condition and seems to have almost no connection with the latter.

SUMMARY

Histochemical tests for alkaline phosphatase and glycogen were employed on the paraffin sections of the skin of the rainbow trout as well as that of the goldfish. The phosphatase activity of the kidney of the goldfish reared under various conditions was measured *in vitro*. The results may be summarized as follows:

1) The alkaline phosphatase relating to the scale formation is demonstrated in the associated cells during the course of development in the rainbow trout and of regeneration in the goldfish.

2) The osteoblasts around normal growing scale and the fibroblasts in the wall of scale pocket both have the active enzyme, while it is not detectable when the growth of scale is retained.

3) The epithelial cells of the goldfish in any conditions show invariable phosphatase activity contrary to those of rainbow trout.

4) Glycogen, common in cartilaginous ossification, could not be demonstrated in the developing stage of the scale of the rainbow trout.

5) In the goldfish reared constantly at about 20°C, the regenerated scale appeared in a few days, on the other hand, it was not regenerated even in four months in the fish reared at about 5°C. When the water temperature was raised, the scale appeared in 12 days. The appearance of regenerated scale seems to be not affected by the nutrition.

6) General course of the development and regeneration of the scale was described. As in the case of bone formation, the fibroblasts, the osteoblasts, and possibly the osteocytes play a part in the formation of the scale.

7) On the appearance of the ridge, further investigation with regard to the possibility of osteoclastic absorption or other enzymic activity of inner attached cells should be made.

8) The calcium salts deposit on the bony layer of scale, but not in the deeper layer of it, which is so indistinguishable from the fibrillary plate that the name the "lower layer of scale" is not always synonymous with the "fibrillary plate".

9) The comparative values of the activity of alkaline phosphatase in the kidney were measured *in vitro*. The value varies according to the enviromental conditions in which fishes are living.

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PLATE I

Explanation of Figures

Fig. 1. A section of the skin of the rainbow trout, 3.3 cm in body-length. Black stained fibroblasts aggregate to form the scale papilla under the epithelium. The black layer of pigment cells is not due to the phosphatase reaction. Gomori's Ca-Co method. × ca. 360. e: epithelium, gc: general corium, f: fibroblasts, m: muscle tissue, p: pigment cell layer, sp: scale papilla

Fig. 2. A section of the skin of the rainbow trout, 3.3 cm in body-length. The thin scale origin appears among the rounded osteoblasts of scale papilla. Gomori's Ca-Co method. × ca. 1200. e: epithelium, gc: general corium, os: osteoblasts, so: scale origin

Fig. 3. A section of the skin of the rainbow trout, 3.3 cm in body-length. The osteoblasts of scale papilla receive reinforcement of marginal fibroblasts. Gomori's Ca-Co method. × ca. 1200. f: fibroblasts of surrounding connective tissue, so: scale origin

Fig. 4. A section of the skin of the rainbow trout, 3.3 cm in body-length. The scale grows horizontally and the ridge appears in the peripheral region. Gomori's Ca-Co method. × ca. 1000. ic: inner attached cell of ridge, os: osteoblast, r: ridge, sb: bony layer of scale

Fig. 5. A section of the skin of the rainbow trout, 3.3 cm in body-length. The osteoblasts begin to degenerate from the central region of scale. Some of them remain in close adherence on the lower surface of the scale. Gomori's Ca-Co method. × ca. 1200. gc: general corium, oc: osteocyte, os: osteoblasts, p: pigment cell layer

Fig. 6. A section of the skin of the rainbow trout, 3.5 cm in body-length. The posterior and anterior portions of two scales in imbricated arrangement. Calcified layer stains also in black. Gomori's Ca-Co method. × ca. 2000. f: fibroblasts, oc: osteocyte, r: ridge, sb: calcified bony layer

Fig. 7. A section of the skin of the rainbow trout, 4.1 cm in body-length. The fibrous laminae are added from the under wall of connective tissue, so that, the scale comes to consist of calcified and uncalcified layers. The uncalcified layer is not always identical with the fibrillary plate. Gomori's Ca-Co method. × ca. 1700. sb: calcified bony layer, sl: uncalcifying bony or fibrous layer

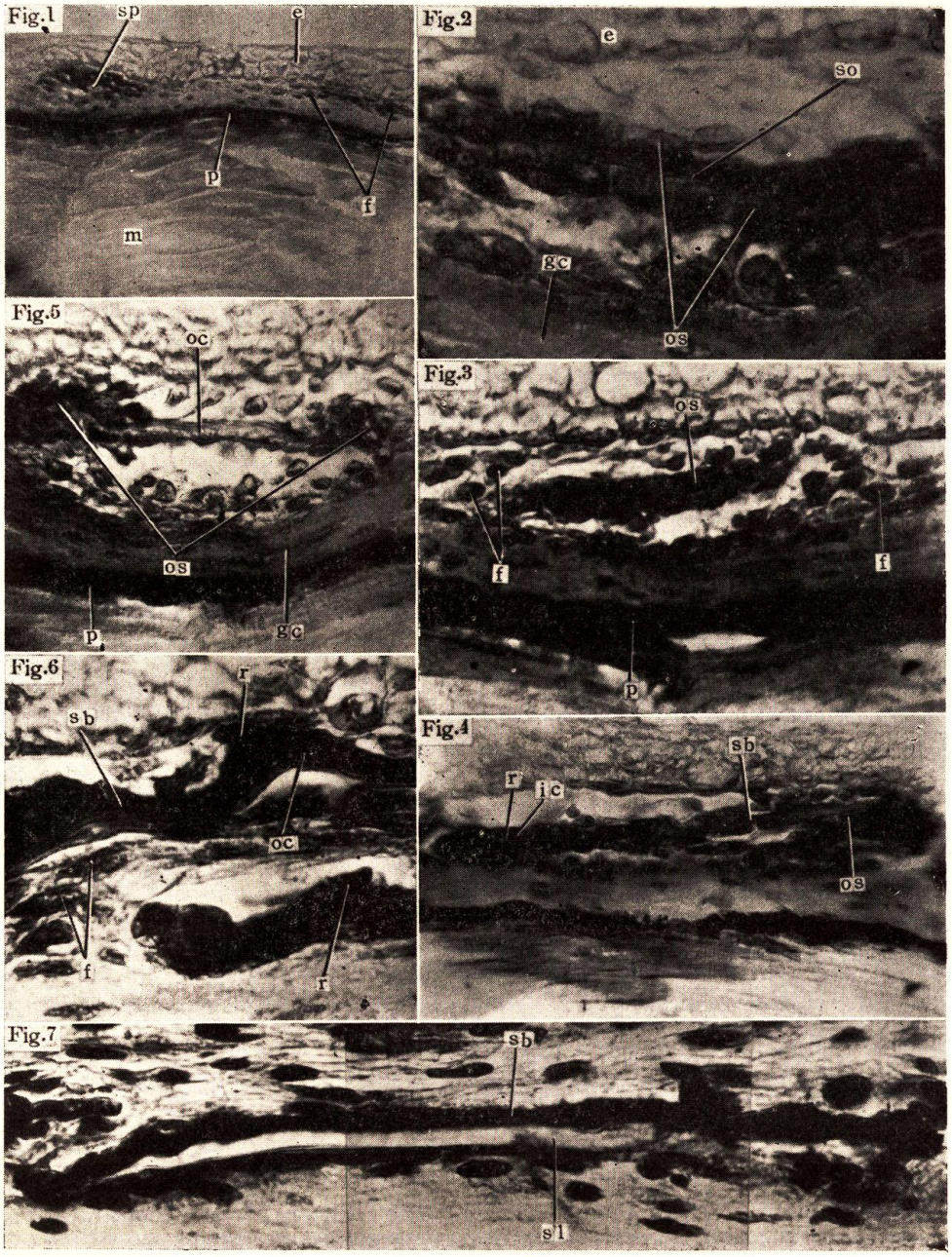

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PLATE II

Explanation of Figures

Fig. 8. A section of the skin of the rainbow trout, 15.5 cm in body-length. The posterior portion of the scale penetrates in the epithelium; alkaline phosphatase is detected in the osteoblasts surrounding directly the growing scale and in fibroblasts in the wall of scale pocket. Gomori's Ca-Co method. × ca. 120

Fig. 9. A section of the operated skin of the goldfish (CS-26). Showing the regenerated epithelium and connective tissue, a neuromast is seen in the former. Alkaline phosphatase is demonstrable only in the epithelium. Gomori's Ca-Co method. × ca. 480. nm: neuromast

Fig. 10. A section of the operated skin of goldfish (CF-50). A similar stage to that in Fig. 9. Delafield's haemetoxylin. × ca. 240

Fig. 11. A section of the operated skin of the goldfish (CF-78). A similar stage to that in Fig. 9. Gomori's Ca-Co method. × ca. 120. or: sites wherein original scales were situated.

Eig. 12. A section of unoperated skin of the goldfish (CF-78). The ontogenetic scales in growth-retaining and associated tissues. Positive reaction for alkaline phosphatase takes place only in the epithelium the same as in regenerated skin. Gomori's Ca-Co method. × ca. 100

Fig. 13. A section of the unoperated skin of the goldfish (WS-83). A similar stage to that in Fig. 12. The epithelium showing positive alkaline phosphatase activity encloses the posterior portion of the scale, but it has no bearing on the growing of scale. Gomori's Ca-Co method. × ca. 200. e: epithelium, s: scale

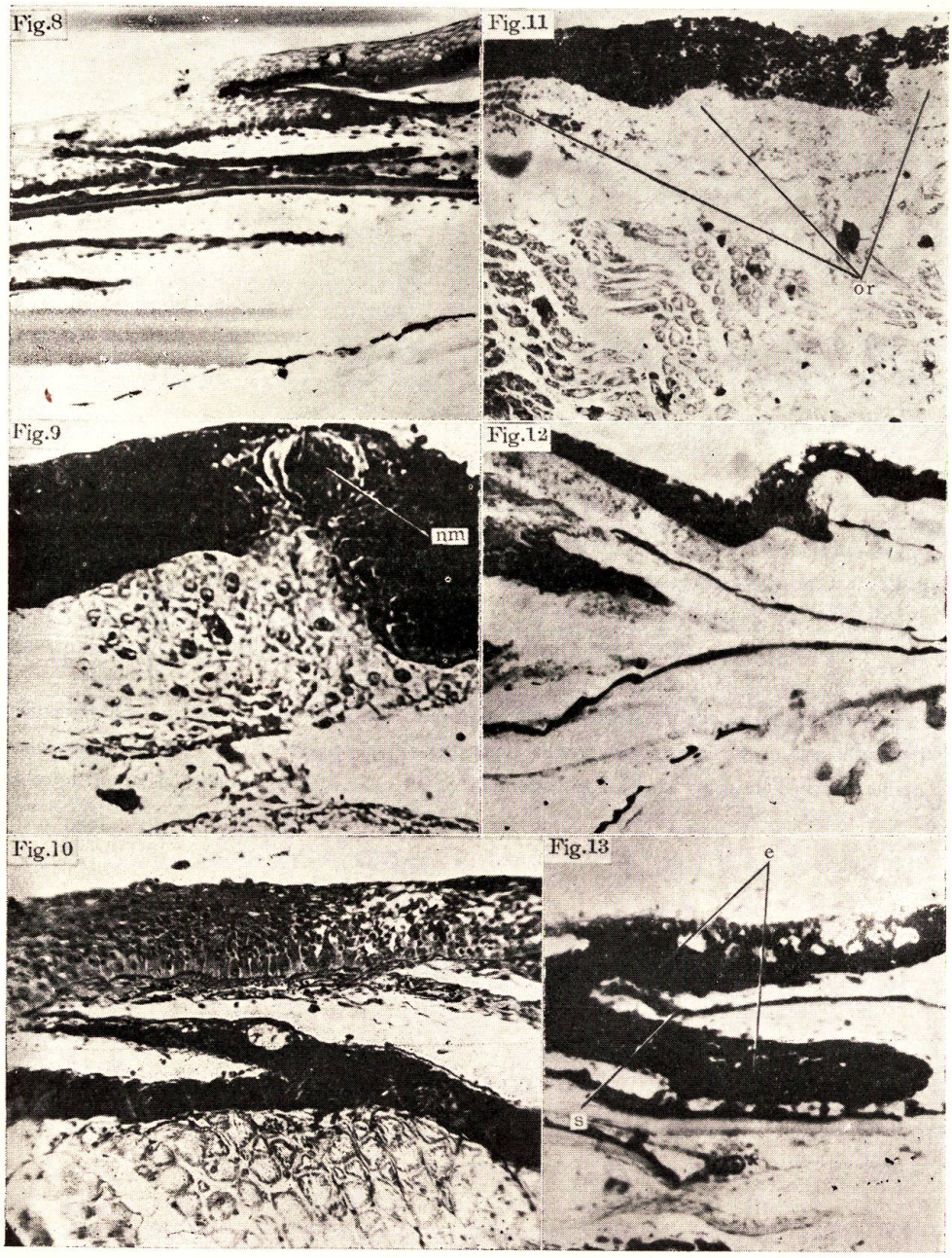

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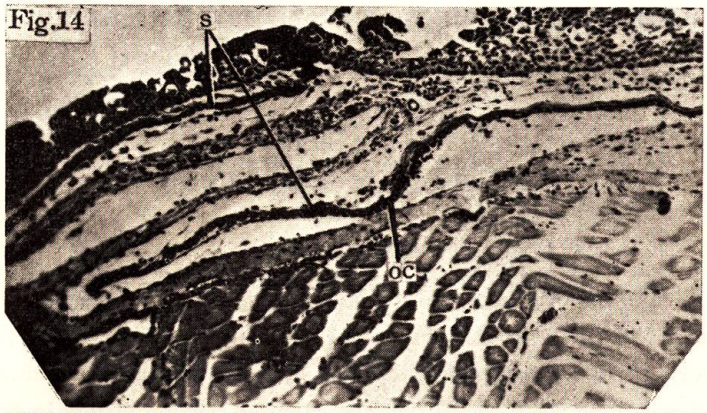
PLATE III

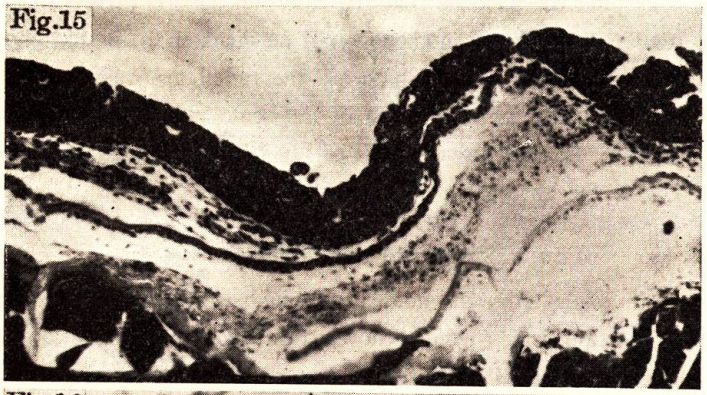
Explanation of Figures

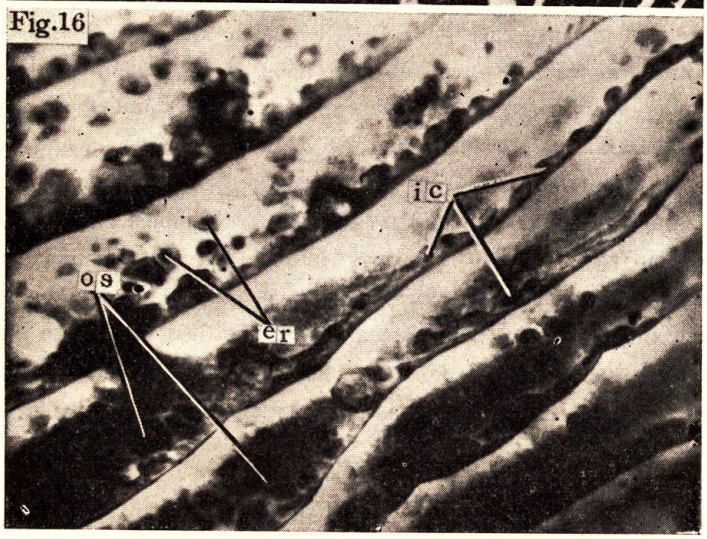
Fig. 14. A section of the operated skin of the goldfish (12 days rearing in WF after 86 days rearing in CF). The regenerating scale and associated tissues. Ehrlich's haematoxylin. × ca. 150. oc: osteocyte, s: regenerating scale

Fig. 15. A same section as in Fig. 14. Alkaline phosphatase is demonstrated in osteoblasts, fibroblasts, and also in epithelium. Gomori's Ca–Co method. × ca. 150

Fig. 16. A flat mount of the regenerated scale taken from the goldfish (WS-50). Transformation of the osteoblasts to the inner attached cells along the ridge and scattered erythrocytes are visible. Mallory's triple stain. × ca. 400. er: erythrocytes, ic: inner attached cells of ridge, os: osteoblasts







J. YAMADA : Alkaline Phosphatase in the Formation of Telost Scale