STUDIES ON THE STRUCTURE AND GROWTH OF THE SCALES IN THE GOLDFISH

JURO YAMADA

Faculty of Fisheries, Hokkaido University, Hakodate, Japan

CONTENTS

I. Introduction 181
II. Definitions 182
III. General description of the scale pattern 184
IV. Development of scales 192
V. Regeneration of scales 198
VI. Absorption of scales 206
VII. Some biochemical aspects of the scale growth 211
VIII. Growth of scales analyzed by means of transplantation 217
IX. General conclusion 222
Literature 224
Explanation of Plates

I. INTRODUCTION

The method of age determination and estimation of life history by means of fish scales has been widely used in population studies of teleost fish since the beginning of this century, when Hoffbauer observed the concentric rings on scales of the carp and thought them to be an indicator of their age. So far, studies on fish scales had been mainly concerned with their phylogenetic and taxonomic meaning but not with their ecological value.

The theory offered by Hoffbauer is based on the assumption that a change in the growth rate of a fish would cause the distance between contiguous ridges on the scales to vary and there would cause an annual ring or a growing zone to form. Many investigations into the determination of the age of fish have been carried out on various species followed this theory, but insufficient fundamental knowledge on the scales has sometimes led workers to erroneous conclusions, because the method was rather empirical but not intimately connected with the mechanism of the development or of the growth of the scale structure. Many arguments have been indulged in, therefore, on the reliability of this method.

In this connection, it was supposed that detailed studies on the morphology of each constituent of the scale structure and on the physiology of the scale

growth would make this method more accurate and would improve the population study of fish as well. This is the reason why the author undertook a comprehensive study of the scales of goldfish (*Carassius auratus L.*). The goldfish was chosen primarily because of the facility in rearing; its growth is rather rapid and the larvae are obtainable with ease. The second reason is its possession of typical cycloid scales which are provided with many concentric ridges and several radial grooves. As the general form and the orientation of the scales are supposed to be influenced by the form of the fish body, the type of the goldfish used in this study is exclusively "Wakin" which is closely similar to the crucian carp in form.

The present paper includes morphological observations on the development, regeneration, and absorption of the scales, and also discussion of some physiological aspects of these phenomena. A teleost scale is formed in the mesodermal connective tissue beneath the epithelium, differing from a placoid scale of selachians which develops as one of the integumental structures such as tooth, spine and nail etc. of ectodermal origin. Accordingly, the studies reported in this paper have been carried out under the assumption that both the morphology and the physiology of the formation or growth of the goldfish scales are much the same as those of a membrane bone. The development, regeneration and absorption of the scales are explained in osteological terms; histochemical tests on alkaline phosphatase, polysaccharides and others, and an injection experiment of parathyroid hormone are also employed, because these substances are supposed to be taking part in the formation and the ossification of the scales. Besides these, some experiments, including the transplantation of scales and the re-examination of once removed scales which were returned into the skin after pictures had been taken, were designed to see the change of scale structure which occurred on the same scales at different stages after a certain period of rearing of the fish.

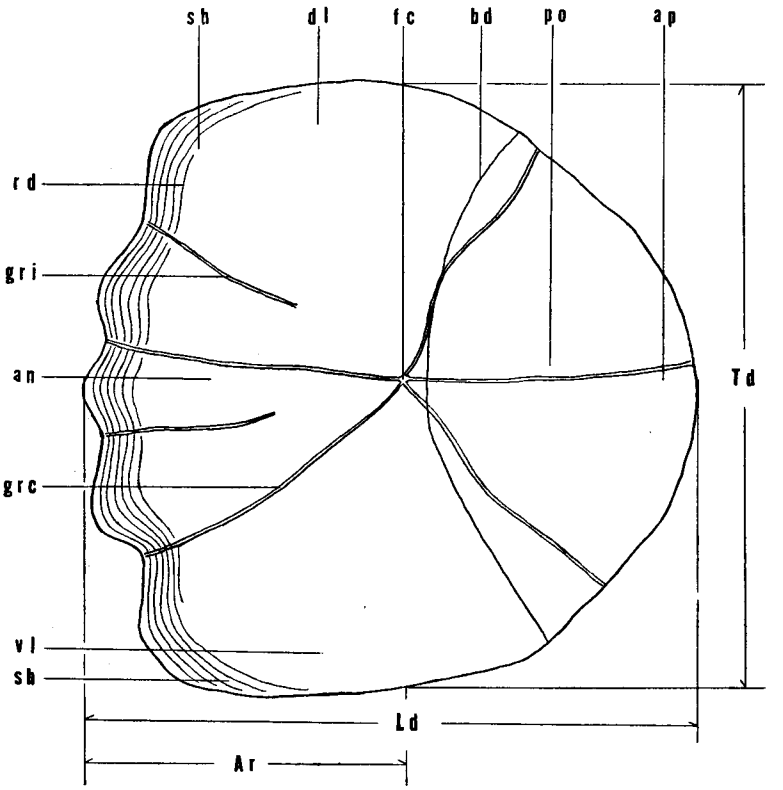
The methods employed in the present study will be described fully in each section.

Before going further, the author wishes to express his sincere gratitude to Professor Saburo Saito of the Faculty of Fisheries, Hokkaido University, for his kindness in directing and encouraging the author throughout the whole course of this work. He is also greatly indebted to Emeritus Professor Tetsuo Inukai, Hokkaido University, for his invaluable suggestions and kind criticism while this paper was in preparation.

II. DEFINITIONS

Clear definitions of the structures and parts which constitute the whole scale are necessary in order to avoid possible confusion because too many names have been employed variously by different authors. In this paper, the following terms will be adopted partly on the recommendation of Kobayashi (1958) (Text-fig. 1).

Anterior part of scale: this is the part anterior to the focus, provided with closely set ridges; called also the "covered" or "unexposed" part, but these



Text-fig. 1. Diagrammatic drawing of a typical goldfish scale
Ar: anterior radius; Ld: longitudinal diameter; Td: transverse diameter; an: anterior part of scale; ap: apex; bd: border between exposed and unexposed parts; dl: dorso-lateral part of scale; fc: focus; grc: complete groove; gri: incomplete groove; po: posterior part of scale; rd: ridge; sh: shoulder; vl: ventro-lateral part of scale

names do not strictly mean the anterior part of scale because the most part of scale is covered with surrounding scales.

Posterior part of scale: the side opposite to the anterior part, provided with rather sparse ridges; the name "exposed" part is also used in the sense described above.

Lateral parts of scale: the upper and lower sides of scale, called respectively dorso-lateral or upper lateral and ventro-lateral or lower lateral.

Shoulders: the two anterior corners of both the lateral sides.

Apex: the posterior-most end of the exposed part.

Focus: variously called nucleus, center or centrum; this is not the geometrical center of the scale but it is the center of its growth.

Ridges: linear elevations of the bony layer of the scale; the term "circuli" or "striae" is most often used. But "ridges" is more suitable because this feature does not necessarily possess concentric circular lines.

Grooves: known by the name "radii" for the reason that these structures appear radially from the focus. Those which start from the focus and reach up to the scale margin are called "complete grooves", whereas others which do not start from the focus or do not reach to the margin are "incomplete grooves".

Longitudinal diameter: the length of the median line passing through the focus and the apex. As the anterior margin shows some jaggedness in large scales, a certain amount of error in measuring the diameter is unavoidable.

Transverse diameter: the length of the line which is drawn at right angles to the longitudinal median line and crosses with it at the focus.

Anterior radius: the anterior half of the longitudinal diameter, limited from focus to anterior margin.

III. GENERAL DESCRIPTION OF THE SCALE PATTERN

Scales of the goldfish vary in form and in superficial structure with the different body parts and with the different body size of the fish. The main part of the body is covered with pentagonal scales which are considered to be standard in form. There are about thirty scales forming the lateral line, above and below which are respectively six and seven rows of scales. It is believed that for one to observe all these scales covering one side of the body surface of individual fish of different body size would be useful in mastering the general features of the scale pattern and in considering the essential nature of the structures which constitute the scale pattern.

Procedure

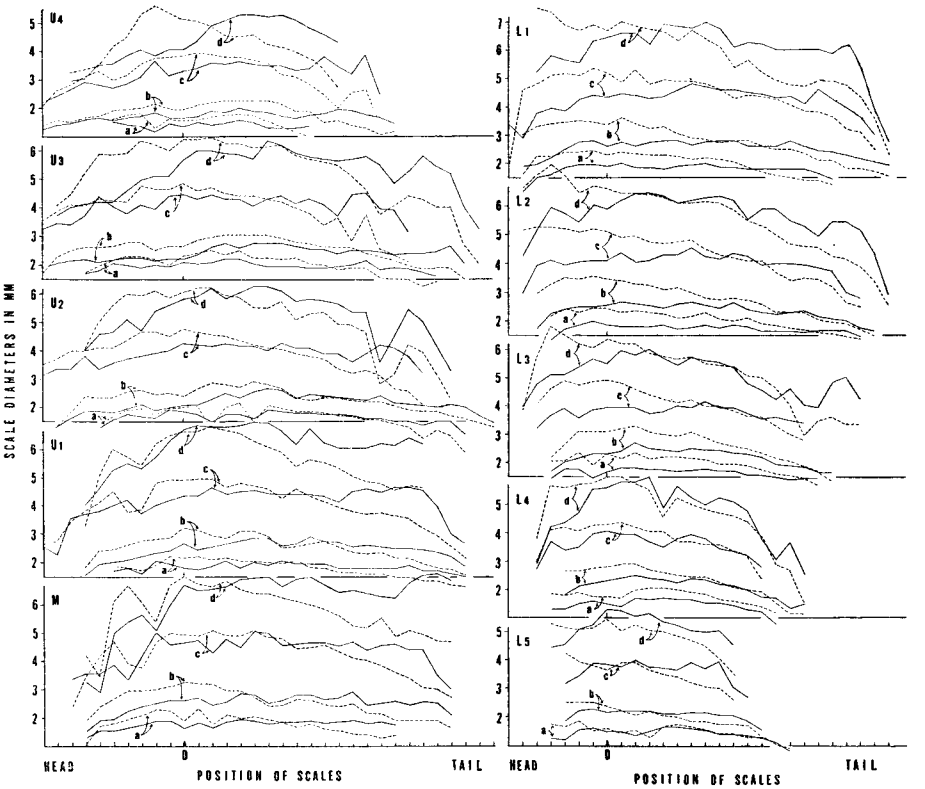
Nearly all scales covering the left side of the body were pulled out from five specimens. Those of the uppermost and lowermost scale rows were omitted because of their smallness and of their irregular form. The fish from which the scales were taken measured respectively 3.8, 5.2, 7.8, 9.8 and 11.3 cm in body length. The scales were arranged on glass slides in regular sequence according to their arrangement on the body surface. Measurements were made on these scales, magnified by means of a shadowgraph, of their longitudinal diameter, transverse diameter, anterior radius, and number of ridges and grooves.

In each individual fish, there are always a considerable number of regenerated scales which can be distinguished easily from ontogenetic ones by their rather numerous radial grooves and by an irregularly formed network of the focal area in which are found no concentric ridges. Further, there are a few extraordinary scales located at the extremities of both anterior and posterior ends of each scale row. The anteriorly located scales are strongly compressed along the longitudinal axis, while the posterior ones are along the transverse. In treating the obtained data, the exception of the above noted uncommon scales was taken into consideration as occasion demanded.

Observations

(1) Longitudinal and transverse diameters

The anteroposterior trends of the two scale diameters are given in Text-

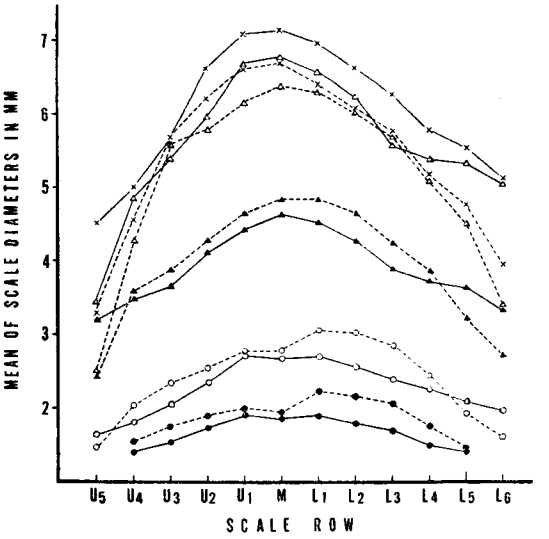


Text-fig. 2. Anteroposterior trend in change of two scale diameters of all the scales in every scale row with four specimens
D: position of the origin of dorsal fin base; U_1: upper first scale row; M: lateral line scale row; L_2: lower second scale row
The specimens are: a, 3.8 cm; b, 5.2 cm; c, 7.8 cm and d, 9.8 cm in body length.
——— longitudinal diameter ——— transverse diameter

fig. 2. Since the number of scales in a given scale row varies individually, the origin of the dorsal fin base (D) was adopted as the standard.

Generally speaking, the transverse diameter is larger than the longitudinal in the anterior part of the body, while in the posterior the relation is reversed. This is clearer in the lower (ventral) half of the body than in the upper (dorsal). The maximum longitudinal diameter is within the part below the dorsal fin while that of the transverse appears more anteriorly. There is at least one point in every row where the two diameters come to be equal. In front of this point, the width of scale is larger than the length while the latter gets ahead of the former behind this point. These points appear to have a tendency to move forward as the fish grow. In other words, the scales grow longitudinally rather than transversally in accordance with the growth of their bearer (see also Text-fig. 3).

In order to compare the scale size amongst the individual rows, the mean



Text-fig. 3. Mean of two scale diameters of scales existing in the space between the origin of dorsal fin and that of anal fin with every scale rows with five specimens

——— longitudinal diameter
------ transverse diameter

● 3.8 cm in body length
○ 5.2 //
▲ 7.8 //
△ 9.8 //
× 11.3 //

of the scale diameters for every scale row was calculated (Text-fig. 3). In this case, because of the fact that the deformed scales in both the anterior and the posterior body parts vary largely in their diameters (Text-fig. 2), only the scales existing in the space between the origin of the dorsal fin and that of the anal fin were used in order to make the comparison accurate. The two diameters, either longitudinal or transverse, show a higher value in the upper first (U_1), lateral line (M) and lower first (L_1) than in other scale rows. In the smaller fish (3.8 and 5.2 cm), the mode of the longitudinal diameter is at U_1, whereas that of the transverse is at L_1. But in the larger fish (7.8, 9.8 and 11.3 cm), the modes, both of the longitudinal and transverse, move towards M. At the same time, it must be noticed that the two diameters are different from each other in growth rate, so the longitudinal which is shorter in the smaller fish tends to surpass the transverse as the fish grows.

(2) *Position of focus*

As the focus is the growth center of a scale, its position in the scale shows the growth rate of each scale-part after the origin of the scale has been established. In this study, the position of the focus in the longitudinal axis of a scale was represented as the ratio of the anterior radius to the longitudinal diameter. A value above 0.50 means that the focus is located posterior to the middle of the longitudinal axis.

Table 1. Frequency of scales of a specimen, 7.8 cm in body length, grouped by series of the ratio of anterior radius to longitudinal diameter, to show the transition of the position of focus in one individual fish

Scale row	Anterior radius / Longitudinal diameter						Total number of scales except the regenerated
	less than 0.35	0.36 ~0.40	0.41 ~0.45	0.46 ~0.50	0.51 ~0.55	more than 0.56	
U_5	1	2	11	6	0	0	20
U_4	2	3	10	7	1	0	23
U_3	1	4	10	10	1	0	26
U_2	2	1	6	10	6	0	25
U_1	2	1	3	9	13	2	30
M	1	0	4	4	17	1	27
L_1	1	1	2	6	14	1	25
L_2	1	1	2	11	9	0	24
L_3	2	1	0	12	5	0	20
L_4	2	1	3	10	1	0	17
L_5	1	5	8	0	0	0	14
L_6	3	8	2	0	0	0	13

First, to see the relative change of this ratio in one specimen, the calculation was made of all scales except the regenerated ones in a specimen of 7.8 cm body length. Table 1 shows the frequency of the scales in several grades of the ratio with every scale row. It is quite clear that the foci of the scales of the lateral line row tend to lie posterior compared with those of the scales of the other rows, and that the tendency decreases, in turn, as the row goes dorsal- as well as ventral-ward. Then, the position of focus was compared individually, in the same way, with the scales of four rows (U_2, U_1, L_1 and L_2). Lateral line scales were omitted in this case because in the smaller fish it was difficult to decide exactly the position of the focus owing to the presence of the opening of the lateral organ. It can be seen in Table 2 that the foci of the scales tend to change their position backward with the growth of the fish body.

These facts reveal that the growth rate of the anterior part of scale exceeds slightly that of the posterior as the scales grow larger. That the lateral line

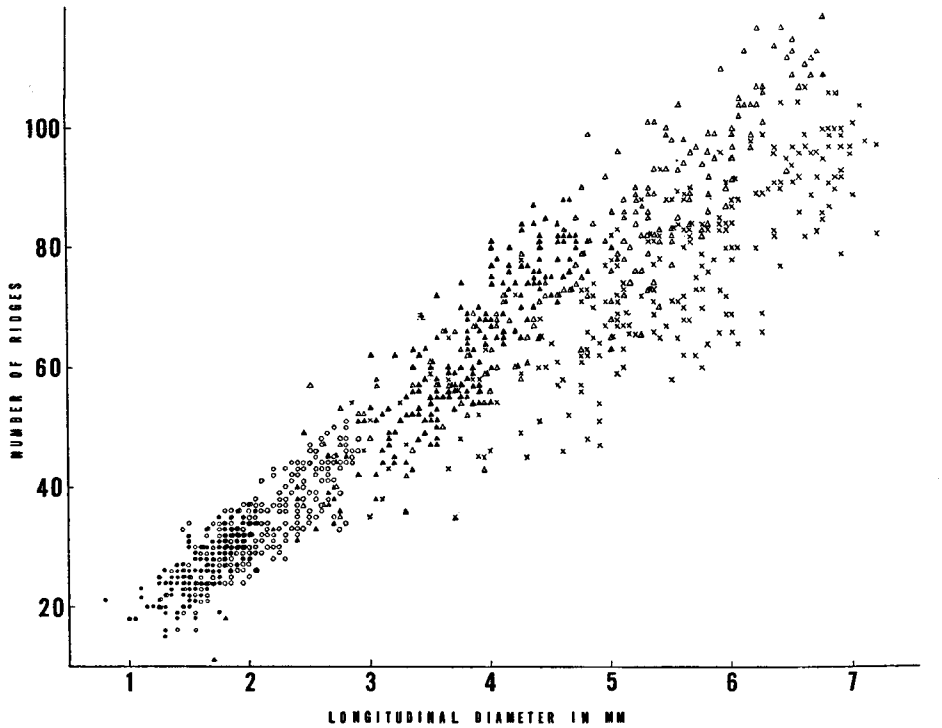
Table 2. Frequency of scales in four scale rows (U_2, U_1, L_1, and L_2) of five specimens grouped according to ratio of anterior radius to longitudinal diameter, to show the transition of the position of focus accompanying the fish growth

Body length (cm)	Anterior radius / Longitudinal diameter						Total number of scales except the regenerated
	less than 0.35	0.36 ~0.40	0.41 ~0.45	0.46 ~0.50	0.51 ~0.55	more than 0.56	
3.8	15	40	33	4	0	0	92
5.2	11	27	50	15	1	0	104
7.8	6	4	13	36	42	3	104
9.8	3	2	6	20	43	5	79
11.3	6	4	17	50	19	0	96

scales show the highest ratio may be concerned with the fact that these scales are the largest in longitudinal diameter among all scales. It should not be considered, however, that the focus appears originally in the real center of the scale when the first ridge is formed, otherwise, it would follow that a ratio less than 0.50 would indicate a higher growth rate in the posterior part of a scale rather than in the anterior at the early stage of growth.

(3) *Number of ridges and their intervals*

As a scale grows larger, ridges appear one by one on the upper surface of scale as linear elevations of bony substance. They increase in number in proportion to the growth of the scale (Text-fig. 4). There exists a certain extent of variation between the number of ridges and the scale size, that is to say, the intervals between ridges are variable in different scales. Since the alteration of ridge intervals is useful in age determination, the number of ridges should be considered with reference to their intervals. In view of this point, it is more reasonable to compare the number of ridges with the anterior radius rather than with the longitudinal diameter. The value obtainable in dividing

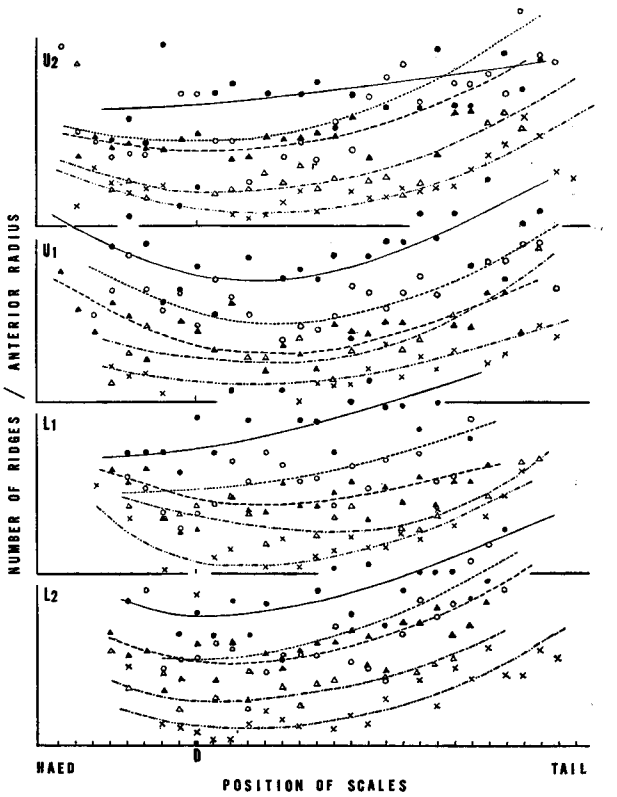


Text-fig. 4. Correlation between number of ridges and longitudinal diameter in all scales of five specimens

● 3.8 cm in body length △ 9.8 cm in body length
○ 5.2 // × 11.3 //
▲ 7.8 //

the number of ridges by the anterior radius represents the density of ridges or the average distance between two adjacent ridges on one scale.

Of all scales in the four rows excepting the regenerated scales, the value was calculated and arranged anteroposteriorly with five specimens (Text-fig. 5). The scales show a large variation in this value with each other. But, on the whole, it may be said, though roughly, that with every row there is a tendency of a sharp decrease in the value in the anterior body part and a gradual increase towards the caudal part. No difference among the four scale rows could be found. Moreover, one can see in every scale row a gradual lowering of these values according to the growth of the fish.



Text-fig. 5. Anteroposterior trend in change of the density of ridges (number of ridge/anterier radius) with four scale rows with five specimens, except the regenerated scales

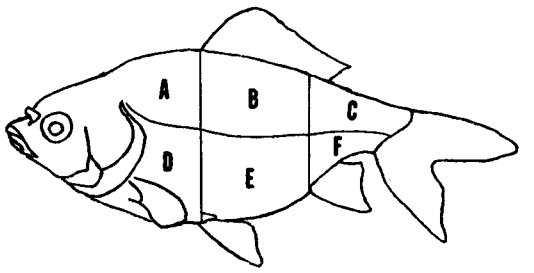
—●— 3.8 cm in body length ·-·-△-·-· 9.8 cm in body length
·····○····· 5.2 〃 —·—×—·— 11.3 〃
—·—▲—·— 7.8 〃

From the above facts, two conclusions can be drawn: 1) The density of ridges varies with the body part, viz., apart from the foremost few scales which are strongly compressed along the body axis, the relative number of ridges is

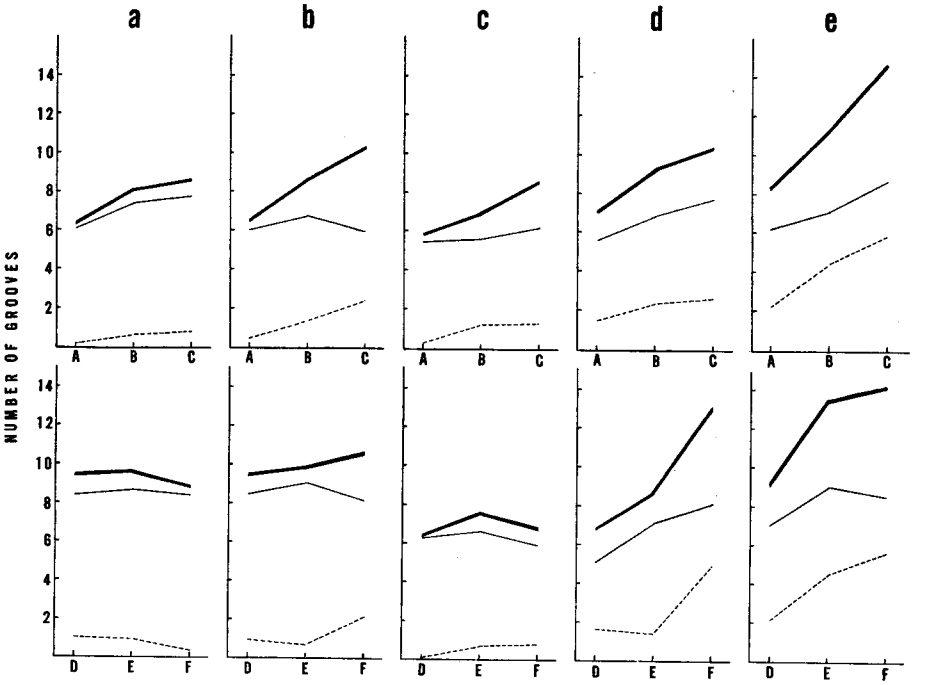
small in the anterior scales whereas there are abundant ridges in those of the caudal part in proportion to their short radius. 2) The number of ridges in a certain width of a scale decreases with the enlargement of the scale, so the intervals between ridges grow larger according to the degree of fish growth. The facts seem to suggest the manner of ridge formation.

(4) *Number of grooves*

In each scale, there are several grooves running radially from the focus to



Text-fig. 6. Showing six parts of body surface divided for comparison of average number of grooves with the scales in each part. The lateral line scales are included in the upper three parts.



Text-fig. 7. Comparison of average number of grooves with the scales in six parts illustrated in Text-fig. 6

a: 3.8 cm in body length
b: 5.2 //
c: 7.8 //
d: 9.8 //
e: 11.3 //

—— total number of grooves
—— complete grooves
—— incomplete grooves

the periphery. They can be called "complete grooves" in contrast to "incomplete grooves" which do not start from the focus or do not reach to the edge. Almost all of the incomplete grooves are those which do not start from the focus. Various theories as to the function of the grooves have been proposed by many authors. The predominant opinion is that they give flexibility to the scales when a fish is in the act of bending the body (Taylor, 1916; Neave, 1940; Wallin, 1957). As a proof of this opinion, Taylor and Wallin showed in the pigfish and the roach, respectively, a gradual increase in number of grooves from the inflexible head part to the most flexible caudal part. In this connection, the relation between the number of grooves and different body parts was also studied in the goldfish. The body was divided into six parts as shown in Text-fig. 6. The average number of both complete and incomplete grooves of the scales within each part, excepting the regenerated scales, is shown in Text-fig. 7. On the whole, the grooves including both the complete and the incomplete have a tendency to increase in number from the head part to the caudal part. They also increase in number with the growth of the body. The figure seems to show that this increase in total number of grooves in parts from head to caudal or with the growth of body should be considered due mainly to the increment of the incomplete grooves and not of the complete.

Consideration

The observations made in this study concerning the change in general shape of the scales with the different body parts and with the different body size can be summarized as follows. 1) The scales in the anterior body part have longer transverse diameter than longitudinal, whereas in the positions following posteriorly they come to show longer longitudinal diameter than transverse. 2) The longest longitudinal appears at a point in the space below the dorsal fin, but the transverse appears more anteriorly. 3) In comparison between the scale rows, the longest longitudinal is shown in the scales of the upper first row in the smaller fish, while the longest transverse is in those of the lower first. In the larger fish, on the other hand, the maxima of both the longitudinal and the transverse appear in the lateral line scales. 4) The rate of the longitudinal growth of the scales is larger than that of the transverse growth.

There is almost no report concerning change in the scale pattern with different body parts of a fish. Only Saito (1956) worked on all the scales of both sides of the body of the crucian carp; he reached conclusions most of which are in accord with the present results obtained in the goldfish. It is quite clear that the general form of the scales is strictly affected by the body-part where they grow. When the body is enlarged with the growth of the fish, the scales covering the body surface would grow in size. Accordingly, the difference in growth rate on a certain body-part may lead to the difference in form of the scales. The fact that the longitudinal diameter of scales tends to surpass the transverse with the growth of the fish shows that the fish grows longitudinally more than transversally. As the relative volume of the viscera is considered to be large in proportion to the whole body in the smaller fish, the bulge of the belly is supposed to cause the comparatively longer transverse diameter in

the lower scale rows in these fish.

The change in position of the focus and in number of ridges should be considered together with the aforementioned facts. The position of the focus moves posteriorly as the scales grow, in other words, the forward growth of the scales exceeds the backward. Moreover, in looking over the whole group of scales of one individual, it is clear that the large ones such as those in the lateral line, except some extremely anterior ones which have been prevented from forward growth by the clavicle, possess a posteriorly placed focus. As a rule, the larger the scale the more ridges there are, but the number of ridges is not strictly in proportion to the anterior radius even on one individual fish. The density of ridges is less in the large scales of the middle body-part which show the comparatively longer anterior radius than in those of the anterior or of the caudal part. The comparison of Text-fig. 2 with Text-fig. 5 will make clear the relation between the transition of the longitudinal diameter of scales and that of the density of ridges. The two are roughly in inverse relation; the body-part where the longitudinal diameter shows its maximum corresponds with the minimum density of ridges. The finding may be interpreted that the ridges formed at a certain length of radius are less in number in the scale which has grown at a higher rate. This idea seems to indicate the significance of the growth rate of scale in respect to the density of ridges.

The number of grooves grows larger anteroposteriorly within the same individual; it also increases all over the body with the growth of the fish. The increment takes place by the appearance of new incomplete grooves. The facts alone are insufficient, however, to lead to a conclusion that the grooves play a role giving flexibility to the scales as Taylor and Neave insists; this will be further discussed on the basis of observations on the structure of grooves.

IV. DEVELOPMENT OF SCALES

Studies on the development of fish scales form the most essential part of the histology and the morphology of growing scales. For this reason, it has long been one of the main subjects of many investigators who have taken a great interest in fish scales. Among many investigations into the histogenesis of scales in various fishes, the early development of scales in the goldfish was studied by Neave (1940), also Pevsner (1926) worked on the crucian carp, *Carassius vulgaris*. Regardless of species used in the studies of this subject, there are some unsolved questions and disagreements in views. The present author himself once studied the development of the scales in the rainbow trout (Yamada and Saito, 1952), and now he admits the necessity to observe it again in the goldfish.

Procedure

The larvae used in this study were obtained from a pond in the campus of the Faculty of Fisheries. They grew about 15 mm in body length within a month after hatching, specimens of the larvae were fixed weekly in Bouin's solution, sectioned by the paraffin embedding method and stained with Delafield's haematoxylin and eosin.

Observation

The first indication of the origin of scale appears as an aggregation of cells which is called "scale papilla" or "cutis papilla." Prior to the appearance of this scale papilla, the skin consists simply of two layers, the outer stratified epithelium and the inner fibrillated corium, between which no tissue layer can be observed (Fig. 1). When the fish grows as large as about 12 mm in body length, there appears a thin layer of connective tissue cells, some of them aggregate in many evenly spread masses at regular intervals to form scale papillae (Fig. 2). The observation of a series of sections showed that the papillae are formed first in the anterior body-part along the presumptive lateral line; thereafter, they rapidly spread over the body surface both longitudinally and transversally.

The cells transformed into the scale papillae are basophilous and larger in size, more rounded in shape than the original connective tissue cells; within each is contained an oval nucleus. The aggregation of these cells comes to be divided into two layers, the upper and the lower, by the appearance of a fine horizontal crack at the middle of the mass (Fig. 3). The cells become thinner losing their cytoplasm and are transformed into spindle-shaped cells lying parallel to the crack. Now, in the crack, a thin homogeneous light-reflective layer appears (Fig. 4). This is the origin of a scale, exactly speaking, the origin of the upper bony layer of a scale. Accordingly, the cells in the papillae should be called "osteoblasts" different to the original connective tissue cells which may be regarded as "fibroblasts." The osteoblasts around the origin of a scale are flattened more and more while losing their cytoplasm, and they finally begin to disappear passing through the stage of naked nuclei which adhered closely to their own product. The degeneration of osteoblasts begins from the central region of the upper side of the just-formed scale (Fig. 5). Thus established scales continue to grow larger while passing through the above described stages pushing down the corium at the anterior and pushing up the epithelium at the posterior, so their growth results in an imbricated oblique arrangement of the scales. The connective tissue left between two scale papillae becomes fibrous and elongates obliquely making itself into a thin fibrous wall which borders two adjacent scales connecting the epithelium to the corium. In this way, each new-born scale comes to be enclosed separately by the connective tissue wall which is called the "scale pocket."

The osteoblasts degenerate completely at the upper surface of the scale except the growing margin, while they remain as a layer at the lower surface and connect to the upper cells around the scale margin which is covered with one or two layers of them (Fig. 6). The lower layer cells also disappear finally, so the lower surface of the scale comes to be in direct contact with the underlying wall of the scale pocket. After this stage, the osteoblasts are seen only at the scale margin; they are contributing to the growth of the scale. Meanwhile, another layer of the scale, the fibrillary plate, is formed beneath the bony layer (Fig. 7). It consists of several layers of fibrous lamellae which are added to the scale one by one with the enlargement of the bony layer. Thus, it is under-

stood that the scale is composed of two different layers, the upper bony layer and the lower fibrillary plate; the former grows horizontally in almost definite thickness while the latter grows both horizontally and vertically by adding successively larger new lamellae.

The ridges, linear concentric rings in upper view, are observed as spicular projections of the bony layer in section (Fig. 7). The tips of the projections invariably lean toward the scale focus. The first ridge appears as early as the time when the lower layer cells still remain at the lower surface of the scale (Fig. 6). There is no special structure which seems to be forming the ridges, but it appears that the ridges are formed one by one as a result of accumulation of bony material which filled the intercellular space between two osteoblasts in contact with the growing ridges. The inner cell subsists still after the degeneration of the outer cell until the outer two or three new ridges develop. The ridge is low and dull when it arises at the outermost periphery of the scale; it gradually increases in height until it finally attains its ordinary height of about 5 μ and its ordinary steepness while two or three new ridges are at the outside. There are often seen naked nuclei attached at the inside of the inner ridges. These facts imply that the inner cells remain at the inside of the just formed ridge and contribute to the growth of the ridges in the consumption of their cytoplasm.

The grooves in section appear as a partial absence of the bony layer (Figs. 8 and 9). The edges of the trough-shaped section show somewhat elevated bony material. Fibrous lamellae of the fibrillary plate become loosened and wave appreciably just under the grooves. There is usually a polygonal cell which entered into the peripheral part of a groove (Fig. 8), but no cellular element can be seen in the groove when the sections passing through the inner part of a scale are observed (Fig. 10, cf. Fig. 2).

In the cross sections of a specimen, 14 mm in body length, it was observed that the scales of five middle scale rows including the lateral line, developed fully along the body axis from anterior to posterior, whereas those of the upper and the lower third rows, just outside of the above mentioned rows, were in the stage of scale papilla. In a fish two months from hatching, 21 mm in body length, the scales had developed fully covering the whole body surface.

Discussion

So far as the author knows, there is no datum concerning the body size of the goldfish in which the scale papillae appear first. The time after hatching or the body size when the scales come to develop cannot be decided exactly because consideration must be given to the fact that the growth rate of young fish is greatly affected by the environmental conditions in which they live; however, the present study showed that the scale papillae do not appear until the fish grows to about 12 mm in body length or until a month has passed after hatching. The papillae are formed first along the lateral line in the anterior half of the body. It has been confirmed by many investigators that the scales in various fishes first appear in the neighbourhood of the lateral line. Particularly, Klaatsch (1890), Hase (1911) and Neave (1936) found respectively in the brown trout,

the perch and the kamloops trout, that the scale formation starts from the anterior and middle region of the body; on the other hand, Paget (1920) as well as Yamada and Saito (1952) showed the first appearance of the papilla in the posterior half in the brown trout and the rainbow trout. The site where the first papillae are formed might be different with the species. But it seems to be sure of all species that they appear first in the neighbourhood of the lateral line and last in the back and belly.

Klaatsch (1890), who first made detailed phylogenetic study of scales from the placoid of selachians to the cycloid of teleosts, pointed out that the scales of the trout are mesodermal in origin and that they consist of two different layers, bone and hardened connective tissue. There have occurred heated controversies related to the dermal origin of teleost scales since the end of the last century. Fach (1937) seems, however, to be the last investigator to insist on the participation of epithelial cells in the formation or growth of the scales. Today, nobody would doubt the fact that the teleost scales develop as one of the completely mesodermal organs. But, concerning the genesis and nature of two constituents of the scale, the bony layer and the fibrillary plate, there are several essential problems still to be solved.

Among many workers who have studied the histogenesis of fish scales, Neave (1936) introduced the osteological concept into the study of the development of the trout scale suggesting that the stages in the laying down of the bony layer are typical of the formation of membrane bone which originates directly in the connective tissue. The present author proved the propriety of Neave's opinion in his previous paper (1956) by applying histochemical tests to the developmental stages of the rainbow trout scale. The process should be essentially the same regardless of the species used, but, so far as the present author has studied, the goldfish seemed to be not as good material as the rainbow trout for observation of the developmental stages of the scales, because the goldfish scales even in germ stage are not soft enough to make good thin slices and further because of the inconspicuousness of the original papilla. But the process could be traced almost satisfactorily from the osteological point of view comparing it with the process observed in the scales of the rainbow trout.

Before the appearance of the scale papilla, the corium is composed of coarse fibre bundles in which few or no cellular elements can be seen. Neave showed in his clear figures that the formation of scale papillae in the trout skin is not caused by the increase of corium cells *in situ* but is based upon the outgrowth of mesenchymes arising at the lateral line region. This may be true of the goldfish because no multiplication phase of fibroblasts was observed in the corium. The scale papilla of the goldfish takes a more depressed form and is larger in size than that of the rainbow trout. The scale itself, therefore, is also larger in size when it comes visible in the papilla. There is no doubt that at first the origin of a scale consists of only the bony or upper layer because the ridges are formed later on it, though Pevsner (1926) in *Carassius* and *Rutilus* or Petrov and Petruschewsky (1929) in *Cyprinus* maintain that the fibrillary plate arises prior to the bony layer.

Concerning the genesis of the bony layer and the fibrillary plate, there are

various opinions expressed by a number of investigators which may be divided roughly into three categories, though each of the investigators has his own shade of difference in meaning. 1) The bony layer is laid down by the upper layer cells of the papilla while the fibrillary plate is formed at the expense of the lower elements (Ussow*, 1897; Nusbaum, 1907; Paget, 1920; Pevsner, 1926). 2) The original cells which form the scale papilla contribute merely to the formation of the upper layer of scale and the fibrillary plate is derived from the underlying connective tissue wall (Klaatsch, 1890; Hase, 1911; Neave, 1936, 1940; Yamada and Saito, 1952; Dietrich, 1953; Yamada, 1956). 3) Opinions which do not fall within the above two. Setna** (1934) is of the opinion that the lower layer cells give rise to the upper cells and on turning around the scale margin each cell stops forming fibres and begins to secrete bony material. On the contrary, Creaser** (1926) supposes the cell migration from the upper to the lower side. Wallin (1957) opposes earlier views; he shows that both the layers are derived from the same germ structure at the periphery and that the bone is transformed to the fibrillary plate. These authors admit the formation of different materials by the same cells.

As is above stated, what is first formed in the papilla is the bony layer and not the fibrillary plate. This structure appears horizontally in the middle of the cell aggregation in the same way as in the formation of membrane bone which takes place in the aggregation of osteoblasts. The theory that the bony layer is the product of only the upper cells seems to be based upon the fact that the lower cells continue to remain in the lower surface of a scale after the degeneration of the upper cells has completed excepting only at the scale margin. Since these lower cells also disappear finally, difficulty arises in interpreting the thickening of the fibrillary plate all over the surface after the disappearance of lower cells, if they act really in forming fibres without taking part in laying down of bone. If that is not the case, the fibrillary plate must be naturally formed by cells of the underlying connective tissue of the scale pocket wall. It was clearly observed in the rainbow trout, however, that the fibrillary plate as well as the bony layer is enclosed by a sheath of osteoblasts at the scale margin; this leads to the impossible thought that the fibrous lamellae must be added through these interposed osteoblasts.

Therefore, the present writer could not help confessing the complicacy of the problem. Recently, however, he noticed that some of the photomicrographs in the previous paper (1952) contain matter useful for the solution of this problem. The older figures are shown again for reference (Figs. 10, 11). In the figures the latest fibrous lamella can be seen in course of formation; it has a connection with the marginal osteoblasts at both the anterior and the posterior margin of the scale. But, it is impossible to consider that the osteoblasts themselves are being transformed to the lamella at the centre of the scale after they have formed bony material at the margin. The following may be more reasonable explanation. The pocket wall provides the scale with a cellular sheath which covers the scale except its upper surface, and at the next stage the sheath

* Cited from Nusbaum (1907). ** Cited from Neave (1940).

turns into osteoblasts at the scale margin and fibrous lamella at the lower surface of the scale. Fibroblasts of the connective tissue, which will be transformed into osteoblasts at the scale margin where there is no scale on them, form or are transformed into fibrous lamella at the lower surface of the scale probably being influenced by the presence of the scale itself. Moreover, the addition of a new fibrous lamella is always preceded by the extension of the bony layer. At the beginning of the scale formation, the papilla is filled with osteoblasts which produce only the bony layer. After the formation of bony material has been completed the lower layer cells degenerate turning into fibrous lamella. The situation is kept at the scale margin as long as the scale grows larger, while at the inner part of the lower surface the fibroblasts produce fibres without passing through the stage of osteoblasts. In this way, the cellular sheath around the scale comes to be composed of marginal osteoblasts and lower fibrous lamella. It is natural, therefore, that an intimate relation exists between the extension of bony layer and the addition of fibrous lamellae. For instance, Paget found in the scales of *Salmo fario* that the thickness of a fibrous lamella varies according to the distance of ridges which correspond to the edge of the lamella. Also Lindahl and Wallin (1955) have shown in the roach scale that in most cases one fibrous lamella is added for every two ridges of bone.

The above interpretation can be applied to the regeneration of scale. When a scale is removed from the scale pocket, the regenerated scale appears in it within a very short period. As the marginal osteoblasts are supposed to be removed together with the scale, the rapid regeneration must be made by the cells proliferated from the pocket wall which normally produce marginal osteoblasts and fibrous lamellae. Nevertheless, they actually produdce first the bony layer followed next by the addition of fibrous lamellae.

The process of ridge formation raises a very important subject on account of their usefulness in the determination of age of fishes. For this reason, many investigators have taken great interest in it and expressed their own opinions. The present study has shown that in section the ridge appears between two osteoblasts and it continues to grow while the inner osteoblast is losing its cytoplasm. So, there is no daubt that some of the osteoblasts which normally produce the bony layer of scale serve in making the ridges to grow. However, the above observation seems to be not sufficient basis for discussion of what induces ridge formation. The problem will be fully discussed later together with other experimental results.

As for the structure and function of the grooves, a detailed description was issued by Neave (1940). He observed in the section to which Mallory's connective tissue stain was applied, that the corresponding parts of the fibrillary plate under the grooves receive anilin blue dye in wedge form whereas the remaining part takes orange G. According to him, hardness of the scale is brought about by the infiltration of ichthylepidin, an albuminoid like collagen, into the fibrillary plate, and the peculiarity in staining at the part just under the grooves is an indication of no deposit of the substance. In considering the fact in connection with the absence of bone at those parts, he considered it as a simple form of ginglymoid arthrosis. As was described in the preceding section, also

Taylor (1916) and Wallin (1957) hold to the idea of the importance of the grooves for the flexibility of the scales. Paget (1920), Pevsner (1926) and Dietrich (1953) on the other hand thought much of the function of the grooves as a canal which serves in transporting cells probably because they found cellular elements in the grooves. The present author would conclude that the function of the grooves is to give flexibility to the scales in the light of the results described in the preceding section—the number of grooves increases along the body axis anteroposteriorly and also according to the scale growth—and in view of their structure observed in section. The cellular elements which were often observed in the grooves may probably participate in the formation of the grooves themselves. The matter will be further considered with respect to the absorption of the scale.

V. REGENERATION OF SCALES

A study of regenerated scales is convenient for elucidating the way of formation of the scale structure or in considering the essential nature of that structure owing to the characteristic features and rapid reappearance of such scales. The regeneration of scales seems to be affected by environmental factors such as water temperature or food supply (Saito and Yamada, 1953; Saito, 1955). Suzuki (1952) reported in the goldfish the complete recovery of the regenerated scales to normal in external appearance within only six months, contrary to the observation by Wunder (1949) that regenerated scales do not return to the original structure even in four years. In this section, there will be presented, in addition to a description of the process of regeneration of scales, some experimental results and a discussion concerning mainly the way of ridge formation, the effect of water temperature and feeding on regeneration, and recovery of the superficial structure of the regenerated scales.

Process of regeneration

Four ontogenetic scales adjacent to each other were removed from the upper first scale row of a goldfish, 6.1 cm in body length, and the corresponding regenerated scales were found to be at different stages of regeneration as shown in Fig. 12. The four regenerated scales (A-D) represent respectively the stages of the 4th, 5th, 6th and 7th week after the removal of the ontogenetic scales. During the period (May-June) the water temperature varied in the range from 10°C to 20°C. The regenerated scales were recognizable in the body surface before the 4th week but they were not hard enough to withstand taking out.

At the 4th week, the regenerated scale (A) was extremely thin and flexible, its longitudinal and transverse diameters showed about 80% of those of the ontogenetic (a) and there were found 3 linear ridges on it. The scale grew to about 90% of original size by the 5th week (b, B) and the number of ridges increased to 6. The regenerated scale (C) on which there were 10 ridges attained nearly the same size as compared with the normal scale (c) by the 6th week. By the 7th week, the regenerated scale (D) which was provided with 13 ridges came finally slightly to exceed the ordinary scale (d) in size. The

regenerated scales are characterized by three things; the wide central area which lacks concentric ridges but shows a grooved network, the ridges set at broad intervals in the outer of the central area, and numerous radial grooves connected with the central network. The ridges are first discontinuous and irregularly waved; the intervals between ridges become smaller and smaller as they are formed outwards arranging themselves as normal concentric ridges.

This is an example of the course of the scale regeneration which has been carried out under the above described conditions. In short, the origins of the regenerated scales were established within 3 weeks after the removal of the ontogenetic scales, thereafter, they grew rapidly during about 4 weeks until they completely recovered their original size. It was also found that the ridges were formed at the extraordinary rate of one ridge every two days during the rapidly growing stage.

The regeneration of a scale takes place essentially in the same manner as the development of an ontogenetic scale as viewed from the histogenetic standpoint. But in the case of regeneration, the formation of a new scale is carried out in almost the whole region of the emptied scale pocket (Fig. 13). A partial destruction of the epithelium which occurred with the removal of the ordinary scale is repaired within only one or two days. Active cells proliferated from the pocket wall gather in the central area of the pocket to form a widely spread plain mass. The new scale, exactly, the bony layer of it, appears in the midst of the cell mass —the developmental process is likewise carried out. However, the bony layer of the regenerated scale is not a continuous layer but is formed as an assemblage of many small platelets. So, the section of the regenerated scale in germ stage shows a horizontal arrangement of discontinuous platelets (Fig. 14). The boundaries between these platelets become later a grooved network in the central area of the scale after the addition of fibrous lamella at the lower surface. The subsequent growth of the newly formed scale which is still a thin and flexible bony structure devoid of fibrillary plate follows the ordinary course as has been observed in the case of the development of the original scales.

In the regenerated scale in surface view, the cellular elements are observed to be more abundant than in the ontogenetic one because of the rapid growth of the former (Fig. 15). Inside of each ridge particularly of the region just within the scale margin there is a row of cells adhering closely to the ridges, while the widely spread osteoblasts occupy the spaces between outer ridges. Between these osteoblasts and the remaining cells at the inside of the ridges a transformation phase can be observed. This furnishes evidence that some of the osteoblasts are transformed to the ridge formative cells as was suggested in the previous section.

Effect of water temperature and feeding on the regeneration of scales

Procedure

Early in winter, when the season causes the growth of fish to cease, twenty-four goldfish about half a year old ranging from 3.5 to 4.5 cm in body length were brought from a nursery. They were reared in the laboratory during the period from December to the following April. The fish 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°C to 22°C and the other two at 4°C to 7°C during the experimental period. One each of the two groups respectively was fed on earthworms and grain food; the other was kept under the starved condition. The fish under experiment therefore consisted of four groups; fed in warm water (WF), starved in warm water (WS), fed in cold water (CF) and starved in cold water (CS). At the beginning of rearing, fifteen scales just below the dorsal fin were removed in three rows including the lateral line from all fish. The regeneration of the scales in each fish was examined at intervals.

The material of this rearing experiment was also used in the observation on the absorption of scale and in the histochemical detection of alkaline phosphatase both of which will be described in the following sections.

Observations

When a scale is removed from the body surface, the corresponding part of epithelium and the marginal osteoblasts are also taken away together with the scale. In all fish in the four groups, the epithelial part recovered in one or two days. Ultra thin scales appeared within two weeks or so in the warm water groups whether fed or starved; on the other hand, no regeneration of scale took place in the cold water groups even after four months (three months in CF). After three months, the water temperature of CF-tank was gradually brought up to 20°C. Then, the fish in that tank regenerated their scales in twelve days. The facts reveal definitely the importance of water temperature in the regeneration of the scales.

As has already been stated, the regenerated scales are considerably smaller in size than the ordinary ones at first. They rapidly grew larger during about two weeks until their growth comes to be checked by the wall of the scale pocket. The regenerated scales which appeared in the warm water groups showed so far no indication of difference in structure between the fed and the starved groups, although those of the starved fish came to be absorbed together with the other ontogenetic scales within fifty days. No absorbed scale could be observed in the fed-in-warm-water group and the cold water groups in which regeneration did not occur. Therefore, the regeneration of the scales may have almost no connection, at least in the early stages, with the nutrition of the fish.

Recovery of the superficial structure of regenerated scales

Procedure

A new method was devised in an attempt to examine change in superficial structure of a growing scale. Its essence is the re-examination of a scale which was once removed from and then inserted again into the body surface. After once removed and after a certain period of rearing of the fish followed the insertion of the scale, the same scale at different growth stages is photographed twice at a definite magnification; the thus obtained two figures are compared and contrasted. It is necessary to take care of the inserted scale lest it fall off. For this reason, soaking of the removed scale in Ringer's solution while the photograph being taken should be avoided, though once tried; it caused the scale to drop off easily. Rather, it would be better to put the scale during the treatment on a clean slide glass without allowing it to get wet. Moreover, after prompt returning of the scale the fish should be kept quiet separately

in a small tank for one night. The presence of the scale should be confirmed before letting the fish free into the rearing pond.

In this experiment, the 5th scale of the upper first row was previously removed from each of nine goldfish in order to obtain the regenerated scale. Every four, six and eight weeks after operation, the newly formed scale was removed from three fish at a time, photographed and quickly returned to the original scale pocket. After about five months of rearing of the fish, these scales were removed and photographed again.

Results and consideration

The general aspect of the experiment is shown in Table 3. Fish No. 6 was lost. In Nos. 3, 5 and 8, the inserted scale had fallen off before the re-examination. Accordingly, out of the nine fish the once-examined scales could be re-examined on five. In all cases, the scales had continued to grow in proportion to the growth of their respective bearers. For example, the comparison of two figures in Nos. 2, 4, and 7 is presented respectively in Figs. 16, 17, and 18; each represents respectively the group of the 4th, 6th, and 8th weeks of the regeneration. There is in each scale a clear boundary which looks like an annulus between the inner old and outer new regions. This must be formed because of retardation of growth of the scale immediately following the operation such as removing and inserting. By means of this boundary one can distinguish easily the scale parts before and after the first examination.

The comparison of the first picture with the corresponding part of the second reveals the following four points: 1) The structure of the regenerated scale remains essentially the same even after about five months of growth. 2) The focal area which shows a grooved network undergoes a little change, viz., the central polygonal platelets come to be filled with a number of minute structures which are irregular elevations of bony material. These structures may be regarded as discontinuous fragments of ridges (Fig. 19). Some of the grooved boundaries between the platelets become indistinct in places and relatively smaller platelets tend to fuse into one. But careful examination of the real scale under microscope shows that the greater part of the network remains unchanged. 3) The radial grooves become clearer; most of them extend to the periphery across

Table 3. Growth of the fish in the period between first and re-examination of the regenerated scales. No. 6 was lost. In Nos. 3, 5, and 8, the once-examined scale was not re-examined. Bracketed number shows the days of regeneration in total.

Fish	First examination				Days from first to re-exam.	Re-examination		
	Days of regener.	Date	Body length (cm)	Body weight (g)		Date	Body length (cm)	Body weight (g)
No. 1	28	Jun. 15	8.2	19	156 (184)	Nov. 18	9.5	36
2	〃	〃	7.9	18	〃	〃	8.4	31
4	42	Jun. 22	9.5	33	149 (191)	〃	10.1	42
7	56	Jul. 6	9.9	33	135 (191)	〃	10.9	49
9	〃	〃	8.0	20	〃	〃	8.4	28

the boundary between old and new parts. A few grooves start newly at the boundary where a few others which start from the focus are interrupted. No groove is formed or disappears in the old part. 4) The arrangement of linear ridges is not altered in the least; the pattern of the already set ridges remains exactly as it was, and new ridges never appear in the focal area excepting some discontinuous irregular fragments as mentioned before.

It may safely be concluded from the above facts that the regenerated scale can by no means regain a superficial structure like that of the previous ontogenetic scale at least within six months of regeneration, although it is undeniable that a part of the grooved network disappears. The author would like to state, however, that the recovery could not take place at an indefinite future time beyond six months because the cellular elements which act in elaboration of the superficial structure should be considered to degenerate at an early stage of regeneration. When the scales were first examined, even after four weeks of regeneration, their essential pattern was found to have been already established. A little alteration in the focal area after that is nothing but the elaboration of the superficial structure by a deposit of more bony material. The appearance of fragmental elevations of bony material in the central platelets is evidence of the elaboration. Properly, these minute discontinuous fragments are expected to become usual linear ridges. In the regenerated scales, because the focal grooved area is formed instantly in a relatively wide space as a gathering of a number of bony platelets, none of them excepting the most external ones shows any directional growth owing to the reciprocal restriction. This is supposed to be the reason why the fragments appear in the focal area instead of linear ridges.

Fig. 20 shows the scale taken from fish No. 9 in which the scale was inserted obliquely being shifted from the original situation after the first examination. Probably owing to the fact that the scale had been strongly pushed against the pocket wall especially at the dorso-lateral margin, the figure (b) indicates a trace of absorption of the scale covering a considerably wide area at right. It may be considered that the scale, after the absorption had ceased, continued to grow according to the growth of the fish. In this scale, most of the grooves extend to the newly developed area keeping the changed situation, but the newly formed ridges stand out in sharp contrast to those of the older area in their direction of running; the new ridges do not grow parallel with the changed older ones but they keep the ordinary direction (compare with a). As a regenerated scale in general manifests much of the pattern of the previous ontogenetic scale as for the arrangement of ridges, consideration of the above observation may lead to the thought that the pattern of a certain scale is determined by the general form of its scale pocket and that the pattern determined by the scale pocket may not be altered easily.

Here, the possible influence of the operation, removal and insertion, on the natural growth of the scale must be considered for fear that the scale structure might be different if the scale were not taken out of the body. Most of the cells surrounding the regenerating scale are supposedly taken out together with the scale itself. It is quite possible to consider that they endure the treatment and

survive after being returned to the original pocket from the fact that a little alteration of the superficial structure does occur in the focal area. There is of course some sign of retardation of scale growth just after the treatment, but it would not seem to indicate a serious influence on the alteration of the pattern of the scale which was once taken out from the body.

Discussion

When a scale is removed from a scale pocket, a newly regenerated scale replaces it within a short period. The process of scale regeneration traces essentially the same course as that of the ontogenetic development in the young fish. Since it is carried out in the wide space of the scale pocket, the bony layer of the regenerated scale is formed as an aggregation of a number of polygonal platelets, not as a continuous plate. This was first pointed out by Neave (1940). The boundaries of these platelets remain to form a grooved network in the focal area. The fibrillary plate is added from the underlying pocket wall after the bony layer has been laid down.

The concentric ridges are set at first short and irregular at wide intervals but they change gradually to closely set continuous ridges according to the outward growth of the scale. This seems to show that the ridges are formed with difficulty while the scale is growing rapidly, but they come to get close to each other when the growth rate declines. However, it is interesting to point out that the time required for inducing the ridges in such a rapidly growing stage is very short; three or four ridges are formed within a week. In the central platelets the linear ridges are never formed but their rudiments lie scattered taking the form of a number of minute fragments. The irregular appearance of these fragments reminds one of intercellular spaces furnishing a suggestion on the manner of ridge formation.

Many workers recognized the cells attached closely inside of ridges and thought that they introduced the ridges. Klaatsch differentiated the upper cell layer of scale into the deeper and the superficial cells; the former supply the foundation of the bony layer while the latter elaborate the relief of the scale surface. Ussow agreed with him for the most part. Nusbaum as well as Neave (1936) considered differently that the cells attached inside of ridges may have an osteolytic property so the ridges are formed as much by a hollowing out behind as by a building up in front. Paget believed that the cells in question were descendants of the upper layer cells and spent their plasma in the formation of ridges leaving their nuclei inside of their own product. According to Setna, ridges are formed in a damming up of the scale margin being obstructed by the wall of the scale pocket. Neave (1940) later reconsidering his previous opinion, regarded the cells in question as osteocytes which were not buried in the bone owing to its thinness. He insisted that ridges are not the product of special cells because the superficial cells lie independently of the underlying just formed ridge but that the ridges are formed when the scale growth comes to be stagnant as a partial thickening of excessive bone formative substance which is ordinarily utilized by the growing scale margin. Dietrich follows Neave's later opinion

but she seems to admit the participation of special cells since her description is similar to that of Klaatsch. Wallin (1957) is of the opinion that normal ridges are formed only when the epidermis or the denser dermis in the scale pocket, not the scale pocket itself, forms a greater obstacle or pressure resistant to the spreading of the scale; on reaching a threshold value, periodically, the pressure induces the formation of a ridge which temporarily removes the pressure. The variety of opinion suggests the difficulty in solution of the problem.

At this point, a description on the arrangement of ridges of typical ontogenetic scale of the goldfish is required in considering the origin of ridges. In the anterior part of the scale the ridges are set close to each other parallel to the margin, while they run obliquely in the upper and lower lateral parts at a little angle to the margin. Several marginal ridges thus come to be interrupted, in turn, by the intersecting lateral margin. On this account, the ridges in the lateral parts are fewer in number and the intervals between them are wider than those in the anterior part. In the posterior part, the ridges are reduced in number to less than half of those in the lateral because some the lateral ridges come to be broken off at the border between the unexposed and exposed parts. Thus, both ends of each of the remaining few ridges meet with each other in V-shape at a point on the median line of the posterior field. There, of course, the intervals between ridges are the largest in inverse proportion to the smallest number.

Setna's view is unreasonable because the ridges of the regenerated scale are formed before the scale margin comes in contact with the pocket wall and further because they do not always run parallel with the margin. There is no doubt but that some of the osteoblasts, even if they are not specially differentiated cells as Neave insists, take part in the ridge formation. The problem is, however, in what way such an arrangement of cells is brought about.

It has already been stated that in section the ridge seems to appear between two osteoblasts so as to fill the intercellular space with bony material. As a matter of fact, however, the ridges in surface view are independent of boundaries among upper layer cells when they have just arisen at the outermost margin of the scale, that is to say, the marginal osteoblasts are not regularly arranged along the ridge but they lie across it (Neave, 1940; Wallin 1957). Therefore, it can scarcely be said that the arrangement of upper osteoblasts is responsible for the induction of ridges. Rather, it should be considered that some of the cells remain in an arrangement at the inside of ridges only to make them grow. Therefore, the essential factor to cause ridges to form should be attributed to another cause.

As was previously described, only irregular fragments are laid down in the central platelets of the regenerated scales instead of linear ridges. This suggests the absence of any factor to induce the origin of linear ridges on these platelets which cannot grow outward because of the presence of marginal platelets. Accordingly, the growth of the bone in a certain direction is supposed to induce the continuous linear arrangement of ridges on the surface of the growing scale. If the direction of running of the ridges indicates the direction of scale growth, the ridges must be set in almost perfect concentric rings because the scale is

believed to grow equally in every direction even though the anterior growth rate is slightly larger than the posterior. The ridges, however, do not really form concentric rings but run at a slightly outward angle to the margin in the lateral parts of the scale. Moreover, the arrangement of ridges in a certain scale reappears even defective in the pattern of the regenerated scale which was formed in the same position (Saito and Yamada, 1953). In the example of fish No. 9 there has been shown the inalterbility of the scale pattern of the regenerated scale which is supposed to be determined by the general form of the scale pocket.

Judging from the facts thus observed, Wallin's view seems to be very interesting. He says on the arrangement of cells, "an obstacle in the direction of the growth of the scale (dermis, inactive preparatory stage of the scale forming cells, or another factor) causes in the assemblage of cells a pressure between the obstacle in question and the calcified scale on the inside." The present author would like to consider that the "pressure" causes a part of yet uncalcified soft bone to form a fold which develops into a complete ridge with the help of inner attached cells, hereupon, the cells are considered to be caught by the fold. The scale grows larger pushing against the surrounding tissues of the scale pocket at the anterior as well as the lateral margin and against the epithelium at the posterior. As the base of the pocket wall connects with the thick fibrous corium at the anterior, there the largest pressure may be formed. The pressure may gradually decrease along the lateral margin of the scale. At the apex of the scale, where it comes in contact with the epithelium through some marginal osteoblasts, the least pressure may be formed. The variance of the pressure at different parts thus results in scales with closely paralleled ridges at the anterior part, obliquely running ridges with comparatively wider intervals at the lateral and extremely few ridges at the posterior.

The re-examination of the same scale showed that the regenerated scale never recovers the superficial structure characteristic of the ontogenetic scale. This would be theoretically reasonable because it is difficult to consider that the ridges could grow centripetally. The superficial structure of the regenerated scale is incapable of being altered after it has been established by the upper osteoblasts which disappear sooner. Therefore, the author agrees with Wunder and he cannot help doubting the statement of Suzuki who insists the complete recovery of the superficial structure of the regenerated scales within only six months. In addition to Suzuki's observation, Kobayashi and Kawai (1960) reported in their study of the regeneration of scales in the mud-loach that the central area which is devoid of ridges and grooves grows smaller with the lapse of time. Since the relative width of the central area is naturally considered to become smaller with the growth of the regenerated scale, it seems necessary to compare the width of the area with the total width of the ordinary scale not with that of the regenerated scale. According to Okabe's observation made in our laboratory (unpublished), the ratio of the grooved area of the regenerated goldfish scale, taken monthly during six months, to the total area of the ordinary scale was about 0.6~0.7 in longitudinal diameter and 0.4~0.5 in transverse diameter; there was observed no lowering of the ratio throughout the 6 months period.

Saito and Yamada (1953) reported on the scales of the crucian carp that the

regeneration takes place faster in summer than it does in winter. Further, Saito (1955) proved no influence by rearing with different foods upon the superficial structure of the regenerated scales. The rearing experiment in the present study showed that water temperature is the principal factor in inducing regenerated scales; an active metabolism in a high water temperature accelerates the regeneration of the lost scales, even if the condition forces the fish to absorb other normal scales. This suggests the necessity of active metabolism of the fish not only for the regeneration but also for the growth of the scales. However, the normal scales cannot grow without the actual growth of the fish body.

VI. ABSORPTION OF SCALES

It is well known that the absorption of scales occurs in nature as a result of severe exhaustion of fish due to an intense activity such as spawning. Absorption of scales of the goldfish can be induced when the fish is reared under malnutritive conditions or when some artificial treatment is used. In the rearing experiment described in the foregoing section some phenomena related to the absorption of scales in both the regenerated and the ontogenetic could be observed. The absorbed scales were also obtained experimentally by means of the modified re-examination method. The process of the absorption and its cause will be discussed in comparison with the formation of the scale structure in general.

Procedure

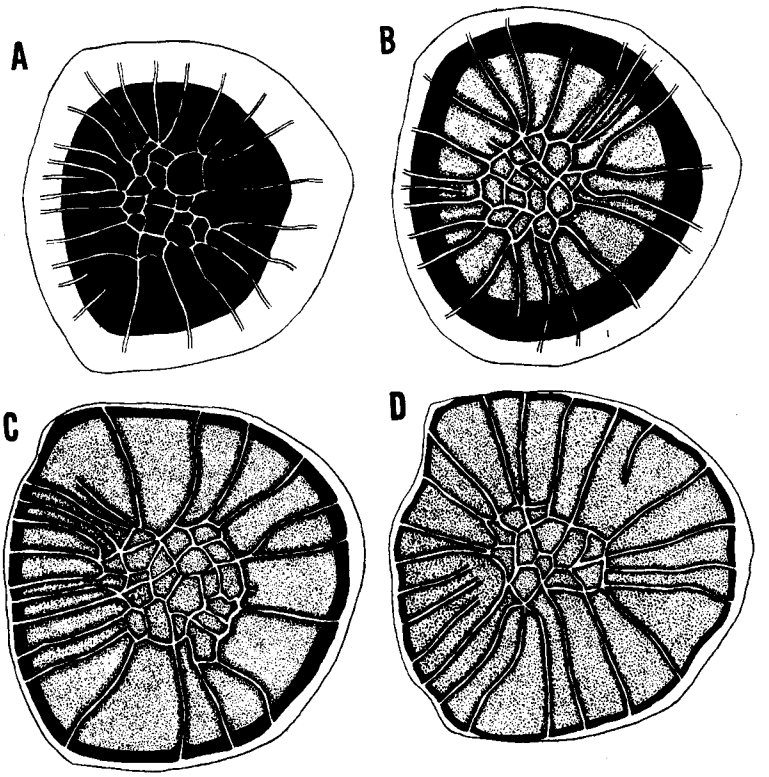
The scales under absorption were obtained from some of the fish during the rearing experiment described above in the previous section (p. 199). The scales were stained by Stoeltzner's method for the detection of the deposit of calcium salts: A fresh scale is rinsed in 2% solution of cobalt chloride for 5 minutes, so the deposit of calcium salts is changed to cobalt salts. After thorough washing in distilled water, the scale is transferred into a dilute solution of yellow ammonium sulphide, about 2 minutes; thus the site of calcium salts can be demonstrated as black precipitate of cobalt sulphide.

The essential point of the re-examination method has already been presented (p. 200), but the procedure was slightly modified in this study. 1) One scale each was removed from the middle body-part of three fish; at the anterior part of each scale a V-shaped piece was cut off. After the operation the scale which has lost most of the anterior part was returned to the original scale pocket. 2) Scales taken from another three fish were rinsed in 70% alcohol for just a short while. After washing in water they were returned promptly to the original places as usual. The scales were re-examined in the usual way after a certain period of rearing of the fish.

Results

A typical ontogenetic scale removed at the outset of the rearing and treated by Stoeltzner's method shows a deposit of calcium salts covering almost the whole surface excepting the outermost margin. The uncalcified margin in which one or two ridges are usually counted is somewhat narrower at the anterior part than at the posterior. Just inside of this margin, an intensely stained band borders

the central calcified area, and along each side of each groove are areas stained also in dark black although the groove itself is never stained (Fig. 21). This must be evidence that in the normally growing scale the just calcified margin as well as the areas along the grooves has more deposit of calcium salts than other calcified area while the outermost margin is not yet calcified.



Text-fig. 8. Diagrammatic drawings showing the order of decalcification in four stages of regenerated scale
A: 26 days of regeneration in WF
B: 50 days of regeneration in WF
C: 78 days of regeneration in WF
D: 50 days of regeneration in WS

In the warm water groups, the regenerated scales appear in a few days; they grow larger rapidly to such an extent that they attain nearly an equal size and a similar shape with the original ontogenetic scale. If these scales are stained by Stoeltzner's method, they show a relatively narrower central area stained uniformly in dark black and a wider stainless margin in comparison with the same parts of the ontogenetic scales. Such a peculiar staining of the freshly regenerated scales gradually changes to that of the ordinary scales. Take the scale at the 50th day of regeneration in the WF group for instance, the calcified zone spreads up nearly to the periphery and the colour tone of the central area

has become pale except the margin of the grooves. After 78 days, the width of the marginal dark stained band as well as that of the outermost stainless zone becomes so thin that the greater part of the scale is covered uniformly by a pale stained area (Text-fig. 8, A-C). The above observation seems to show the disappearance of the once deposited calcium salts in the central area. The process develops faster in the WS group than in the WF group. For example, in the regenerated scale taken from a fish in the WS group of 50 days rearing, there can be found not only almost no dark stained band at the periphery but also a sign of absorption occurring at the shoulders (Text-fig. 8, D). On the other hand, in the fish of the CF or CS, no regenerated scale appeared, the ontogenetic scales were never absorbed and their mode in staining showed as much the preservation of calcium salts in marginal and radial areas as did the scales taken at the outset of rearing. From the results obtained here, it may be concluded that a part of deposited calcium salts is removed prior to the actual absorption of the scale and that the process is accelerated in warm circumstances probably because of the active metabolism of the fish.

Normally, the fibrillary plate is formed at the inside of the outermost margin which consists of only the bony layer. But the corresponding section of the absorbed edge represents the inverse relation (Fig. 22). Moreover, a crack parallel with the absorbed margin is often observed on the surface of the exposed fibrillary plate which has been left behind free as a result of the absorption of the upper bony layer. In some cases the absorption of that layer takes place also on the central focal area (Fig. 23).

In the foregoing account, the absorption of the scale may be considered to consist of three processes in a broad sense, viz., the dissolution of calcium salts, the erosion of decalcified bone and the mechanical destruction of the fibrillary plate.

It was frequently observed in the regenerated scales that a certain ridge is not cut by a groove but continues across it (Fig. 24). This is quite curious because the ridges are linear elevations of bony layer while the grooves manifest a part devoid of bone. The grooves, aside from the absorbed portion, never reach up to the outermost margin, fading out in the uncalcified zone. It is quite likely, therefore, that the grooves extend as the result of the absorption of once formed bone matrix. The regenerated scales grow so rapidly until they become restricted by the scale pocket that the above phenomenon might be caused by the elongation of the groove unproportionate to the accelerated laying down of bony material. As may be seen in the figure, a part of the osteoblasts enclosing the scale margin enter into the groove. If these cells do take part in elongation of the groove, they should certainly have an osteolytic property.

Thus, the two phenomena, the absorption of the scale and the elongation of the grooves, may be considered on a common basis, that is, the erosion of bone. In this respect, the osteoclastic absorption of bone which is known as occurring in general osseous tissues comes to one's mind; the possibility of the idea will be dicussed later.

The scale from which the anterior part was cut off in V-shape shows a pair of sharp projected shoulders. The scale planted into the original scale pocket

after having been submitted such an operation was strongly absorbed particularly at the shoulders. This occurred equally in the three scales treated in that way from three individual fish. An example is shown in Table 4 and Fig. 25. The lost part of the scale is characteristic of a partial regeneration and not of an extension from the edge of the remaining part. The trace of the absorption may be seen at the scale margin extending from the shoulders to both the lateral sides and also in some of the central area. Along the ventro-lateral margin (left) is a new growth zone which shows slight growth after the absorption ceased.

Table 4. Growth of a fish used in the experiment for the examination of the fate of a scale in which the anterior part was cut off in V-shape

	Operation	Examination	Days in rearing
Date	Aug. 30	Nov. 10	72
Body length	9.9 cm	11.0 cm	
Body weight	38 g	53 g	

Table 5. Growth of a fish used in the experiment for the examination of the fate of a scale in which the cellular elements were killed with alcohol

	Operation	Examination	Days in rearing
Date	Jul. 30	Sept. 19	78
Body length	7.5 cm	8.2 cm	
Body weight	15 g	22 g	

In another experiment with three other fish, in which the cellular elements were killed by treating the scales with alcohol before insertion, the scales showed without exception a strong absorption at both the lateral parts. The result shown in Table 5 and Fig. 26 is an example from the three cases. At the anterior margin of this scale the extent of loss due to absorption is rather smaller as compared with the lateral margin, and a new growth zone develops discontinuously with the absorbed margin only at the anterior. No growth zone can be seen at any other part.

With another three fish, one of the scales was planted without receiving such operations as control for the above two experiments. Since the three scales continued to grow without showing any sign of absorption in this case (Fig. 27), it is obvious that the treatments, cutting off a part of scale or rinsing a scale in alcohol, caused the artificial absorption. It should be noticed that unlike the ordinary absorption which is caused by some environmental factors, other scales of the same individual fish are still growing larger while the scale in question is being absorbed. In the former experiment, the pocket wall which was stimulated by the sharpened tips of the shoulders would probably give rise to the absorption of the scale. It has been seen in Fig. 20 that such a stimulation caused the absorption of the regenerated scale at the part where a strong pressure may have been formed. It is a matter of course that the part lost in the V-shaped cutting was repaired by a partial regeneration because edge of the remaining part has no peripheral osteoblast. The junction between the regener-

ated and the remaining parts was also absorbed considerably. This would be caused in the necessity of joining the old part to the newly formed part. In the latter experiment, on the other hand, the scale was planted without hurt but the marginal osteoblasts were killed with alcohol. As the scale was washed before planting, it is impossible to suppose any effect of alcohol in stimulating the pocket wall. The dead scale having no power to grow by itself is nothing but an obstacle to the scale pocket, so the strongly absorbed scale may be an expression of the effort of the pocket which worked to eliminate the obstacle.

Discussion

Ichikawa (1953) reported similar results to the above on the disappearance of calcium salts prior to the destruction of the scales of the carp under starvation. On the surface of those scales he observed a remarkably thinner calcified layer compared with that of normal scale and considered that a mechanical breaking would occur on the softened scale due to the absorption of calcium salts from the fibrillary plate. The dissolution of a part of deposited calcium salts is taking place even under normal condition, but it is accelerated in warm circumstance with insufficient nutrition probably because of the active metabolism of the fish. Evidence for this statement is shown in the fact that the actively calcifying zone gives a stronger reaction being stained darker than the central calcified zone. Wallin (1957) proposed in his study of the roach that the width of the osseoid zone (the outermost uncalcified zone) or of the zone of calcification (dark stained zone) tells whether the fish is actually in the growing state or not, because he observed the fact that both the zones increase in width when the scales are growing rapidly. The present observation on the transition in staining reaction of the regenerated scales confirms his proposition. It was also proved that scleroprotein, the ground substance of bone, is produced by the active osteoblasts at the scale margin while calcium salts secondarily deposit on it at just inside of the margin. According to another report of Ichikawa (1956), a part of the deposit of calcium salts in goldfish scales is considered to be replaced gradually by new elements since he found a deposit of injected radioactive strontium in the inner part of the scales. Therefore, it seems probable that there is always a repetition in depositing and dissolving calcium salts over the whole surface of the scales and that a turnover of calcium ions is always taking place between the scales and the surrounding tissue fluids.

The idea of mechanical breaking proposed by Ichikawa may be applied only to the fibrillary plate but not to bone. The term "mechanical" seems to imply a rubbing action of the shrinking scale pocket, but the experimental results showed that the absorption of scale can be induced in the growing fish. Osteoclasts, usually multinucleated giant cells having an osteolytic function, are known in general osseous tissues especially where the absorption is taking place. The nature of their function or the stimuli which cause them to form still remain as unsolved questions. They are regarded as arising from the fusion of relatively immobile connective tissue cells or of mobile cells such as macrophage or even from any cells, and such transformation of cells is said to be reversible (Hancox, 1949). The formation of osteoclasts in scale pockets from osteoblasts or from

fibroblasts under some stimulation appears therefore to be quite likely. It was found in this study that the stimulation which caused the scale to be absorbed was a strong pressure against the scale pocket in one case or the killing of the cellular elements of the scale in another case. The absorption brought about by starvation is similar to the former case.

The absorption of bony layer occurs also in the focal area. According to Yasuda (1958), scales of the crucian carp reared under a mal-nutritive condition for three years showed in the central area a network like that of the regenerated scales. As he did not illustrate the scales the present author cannot offer any comment about that, but he supposes that the partial absorption which occurred in the focal area of those scales probably presented such a network, so they should be different from the regenerated scales in structure because the former ones are caused in the partial destruction of bone while the latter ones consist of complete bone which only lacks linear ridges.

VII. SOME BIOCHEMICAL ASPECTS OF THE SCALE GROWTH

Alkaline phosphatase and glycogen

The protein constituent of teleost scales is reported to be composed 76% of collagen and 24% of ichthylepidin; the latter is also an albuminoid intermediate between collagen and keratin (Green and Tower, 1901). In general in the osteogenic process, alkaline phosphatase is known to play an important role in producing protein and the subsequent ossification. The occurrence of a considerable amount of glycogen prior to the calcification has also been recognized in many osseous tissues. Since it was interesting to ascertain whether or no alkaline phosphatase and glycogen exist in the scale forming cells, as in other osseous tissues, histochemical tests for those substances were applied to tissue sections of goldfish skin.

Procedure

The materials used in this study were obtained also from the fish which were being reared under the various conditions to investigate the regeneration of scales (p. 199). Small pieces of skin taken from these fish were fixed and embedded in paraffin. As fixatives, Bouin's and Helly's fluids were used 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 respectively. In the histochemical test for alkaline phosphatase, use was made of Gomori's calcium-cobalt technique, sodium-β-glycerophosphate buffered pH at 9.0 as the substrate. For the demonstration of glycogen, Bauer's cromic acid-Schiff reaction and Best's ammonia carmine reagent were used on the collodionized tissue sections. 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. Besides these, the measurement of hydrolytic power of alkaline phosphatase in the kidneys of the fish under the experimental rearing conditions was performed *in vitro* to find out any possible relationship between the enzymic activities of the kidneys and the environmental factors.

Observations

As has been stated already, the rearing was carried on dividing the fish into four groups; two groups in warm water (20°C) and two groups in cold water (5°C) respectively with or without food supply. Probably because of the smallness of the tanks neither the growth of the bodies nor that of the scales could be observed in the groups during the experimental period.

The histochemical test for alkaline phosphatase applied to the skin of these fish proved that the enzyme exists only in the epithelial cells but not in the cells or tissues surrounding the scales (Fig. 28). There was observed no difference in staining reaction with the fish under different rearing conditions. It has already been mentioned that no regeneration of scale occurred in the cold groups even after four months of rearing contrary to the rapid appearance of the regenerated scales in the warm water groups (see p. 200).

On certain days after the removal of scale, the corresponding skin pieces in which no regenerated scale was observed were examined in the same way. After 26 days, for example, the operated area is covered completely with regenerated epithelial cells including a neuromast under which is an accumulation of multiplied fibroblast; the enzyme is detected only in the epithelial cells but not in the fibroblasts (Fig. 29). Similarly, after 78 days, the scale pockets are filled with cells, but in them no alkaline phosphatase can be proved (Fig. 30). These fish regenerated the lost scales when the water temperature was raised to 20°C. Both the fibroblasts in the wall of the scale pocket and the osteoblasts which adhered to the newly formed scales showed a clearly positive reaction of the enzyme (Fig. 31).

Therefore, there is no doubt but that the occurrence of alkaline phosphatase in the scale-forming cells is a necessary factor to induce the regeneration of scales and probably to make them grow. The absence of the enzyme in the cells associated with the ontogenetic scales being in restrained growth is very suggestive of its importance in producing the proteins which constitute the ground substance of the scales.

Table 6. Alkaline phosphatase activity of the kidneys of the fish reared for 50 days under various conditions. Eighteen hours incubation at 36°C in a substrate containing sodium-β-glycerophosphate in veronal buffer adjusted pH at 9.0. Colorimetric measurement was performed by Youngburg & Youngburg's method. WF: warm water (20°C) with food supply; WS: warm water without food supply; CF: cold water (5°C) with food supply; CS: cold water without food supply

Condition of rearing	Figh		Org.-P in substrate	Inorg.-P liberated per 10 mg of kidney, less than control	Org.-P hydrolysed
	Body length (cm)	Body weight (g)	(mg)	(mg)	(%)
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

As the appearance of regenerated scales was assumed to be influenced by water temperature, it was questioned whether any correlation exists between the phosphatase activity and the environmental conditions. In this account, the kidney was chosen for a study of the activity of the enzyme because it was supposed to possess rather abundant alkaline phosphatase and to be sensitive to the metabolic activity of the fish. Measurements were made on the kidneys obtained from fish at 50 days of rearing in each group. The water-extracted enzyme was added to a substrate containing a known amount of phosphoric ester, and after incubation for 18 hours at 36°C liberated inorganic phosphate was measured by means of a photoelectrocolorimeter. The result is shown in Table 6. The fatness of the fish in the warm groups is conspicuously lower than that of those in the cold; the enzymic activity of the kidney decreases in the order of WF, WS, CF and CS. This is evidence showing an intense metabolic activity of the fish in comparatively higher water temperature. Also feeding seems to have considerable, though less, influence on the metabolism of the fish. The difficulty of scale regeneration in the fish at low temperature is supposed to be caused by the weakness to synthesize intracellular alkaline phosphatase due to the inactivation of internal metabolism.

As to the histochemical detection of glycogen, both the methods yielded negative results even in the cells associated with the regenerating scales in which the presence of alkaline phosphatase was proved.

Polysaccharides and ribonucleic acid

The occurrence of mucopolysaccharides in the form of chondroitin sulphate has been known in various hardened tissues in connection with the calcification (Logan, 1935; Partridge, 1948; Dziewiatkowsky; 1951; Bradbury, 1958). Besides the presence of alkaline phosphatase, it is reported that a high content of ribonucleic acid (RNA) is generally found in protein-forming cells and that there is a direct correlation between the content of RNA of the cells and their capacity for synthesizing proteins (Brachet, 1950). Histochemical examinations for these substances were thus attempted to the normal and the regenerated scales.

Procedure

Pieces of the goldfish skin containing either normal or regenerated scales were fixed in Allen-Bouin's and Carnoy's fluids or in lead acetate-formalin mixture after Lison-Sylvén (Glick, 1949). Sections prepared by paraffin method were treated with McManus' periodic acid-Schiff (PAS) procedure to demonstrate polysaccharides. The sections were also stained with toluidine blue to render metachromasia visible. Staining with methyl green-pyronine after Unna-Pappenheim was employed for the identification of RNA.

Results

Both the bony layer and fibrillary plate show a strong reaction to the PAS regardless of the state of calcification. Fig. 14 shows a section of a regenerated scale to which the PAS was applied. The reaction is negative at the part of the fibrillary plate just under the grooves. The difference in intensity of the reaction cannot be identified as due to the two different layers. The sections of a normal scale stained with toluidine blue show neither orthochromasia nor metachromasia

excepting only the lowest (newest) fibrous lamella which shows orthochromasia. When the sections of the scale which had been decalcified with HCl were treated with toluidine blue, the bony layer manifested metachromatic staining. Similarly, the regenerated scale in germ stage which is not yet calcified also shows metachromasia. This proves in the bony layer the presence of acid mucopolysaccharide bounded by calcium ions.

The active osteoblasts around the rapidly growing regenerated scales show feeble pyroninophilia (Fig. 32); the staining reaction disappears when the section is treated with 5% trichloroacetic acid in order to remove RNA from it. On the contrary, the osteoblasts enclosing the periphery of the normal ontogenetic scales do not show such an affinity with pyronine. These findings indicate that the contents of RNA in the scale-forming cells is intimately connected with their ability to synthesize proteins.

Parathyroid hormone

Parathyroid hormone is known to control the concentration of blood calcium in higher vertebrates. Parathyroidectomy results in lowering the serum calcium level (Collip, 1925). Hypertrophy of the glands affects skeletal tissues by causing removal of calcium salts from them and the implantation or the tissue culture of the gland in contact with bone induces the absorption of the bone (Barnicot, 1948; Gaillard, 1955). The presence of parathyroid glands in fish is not so far confirmed. Only Schereschewsky* (1940) found in *Lebistes* a glandular epithelium, at the isthmus between first gill arches, which he insisted was the parathyroid. For this reason, an injection experiment of the hormone prepared from horses was performed in an attempt to ascertain the possible effect of the hormone on the fish scales.

Procedure

Parathyroid hormone was prepared according to Tweedy's procedure (Tweedy, 1930) from the glands taken from horses.** From 40 g of the glands 16.5 mg of gray amorphous powder was obtained. Ringer's solution containing 0.3 mg/ml was injected everyday for 42 days into three fish so as to correspond to 0.1 mg per 100 g of the body weight at a time, and their scales were examined weekly by applying Stoeltzner's method.

Result

The fish as well as their scales continued to grow normally during the experimental period as compared with the control. The absorption of their scales could not be observed. The regeneration of the scales which were removed for weekly examination took place as usual, and no difference in the manner of calcification was observed in comparison with those of the control fish. After all, no effect on the scales of the repeated injection could be observed.

Discussion

The presence of alkaline phosphatase, acid polysaccharide and ribonucleic

* According to Kawamoto (1956).

** The collection of the glands and the extraction of the active substance were performed through the aid of Mr. T. Fukazawa of the Faculty of Agriculture, Hokkaido University, Mr. H. Mori of the Ebetsu Health Center, and Mr. S. Nakatsubo of the Sansho Pharmaceutical Co. Ltd., to whom the writer expresses his heartfelt thanks.

acid was proved in the goldfish scales and the associated cells. This suggests that the scales, at least their bony layer, is formed by a biochemical mechanism much the same as that of other osseous tissues, although no glycogen was proved and no effect of parathyroid hormone could be observed.

The mechanism of the hardening of osseous tissues is not so far quite solved. Robison and his collaborators (Robison, 1923; Robison and Soames, 1924; Martland and Robison, 1924; Fell and Robison, 1933; 1934) offered an explanation of it from the evidence that increase in amount of alkaline phosphatase and glycogen is recognizable in the hypertrophic cartilage cells. They believed that alkaline phosphatase is concerned in the process of ossification by exerting effect on phosphoric esters in the blood, thus bringing about a local increase in the concentrations 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. It was supposed that hexosemonophosphoric esters liberated in the phosphorolytic process of glycogen may be the substrate for phosphatase (Harris, 1932). Nowadays, however, since it is known that bone salt is not a simple form of calcium phosphate but that it consists of submicroscopic crystals of hydroxyapatite $[Ca_3(PO_4)_2]_3 \cdot Ca(OH)_2$ (Hendricks and Hill*, 1950), the process of the deposition of calcium salt is considered to be not so simple as described above. At any rate, there is no doubt but that alkaline phosphatase plays an important role in the deposition of the calcium salt by liberating phosphate ions. There are a few reports concerning alkaline phosphatase related to fish scales (Roche *et al*, 1940; Wallin, 1957). Roche *et al* found that the activity of the enzyme in *Clupea pilchardus* varied according to seasons, that is, the activity in the period from May to July rises up to ten or twenty times that in winter. Wallin failed to demonstrate the enzyme histochemically in the scale-forming cells of the roach and concluded that alkaline phosphatase has no bearing on the formation of the scales. He considered that Roche *et al* had measured an activity of the enzyme in the epithelium because he found strong activity thereof in the epithelium. The invariable activity of alkaline phosphatase in the epithelial cells of the goldfish was proved also in the present study. It was shown distinctly that the osteoblasts around the regenerated scale which is in an actively growing stage contain a considerable amount of the enzyme. Further, the occurrence of alkaline phosphatase in the original papilla cells was recognized in the rainbow trout, in spite of the absence of this enzyme in the epithelium of this species (Yamada, 1956). Wallin's failure is probably due to the fact that he applied the reaction to cells which were in a state either inactive or active but not sufficiently sensitive to react. This interpretation may be applied to the explanation of the negative reaction of alkaline phosphatase as well as RNA in the normal ontogenetic scales. Lφvtrup (1959) found in a study of amphibian embryogenesis that the activity of alkaline phosphatase was retarded or blocked at low temperatures when the morphological development was delayed or stopped. The difficulty of scale regeneration at low temperature is thus a result of a low chemical activity which is reflected in the

* Cited from Maximov and Bloom (1952).

facts that the enzyme was histochemically absent in the associated tissues and that the kidney showed a low enzymic activity *in vitro* in case of the fish kept at a low temperature.

The absence of glycogen in the scale-forming osteoblasts at any stage seems strange because glycogen is generally found as the source of the substrate for alkaline phosphatase in every osseous tissue which shows the occurrence of the enzyme. Wallin also could not detect glycogen. The fact is unexplainable so far the methods for glycogen were correctly employed. Only it may be possible to consider that phosphate ester already decomposed is carried by humours into the areas where the scales are growing.

The positive reaction of PAS in both the bony layer and the fibrillary plate suggests the presence of mucopolysaccharides as a cementing substance among collagen fibrils. As the reaction is not influenced by the state of calcification this type of polysaccharides may probably have no connection with the calcification. The negative reaction at the part of the fibrillary plate just under the grooves is interesting in considering the possible function of the grooves. A metachromatic staining in addition to the PAS on the decalcified bony layer and on the regenerated scales implies the presence of acid polysaccharides, especially of chondroitin sulfate, as it was reported that metachromasia is weakened *in vitro* when calcium ions are added to chondroitin sulfate (Levine and Schubert, 1952). The fibrillary plate does not show metachromasia; this suggests some other calcifying mechanism in this structure differing from that in the bony layer. Wallin is of the same opinion as he found many large crystals, the so-called Mandlian bodies, in the fibrillary plate. Kato (1953) proved that the Mandlian bodies are crystals of calcium oxalate which were thought to be rare in the animal kingdom. This together with the fact that it lacks chondroitin sulfate, makes the fibrillary plate to be conspicuous among calcified tissues. The orthochromasia of the newest fibrous lamella shows its basophilic nature which is soon lost probably on account of what was considered by Neave as an infiltration of ichthylepidin.

A high concentration of alkaline phosphatase and RNA in the osteoblasts around the regenerated scales is evidence of active protein synthesis, that is, the formation of collagen fibrils, occurring in these cells. It is still uncertain in what way these substances participate in the synthesis of proteins. It may be said without doubt that the occurrence of these substances in the scale-forming cells is a necessary condition for the growth of the scales. Concerning the formation of collagen fibrils in fibrogenic cells, for reference, Jackson (1954) reported in a bone rudiment of fowl embryo that the cytoplasm of the osteoblasts is packed with long submicroscopic filaments which may become extracellular fibrils of the bone matrix. It was also found by him (1955) that there are present in fibrogenic cells some cytoplasmic granules which contain polysaccharide protein components and give alkaline phosphatase reaction but no specific staining for nucleic acids. He assumes the function of the granules that they may secrete the ground substance of bone, they may synthesize the collagen fibrils, or they may contain precursors for the complex extracellular material.

Wallin made an injection experiment on the roach using AT 10, an anti-

tetanic drug which acts similarly to the parathyroid; he found that it caused a conspicuous rise in the content of serum calcium within 48 hours after injection. He concluded in his account that there is no essential difference between the fish and higher animals in reacting to a preparation of the character of the parathyroid hormone. Since he did not make any comment about the effect of the substance on the scales and since in the present study the measurement of serum calcium was not made, it is impossible to compare the two results with each other. However, as Wallin made research on this problem in his study of fish scales, the present author supposes that he could not observe any positive effect of AT 10 on the scales. In the present case, on the other hand, it is uncertain whether such a rise of serum calcium level might be caused to occur or not. After all, the growth of the scales was by no means affected by the injection of the hormone prepared from the glands of horses.

VIII. GROWTH OF SCALES ANALYZED BY MEANS OF TRANSPLANTATION

The transplantation of scales in the goldfish has been undertaken with success by several workers. Mori (1931) observed the transformation of the ordinary scales when they were transplanted into the lateral line. Goodrich and Nichols (1933) described the effect on chromatophores and the tissue reactions following after auto- or homoioplastic transplantation of scales among various types of goldfish. Besides these works on the transplantation of an individual scale, there are other studies concerning the transplantation of a patch of skin including several scales (Sauter, 1934; Nardi, 1935). But there is no report which analyzed the appearance of the superficial pattern of scales by means of transplantation. In this section, the observation on the growth of transplanted scales which was traced according to the re-examination method will be presented.

Procedure

A general account of the experiment is shown in Table 7. A certain scale in each of nine fish was removed from its scale pocket and another smaller scale taken from the same individual (autoplastic transplantation) in Nos. 1-6, or from another smaller individual (homoioplastic transplantation) in Nos. 7-9, was transplanted into that scale pocket. The ordinary and donated scales were photographed, the latter one before planting. The transplanted scales were chosen so as to be rather smaller in Nos. 1-3, moderately smaller in Nos. 4-6 and remarkably smaller in Nos. 7-9 than the ordinary scales. After a certain period of rearing of the fish, the transplanted scales were removed and photographed again. Then the obtained three figures from one fish, that of the ordinary scale, the transplanted scale before planting and the same scale after rearing, were compared with each other. A tracing paper on which was copied the outline of a figure was employed in the comparison putting the paters one upon another figures.

Results

The fish may be divided into three groups, in each of which are three fish,

Table 7. Growth of the fish used in the experiment of scale transplantation, and the comparison in size of the ordinary and the transplanted scales. Autoplastic transplantation in Nos. 1–6. Homoioplastic transplantation in Nos. 7–9, the transplanted scales were taken from a fish, 4.3 cm in body length.

Fish	Rearing period	Days in rearing	Before rearing		After rearing		Diameters of ordinary scale		Diameters of transpl. scale		l/L	t/T
			B.L.	B.W.	B.L.	B.W.	Long. (L)	Trans. (T)	Long. (l)	Trans. (t)		
			cm	g	cm	g	mm	mm	mm	mm		
1	May 14 Nov. 18	188	9.8	36	10.8	54	5.9	6.8	5.8	5.3	0.98	0.79
2	May 14 Nov. 18	188	9.8	35	10.5	48	6.1	5.9	5.8	5.0	0.95	0.85
3	May 14 Sept. 19	128	8.1	18	9.1	30	4.7	4.6	4.7	4.2	1.00	0.92
4	Aug. 29 Nov. 10	73	9.6	36	10.3	48	6.0	6.0	5.0	4.9	0.83	0.81
5	May 15 Nov. 18	187	8.2	19	9.0	26	4.5	4.6	3.4	3.6	0.76	0.77
6	Sept. 1 Nov. 10	71	8.8	31	9.2	33	5.4	6.5	4.3	3.6	0.79	0.75
7	Jul. 3 Nov. 18	138	7.3	14	8.3	26	4.2	4.4	2.0	2.5	0.48	0.57
8	Jul. 3 Nov. 18	138	6.9	11	7.9	21	3.4	4.1	2.0	2.4	0.58	0.59
9	Jul. 3 Nov. 18	138	6.2	8	8.1	21	2.8	3.9	2.0	2.4	0.70	0.62

according to the ratio of the two diameters of an ordinary scale to that of the transplanted scale. The results will be described in turn for each group.

No. 1 - No. 3 (Figs. 33-35): In each fish, the ordinary scale was the 6th of the upper first scale row while the transplanted scale was the one from the 20th of the same row, so the latter is nearly the same as the former in longitudinal diamter but is slightly smaller in transverse. Therefore, the transplanted scale showed a larger growth after rearing of the fish at both the lateral margins than at the anterior where the width of the extension is variable in detail, viz., the donated scale has a tendency to approach the ordinary scale in form adapting itself to the general form of the scale pocket of the host. Table 8 shows the

Table 8. Width of growth and number of ridges formed after the transplantations at the anterior and the lateral parts of the transplanted scales in Nos. 1-3. W: width of growth; R: number of ridges

Fish	Anterior growth				Lateral growth			
	Max.		Min.		Upper		Lower	
	W. (mm)	R.	W. (mm)	R.	W. (mm)	R.	W. (mm)	R.
No. 1	0.36	17	0.15	8	0.53	13	0.49	12
2	0.19	7	0.11	5	0.37	11	0.43	12
3	0.28	11	0.07	2	0.20	3	0.50	12

width of growth at different parts and the number of ridges formed in the corresponding parts. It is obvious from the table that the scales showed a better growth at the margin where the scale pockets supposedly offered them more space to grow. The ridges were formed almost in proportion to the width of growth. The distance between ridges is wider at the lateral margin than at the anterior, but this is common in usual goldfish scales and is not limited particularly to the transplanted ones. Rather, it should be emphasized that the space in the scale pocket exerted almost no effect on the spacing of ridges though it allowed a better growth of the scale at the lateral part. The examination by making use of tracing paper proved that the transplanted scales in No. 2 and No. 3 barely attained approximately the same size and form as the ordinary scale after 6 or 4 months, despite the fact that the transplanted scales should grow larger than the ordinary scales because the fish showed a considerable increase either in body length or in body weight during the period (Table 7). Accordingly, it can hardly be said that the transplanted scales grew faster than other scales of the same fish even though such scales are not restricted by the wall of the scale pocket. The position of the grooves in the donated scales was not altered; they extend straight outward keeping their ordinary situation. This seems to indicate that the scale pocket has no definite pattern as to the induction of the grooves and that the osteogenic cells may easily be transformed into osteolytic cells.

No. 4 - No. 6 (Figs. 36-38): The ordinary scale removed was the 6th of the upper first scale row while the donated scale was taken from the same position of the upper 4th row; the latter corresponded to about 70~80% of the former in longitudinal or transverse diameter. As the experiment failed in No. 4 and No. 6, two other fish were recruited in the course of rearing. In No. 5, which was reared for a longer period than the other two, the transplanted scale showed a marked growth around the margin particularly at the ventro-lateral probably because the scale was planted in a pocket biased dorsalward (Fig. 3~~8~~). Intervals between ridges in the ventro-lateral field where was found the largest growth, were distinctly wider than those in the inner part of that field and also in the corresponding part of the ordinary scale, while in the anterior and dorso-lateral fields, the ridges lie in uniform spacing being unaltered. The innermost one of the widely set ridges in the ventro-lateral field is somewhat disturbed. It reminds one of an incomplete ridge which is generally seen in the inner region of regenerated scales. Just inside of the ridge, there can be seen a trace of absorption which occurred along the old margin of the transplanted scale. In short, a considerable gap in the scale pocket around the transplanted scale induced a larger growth, and the accelerated growth affected the arrangement of ridges only at the ventro-lateral field where the largest growth was obtained accompanied with a partial absorption of the inner part of the field. With the other two recruited fish, the rearing period was short and the transplanted scales showed little growth. The result obtained from the scale of No. 4 seems to be quite similar to that from No. 2 or No. 3, viz., the scale gets close to the ordinary scale in outline but being still smaller than the ordinary scale and the arrangement of the newly formed ridges is not affected by the larger scale pocket (Fig. 3~~7~~). Fish No. 6 showed almost no growth of the body owing to the

injurious effect of a wooden rearing tank to such an extent that this and another fish were kept whilst the latter died early. The transplanted scale also showed practically no growth (Fig. 39). Both in No. 4 and No. 6, absorption of the old margin could not be found. Therefore, it may be said that the transplanted scales in these fish grew in proportion to the growth of the bodies without showing any partial absorption along the old margin.

No. 7 - No. 9 (Figs. 39-41): The 6th scale of the upper first row was removed from three fish and into the scale pocket respectively, was transplanted a scale taken from nearly the same position of another fish, 4.3 cm in body length. The donated scales showed equivalent to 50~60% of the ordinary scales in longitudinal and transverse diameters. In all cases, the transplanted scales presented the same results. The new growth zone of these scales showed obviously a superficial pattern characteristic of regenerated scales. So, the scales take an appearance of a regenerated scale on which the transplanted scale was placed, but no polygonal platelets can be seen in the central region and the number of grooves is not so great as compared with a typical regenerated scale. The examination of these scales under microscope with various focusing proved that the two zones, inner transplanted zone and outer newly formed zone, are in the same horizontal level, suggesting that the scale pocket into which was inserted the smaller scale did not induce another new scale, that is, these curious scales would be formed in cooperation of the transplanted scale with the scale-forming tissue of the host. The margin as well as the surface of the transplanted zone presents a sign of severe absorption. The scales may be regarded, therefore, as an exaggeration of the one obtained in fish No. 5. Each scale has surpassed in growth the size of the ordinary scale.

Discussion

It may be said generally, in summarising the observations obtained with No. 1 - No. 9, that if the scale pocket is larger in comparison with the transplanted scale a larger growth is induced. However, the transplanted scales do not always grow rapidly until they come to be restricted by the pocket wall. Examples of such a situation may be seen in Nos. 2, 3, 4 and 6. In these scales, the ridges were formed closely with each other their ordinary arrangement being unaltered. In Nos. 5, 7, 8 and 9, on the other hand, the growth zone of the transplanted scales showed widely set ridges. They all grew beyond the size of the ordinary scales in spite of the fact that they were remarkably smaller than the ordinary scales when they were transplanted. This shows unusually rapid growth of these transplanted scales. It must be noticed here that there always occurred a partial absorption of the transplanted scale in connection with the rapid growth. According to Goodrich and Nichols, who studied the homoiotransplantation of scales among several varieties of the goldfish, the absorption of the transplanted scales represents the degree of incorporation between the scales and their hosts. The absorption which occurred in the above mentioned scales of the present experiments may be interpreted in this light. When the transplanted scale is not so small in size compared with the ordinary scale, the scale pocket reacts in receiving the new scale as if it were an ordinary one, and the scale grows larger keeping in

step with other scales of the same fish. So, it is reasonable that the transplanted scale in No. 6 showed almost no growth because practically the fish did not grow. In such a case, the ridges are formed at usual intervals being not influenced by the wider pocket. On the contrary, when the transplanted scale is considerably smaller and is incompatible with the scale pocket which properly has ability to form an entirely new scale, it comes to be partially absorbed. It appears, therefore, that the absorption of the non-compatible scale is required for the reconstruction or reorganization of the scale-forming cells in the pocket from which the ordinary scale has been lost, and only in this reorganized cell mass around the transplanted scale can its rapid growth be introduced. The ridges are hardly formed in such a rapidly growing zone, but they come gradually to be formed in contiguity when the growth slackens.

Here arises a question, viz., the rapid growth accompanied by the absorption of the transplanted scale in Nos. 7-9 might be caused by homoiotransplantation and not by the smallness of the scale, because in Nos. 1-6 the scale was taken from the same individual whereas in Nos. 7-9 from another. Goodrich and Nichols reported on homoiotransplants in various combinations among different types of goldfish, that the dissolution of scale occurred in 9 out of 85 scales when the transplants were made from the type gold to gold in contrast to none in the case of autotransplants. Therefore, the question seems not to be groundless. At any rate, it does not matter so far the absorption is cosidered as a representation of incompatibility between the transplanted scale and the scale pocket.

The author interpreted in a foregoing section the arrangement of ridges, borrowing from the opinion expressed by Wallin; viz., it seems to be brought about by a pressure which is formed between a growing scale and the surrounding tissues and that pressure decreases from the anterior to the apex. This interpretation would be correct as to the arc-shaped bending of ridges and it should be further supplemented concerning the spacing of ridges in the light of the results obtained in the transplantation experiments; that is to say, the distance between ridges or the number of ridges formed in a definite width of the scale is determined exclusively by the growth rate of the scale itself. Rapid extension of the scale-forming cells or tissues prior to the actual growth of the scale is supposed to reduce the pressure formed between the tissues and the scale. In this respect, Neave's opinion—the ridges are formed in a partial accumulation of excessive bony substance which is normally utilized in extending the scale margin—may be acceptable. As the rapid growth of scales is necessarily brought about by the rapid growth of fish body, excepting the case of regeneration of scales, the distance between ridges may be generally regarded as an indication of the growth rate of fish. In this connection, the feeding experiment on the rainbow trout reported by Gray and Setna (1930) is very suggestive. They obtained scales with abnormally wide intervals between ridges even during winter by supplying the fish with abundant food and scales with abnormally narrow rings by supplying scanty diet. They suggested in view of such results that the width of ridges is closely associated with the growth rate of fish.

On account of the facts presented here, it is incorrect to consider the growth of scale separately from the growth of the fish since the scale is not a wast product

of metabolism such as a nail or hair a view frequently held.

IX. GENERAL CONCLUSION

The structure and growth of the goldfish scales have been examined from various aspects and discussions based on each examination have been presented separately. So, it will be needed here to offer a collective consideration on the formation or growth mechanism of each of the constituents of the scale, and grounded upon such consideration the usefulness of the scale for age determination should be determined.

The goldfish scale just like other teleost scales is composed of two different layers, the upper bony layer and the lower fibrillary plate. The bony layer bears on its surface a number of concentric ridges which are linear elevations homogeneous with the bony layer, while the fibrillary plate is built up of several thin lamellae. The former increases in width by its marginal growth in almost uniform thickness, but the latter grows both in width and in thickness by the addition of successively larger new lamellae. Calcium salts deposit on the bony layer and the intimate lamellae of the fibrillary plate. The development or extension of the bony layer, which corresponds morphologically as well as physiologically with that of a membrane bone in general, occurs always in precedence of and is necessarily accompanied with the addition of new fibrous lamellae. The scale originates from an aggregation of osteoblasts which have been derived from fibroblasts which migrated from the lateral line region. The osteoblasts which are divided into two layers by their own product, the origin of the bony layer, come to behave in different ways; the upper layer cells degenerate sooner with some of them remaining inside of the ridges whereas the lower ones remain longer forming a fibrous lamella. This situation is kept at the scale margin as long as the scale grows larger. On the other hand, at the lower central region of the scale is formed a new fibrous lamella in expense of underlying fibroblasts which do not pass through the stage of osteoblasts. The cellular sheath surrounding the growing scale is thus composed of marginal osteoblasts and a central fibrous lamella, and it takes part in making the scale grow while another new sheath is prepared. It is natural, therefore, that a close relation is found between the growth of the bony layer and that of the fibrillary plate, so the scale may be regarded as having a distinctive structure in this respect among skeletal tissues. The peculiality of the fibrillary plate is also found in the fact that it is lacking in acid polysaccharide although calcium salts are deposited on it.

The growth of the scale is in proportion to the growth of the fish body; regional differences in the extension of the body surface lead to the variable forms of the scales which have adapted themselves to their respective scale pockets. The width of growth in the anterior part of the scale is slightly larger than in the posterior judged from the transition in position of the focus.

The origin of the ridges is considered to be a fold of bony substance formed as a result of an obstacle of surrounding cells or tissues of the scale, not by the restrictive action of the scale pocket upon the growing scale. The fold grows into a complete ridge in increasing its height in consumption of the cytoplasm of a row of osteoblasts caught by the fold. The fold hardly occurs when the scale is

growing rapidly, and the rapid growth of the scale, except in the case of the regeneration, becomes possible only when the body grows rapidly. On the contrary, retarded growth of the scale which follows retarded growth of the fish induces on its surface the formation of closely set ridges. After all, it would be safe to say that the growth rate of the fish exerts effect upon the spacing of ridges which are being formed on its scales.

It can generally be said that the grooves in the scale are increased in number with the growth of the scale by the appearance of incomplete grooves. They extend outward probably in dissolving once-formed bony material at the scale margin. Their function is supposed to be giving flexibility to the scale judging from their histological structure and from the fact that the number of grooves of the scales covering the flexible part of the body is greater than in those of other parts. The scale pocket has no special structure to effect the pattern of arrangement of the grooves.

When a scale pocket loses its ontogenetic scale, it is immediately supplied with a new regenerated scale, and when a scale becomes obstacle for the scale pocket, it is absorbed until the obstacle is removed. The facility in producing or resorbing of the scale suggests the possible mutual conversion between osteogenic and osteolytic cells. This explanation is also applicable to the appearance or disappearance of the grooves. The regenerated scale never recovers its superficial pattern characteristic of the original ontogenetic scale. This is reasonable from the viewpoint of the mechanism of ridge formation. A high metabolism rate of the fish in a high water temperature accelerates the regeneration of the scale. One of the reasons is considered to be related to the ability to synthesize intracellular alkaline phosphatase in the scale-forming cells.

From the facts considered above, it may generally be accepted that the so-called "winter ring" or "annulus" is formed from autumn to winter with decline in the growth rate of scale, and a zone marked by wide intervals of ridges appears from spring to summer due to increase in the growth rate. But it should be emphasized that winter or summer as such does not necessarily have any effect on the scales to make the ridge intervals narrow or wide, because the spacing of ridges is in no direct correlation with the seasons but it only reflects the growth rate of the scales or of the body. A suitable water temperature with sufficient nutrition would allow the fish to grow fully, but a higher temperature with insufficient nutrition would not only arrest the growth of the fish but also would sometimes cause reduction in the weight of the fish. At that time, narrow ridge intervals like a winter ring or a disturbance of ridges formed after the resorption of the scales, both likely to be mistaken as a year ring, may occur even in summer.

In conclusion, it may be said generally that the scales are useful for the age determination of the goldfish so far as the fish shows annual periodicity in its growth rate. In this connection, a careful consideration of the ecology of the fish or of the environment should be required in order that one may avoid any errors which are thought to be probable in scale reading. This conclusion may be applied not only for the goldfish but also for other teleosts since all teleost scales are considered to be essentially the same in structure as well as in growth mechanism.

LITERATURE

Barnicot, N. A. (1948). The local action of the parathyroid and other tissues on bone in intracerebral grafts. *Jour. Anat.* 82, 233–241.

Brachet, J. (1950). *Chemical embryology.* 2nd Edit. 522p. New York; Interscience Publishers.

Bradbury, S. (1958). A cytological and histochemical study of the connective tissue fibres of the leech, *Hirudo medicinalis*. *Quart. Jour. Micr. Sci.* 99(2), 131–142.

Collip, J. B. (1925). The extraction of a parathyroid hormone which will prevent or control parathyroid tetany and which regulates the level of blood calcium. *Jour. Biol. Chem.* 63(2), 395–438.

*Creaser, C. W. (1926). Structure and growth of scales of fishes in relation to the interpretation of their life-history, with special reference to the sunfish *Eupomotis gibbosus* *Univ. Mich. Misc. Pubs.* 17.

Dietrich, M. A. (1953). A histological study of the development of the scales of the large-mouth bass (*Micropterus salmoides*). *Quart. Jour. Micr. Sci.* 94(1), 71–82.

Dziewiatkowsky, D. D. (1951). Isolation of chondroitin sulfate S from articular cartilage of rats. *Jour. Biol. Chem.* 189(1), 187–190.

Fach, M. (1937). Zur Genese der Fischschuppe. *Zeitschr. Anat. Entw. Gesch.* 107, 65–67.

Fell, H. B. & Robison, R. (1933). Glycogen and cartilage. *Nature* 131, 62.

——— & ——— (1934). The development of the calcifying mechanism in avian cartilage and osteoid tissue. *Biochem. Jour.* 28(6), 2243–2252.

Gaillard, P. J. (1955). Parathyroid gland tissue and bone *in vitro*. *Exp. Cell Res. Suppl.* 3, 154–169.

Glick, D. (1949). *Technique of histo- and cytochemistry.* 531p. New York; Interscience Publishers.

Goodrich, H. B. & Nichols, R. (1933). Scale transplantation in the goldfish *Carassius auratus*. *Biol. Bull.* 65(2), 253–265.

Gray, J. & Setna, S. B. (1930). The growth of fish. IV. The effect of food supply on the scales of *Salmo irideus*. *Jour. Exp. Biol.* 8, 55–62.

Green, E. & Tower, R. W. (1901). The organic constituents of the scales of fishes. *Bull. U.S. Fish. Commiss.* 21, 97–102.

Hancox, N. M. (1949). The osteoclast. *Biol. Rev.* 24(4), 448–471.

Harris, H. A. (1932). Glycogen in cartilage. *Nature* 130, 996–997.

Hase, A. (1911). Die morphologische Entwickelung der Ktenoidschuppe. *Anat. Anz.* 40, 337–356.

*Hendricks, S. B. & Hill, W. L. (1950). The nature of bone and phosphate rock. *Proc. Nat. Acad. Sci., Geophysics* 36, 731.

Ichikawa, R. (1956). A study of fish scale by radioactive strontium. Isotope Kenkyu-Riyo Soran, Nippon Genshiryoku Sangyo Kaigi (Congress of Japan Atomic Energy Industry). 551–552. (in Japanese).

——— (1953). Absorption of fish scale caused by starvation. *Rec. Ocean. Works Jap., N.S.* 1(2), 101–104.

Jackson, S. F. (1954). Fibrogenesis in connective tissues. *Nature* 173, 950–951.

——— (1955). Cytoplasmic granules in fibrogenic cells. *Nature* 175, 39–40.

Kato, K. (1953). Calcium oxalate and other calcium salts in fish scale. *Sci. Rep., Saitama Univ., Ser. B.* 1(2), 51–58.

Kawamoto, N. (1956). *Fish physiology.* 319p. Tokyo; Ishizaki Shoten (in Japanese).

Klaatsch, H. (1890). Zur Morphologie der Fischschuppen und zur Geschichite der Hartsubstanzgewebe. *Morph. Jahrb.* 16, 299–258.

Kobayashi, H. (1958). Comparative morphology and hierarchy of fish scales. *Bull. Aiti Gakugei Univ.* 7, 1-104. (in Japanese).

——— & Kawai, M. (1960). Studies on the regenerative scales of fishes. II. On the regenerative scales of mud-loach, *Misgurnus fossilis anguillicaudatus* (*CANTOR*). *Bull. Jap. Soc. Sci. Fish.* 26(3), 207-210. (in Japanese).

Levine, A. & Schubert, M. (1952). Metachromasy of thiazine dyes produced by chondroitin sulfate. *Jour. Amer. Chem. Soc.* 74(1), 91-97.

Lindahl, P. E. & Wallin, O. (1955). On the relationship between the growth structure of the bony and the fibrillary layers of the roach-scale. *Arkiv f. Zool., Ser. 2.* 8(8), 561-563.

Lison, L. (1953). *Histochimie et cytochimie, Principes et méthodes.* 2e Édit., 607p. Paris; Gauthier-Villars.

Logan, M. A. (1935). Composition of cartilage, bone, dentin, and enamel. *Jour. Biol. Chem.* 110(2), 375-389.

Lφvtrup, S. (1959). Utilization of energy sources during amphibian embryogenesis at low temperatures. *Jour. Exp. Zool.* 140(3), 383-394.

Martland, M. & Robison, R. (1924). The possible significance of hexosephosphoric esters in ossification. V. The enzyme in the early stages of bone development. *Biochem. Jour.* 18(6), 1354-1357.

Maximov, A. A. & Bloom, W. (1952). *A textbook of histology.* 6th Edit., 616p. Philadelphia and London; W. S. Saunders Co.

Mori, Y. (1931). On the transformation of ordinary scales into lateral-line scales in the goldfish. *Jour. Fac. Sci., Imp. Univ. Tokyo, Sect. IV. Zool.* 2(3), 185-194.

Nardi, F. (1935). Das Verhalten der Schuppen erwachsener Fische bei Regenerations- und Transplantationsversuchen. *Roux Arch. Entw. Mech.* 133, 621-663.

Neave, F. (1936). The development of scales of *Salmo*. *Trans. Roy. Soc. Can., Sect. V.* 30(3), 55-72.

——— (1940). On the histology and regeneration of the teleost scale. *Quart. Jour. Micr. Sci.* 81(4), 541-568.

Nusbaum, J. (1907). Materialien zur vergleichenden Histologie der Hautdecke der Wirbeltiere. III. Zur Histogenese der Lederhaut und der Cycloid-Schuppen der Knochenfische. *Anat. Anz.* 30, 297-310.

Paget, G. W. (1920). Report on the scales of some teleostean fish with special reference to their method of growth. *Fish. Invest., Ser. 2.* 4(3), 1-28.

Partridge, S. M. (1948). The chemistry of connective tissues. 1. The state of combination of chondroitin sulphate in cartilage. *Biochem. Jour.* 43(3), 387-397.

Petrov, V. V. & Petruschewsky, G. K. (1929). Beiträge zur Kenntnis der Struktur der Schuppen von *Cyprinus carpio L.* *Zool. Anz.* 84, 257-269.

Pevsner, V. (1926). Zur Frage über die Struktur und die Entwickelung der Schuppen einiger Knochenfische. *Zool. Anz.* 68, 303-313.

Robison, R. (1923). The possible significance of hexose phosphoric esters in ossification. *Biochem. Jour.* 17(2), 286-293.

——— & Soames, K. M. (1924). Ditto. II. The phosphoric esterase of ossifying cartilage. *Ibid.* 18(3/4), 740-754.

Roche, J., Collet, J., & Mourgue, M. (1940). Activité phosphatasique et croissance des os dermique (Écailles) chez les poissons (Sélaciens et Téléostéens). *Enzymologia* 8(4), 257-260.

Saito, S. (1955). On the mechanism of the appearance of the scale structure. III. On the regeneration of the scales of the crucian carp, *Carassius carassius* (*L.*), when they are reared with the different food. *Bull. Fac. Fish., Hokkaido Univ.* 5(4), 332-335.

——— (1956). Ditto. IV. A study on the scale structure of the crucian carp, *Carassius carassius* (*L.*), *Ibid.* 7(2), 85-95.

Saito, S. & Yamada, J. (1953). Ditto. II. The scale structure of the crucian carp examined with the regeneration. *Mem. Fac. Agr., Hokkaido Univ.* **1**(4), 555–560. (in Japanese).

Sauter, V. (1934). Regeneration und Transplantation bei erwachsenen Fischen. *Roux Arch. Entw. Mech.* **132**, 1–41.

*Schereschewsky, H. (1940). Über den Bau des Epithelgewebes in der pharyngeal Kiemenregion der Knochenfische. *Doklady Akad. Nauk, USSR., N.S.* **29**, 165–168.

*Setna, S. B. (1934). Development of the trout scale. *Jour. Univ. Bombey (Biol. Sci.)* 2(5), 17–32.

Suzuki, R. (1952). The change in structure of regenerated scales of goldfish. *Jap. Jour. Ichthy.* 2(4/5), 192–195. (in Japanese).

Taylor, H. F. (1916). The structure and growth of the scales of the squetague and the pigfish as indicative of life history. *Bull. Bur. Fish.* 34, 289–330.

Tweedy, W. R. (1930). Studies on the plasma calcium raising principle of bovine parathyroid glands. 1. A method of preparation and some observation on the yield, solubility and stability of the product. *Jour. Biol. Chem.* 88(3), 649–657.

*Ussow, S. A. (1897). Die Entwickelung der Cycloid-Schuppe der Teleostier. *Bull. Soc. Imp. Nat. Moscou* **1897**, 147–148.

Wallin, O. (1956). Mucopolysaccharides and the calcification of the scales of the roach (*Leuciscus rutilus*). *Quart. Jour. Micr. Sci.* **97**(3), 329–332.

——— (1957). On the growth structure and developmental physiology of the scale of the fishes. *Rep. Inst. Freshw. Res., Drottingholm* 38, 385–447.

Wunder, W. (1949). Beobachtungen über Schuppen-regeneration beim Goldfisch (*C. auratus L.*), beim Karpfen (*C. carpio L.*) und bei der Brachse (*Abramis brama L.*). *Roux Arch. Entw. Mech.* **143**, 396–470.

Yamada, J. (1956). On the mechanism of the appearance of the scale structure. V. The possible role of alkaline phosphatase in the formation of the teleost scale. *Bull. Fac. Fish., Hokkaido Univ.* 7(3), 185–201.

——— & Saito, S. (1952). Ditto. I. The early development of the scale in the rainbow trout, *Salmo irideus G. Mem. Fac. Agr., Hokkaido Univ.* 1(3), 354–360. (in Japanese).

Yasuda, H. (1958). Implication of the scale ring. I. The phenomenon of unformed rings on the scales. *Bull. Jap. Soc. Sci. Fish.* 24(8), 613–615. (in Japanese).

(* not available)

Explanation of Plates

PLATE I

Figs. 1-3. Sections through the skin of a goldfish, 12 mm in body length.

1. The skin composed simply of the epithelium and corium. Haematoxylin and eosin. ×250 ep: epithelium; co: corium
2. Two adjacent scale papillae appearing between the epithelium and corium. Haematoxylin and eosin. ×250 sp: scale papilla
3. The scale papillae in which appeared a fine crack. Haematoxylin and eosin. ×320 cr: crack

Figs. 4-6. Sections through the skin of a goldfish, 15 mm in body length.

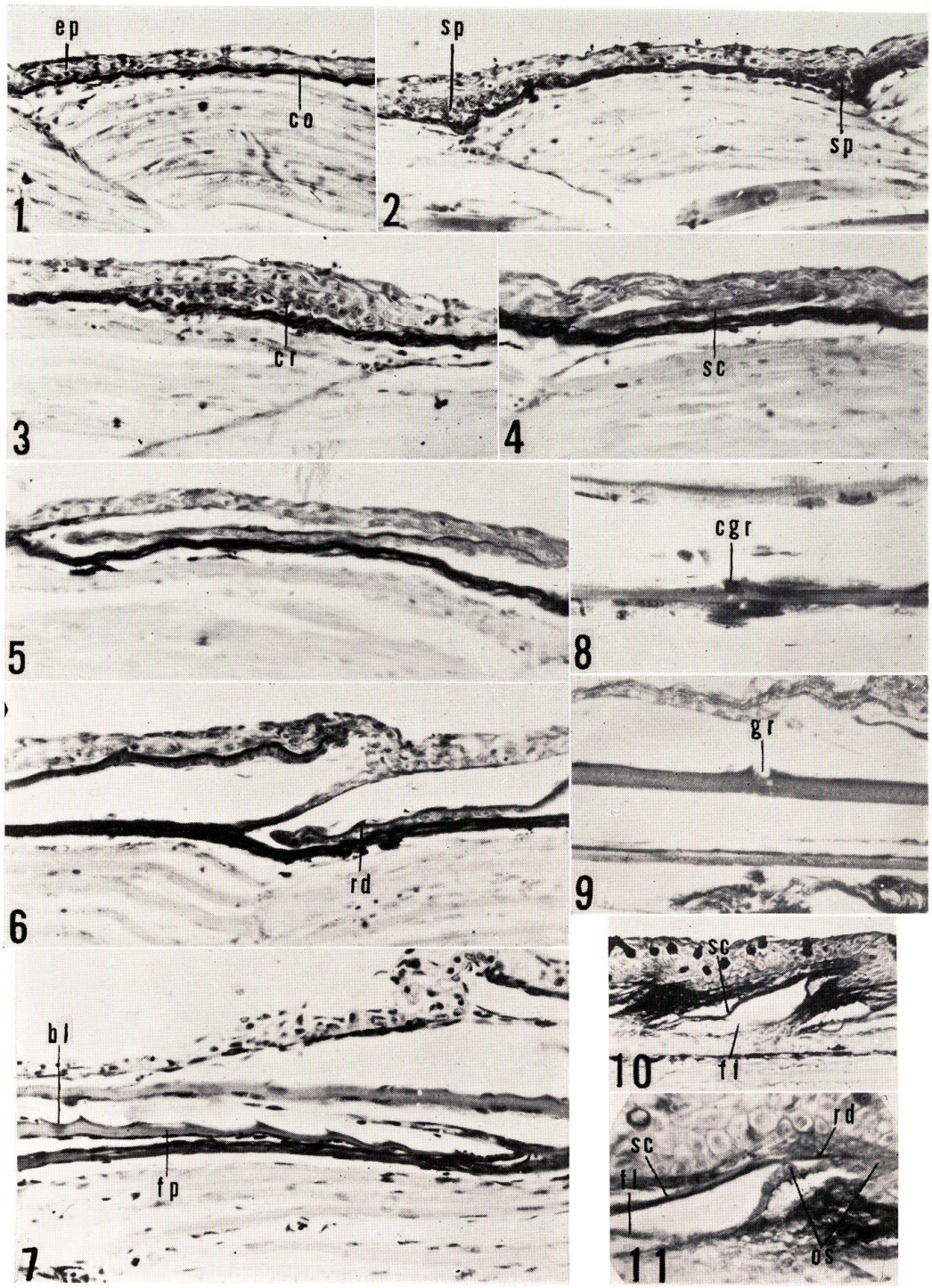
4. The origin of scale enclosed by flattened papilla cells (osteoblasts). Haematoxylin and eosin. ×320 sc: scale origin
5. A stage of the development of scale showing the degeneration of upper osteoblasts. Haematoxylin and eosin. ×320
6. Posterior end and anterior ends of two adjacent scales separated by the wall of scale pocket. Haematoxylin and eosin. ×320 rd: ridge

Figs. 7-9. Sections through the skin of a goldfish, 21 mm in body length.

7. Anterior part of the completely developed scale composed of the bony layer and fibrillary plate. Spicular projections of ridges are seen on the scale surface. Haematoxylin and eosin. ×320 bl: bony layer; fp: fibrillary plate
8. A groove in section at the peripheral part of scale. A cell square in form is visible in the groove. Haematoxylin and eosin. ×800 cgr: cell in groove
9. A groove in section at the inner part of scale. Haematoxylin and eosin. ×320 gr: groove

Figs. 10-11. Sections through the skin of the rainbow trout, 38 mm in body length, to show the manner of formation of fibrous lamella. Haematoxylin.

10. ×250 fl: fibrous lamella; sc: scale
11. ×640 fl: fibrous lamella; os: osteoblasts; rd: ridge; sc: scale



J. Yamada: Studies on the Structure and Growth of the Scales

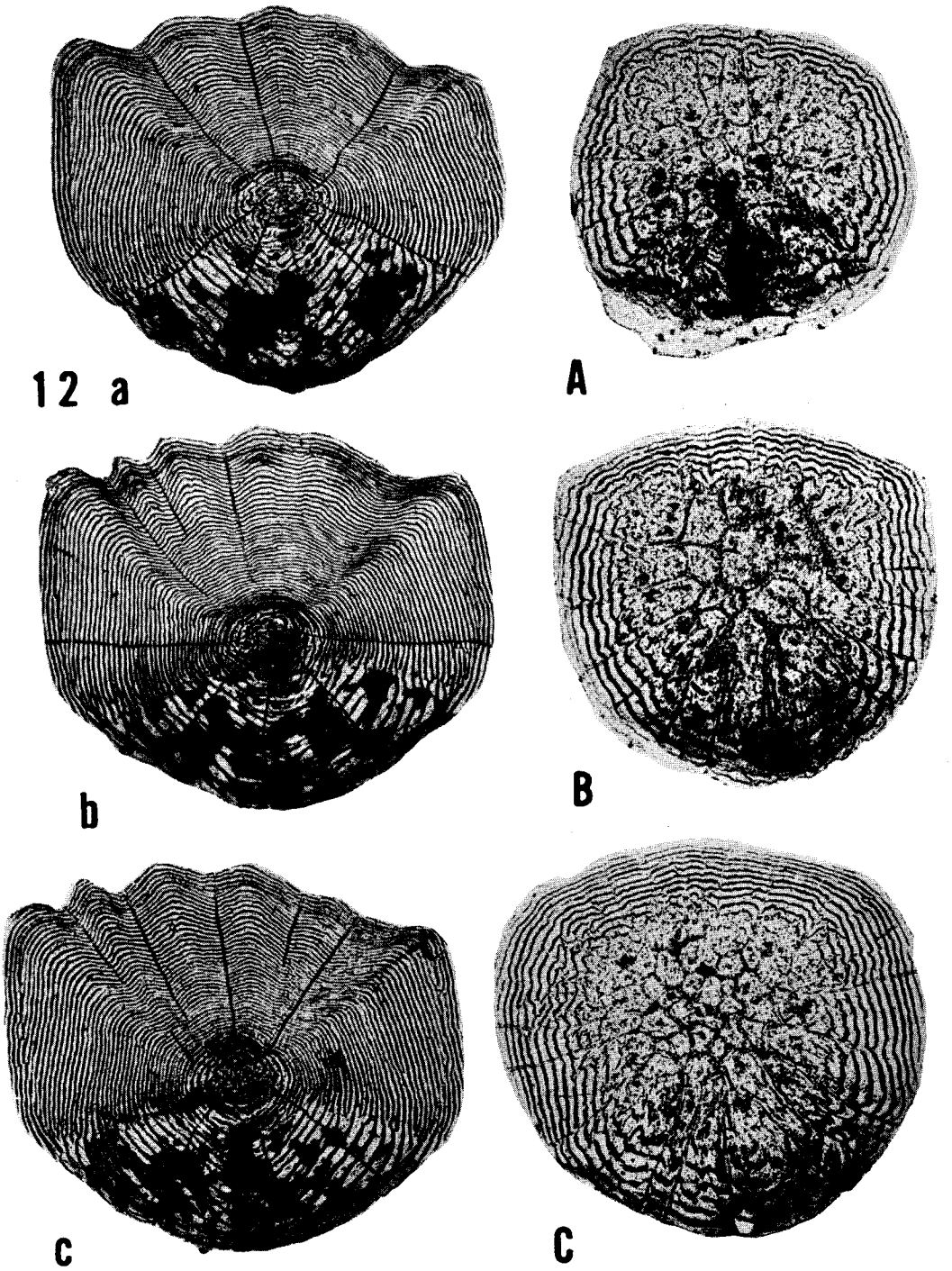
PLATE II

Fig. 12. (continued on Plate III) Four ontogenetic scales (a–d) removed from the upper first scale row of a goldfish, 61 mm in body length, and the corresponding regenerated scales (A–D) at different stages of regeneration. ×18

a, A Ontogenetic scale (a) and the regenerated scale (A) four weeks after the removal of a.

b, B Ontogenetic scale (b) and the regenerated scale (B) five weeks after the removal of b.

c, C Ontogenetic scale (c) and the regenerated scale (C) six weeks after the removal of c.



J. Yamada: Studies on the Structure and Growth of the Scales

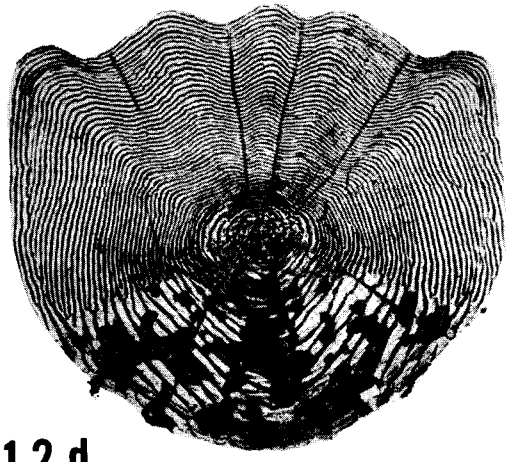
PLATE III

Fig. 12. (continued from Plate II) ×18
d, D Ontogenetic scale (d) and the regenerated scale (D) seven weeks after the removal of d.

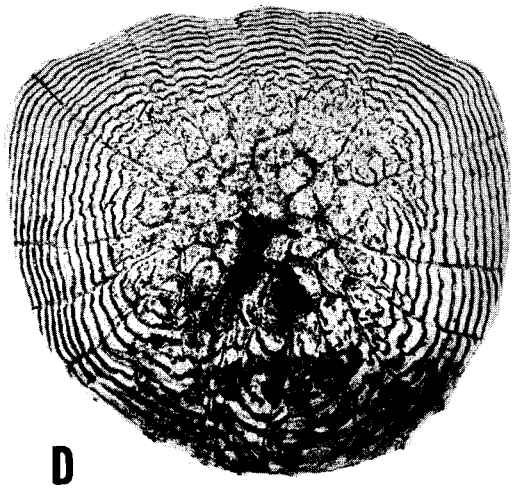
Fig. 13. A section through the regenerating scales. Haematoxylin and eosin. ×120 sc: regenerating scales

Fig. 14. A section through the focal area of a regenerating scale showing discontinuous platelets in horizontal arrangement. PAS. ×250 pl: platelets of regenerating scale

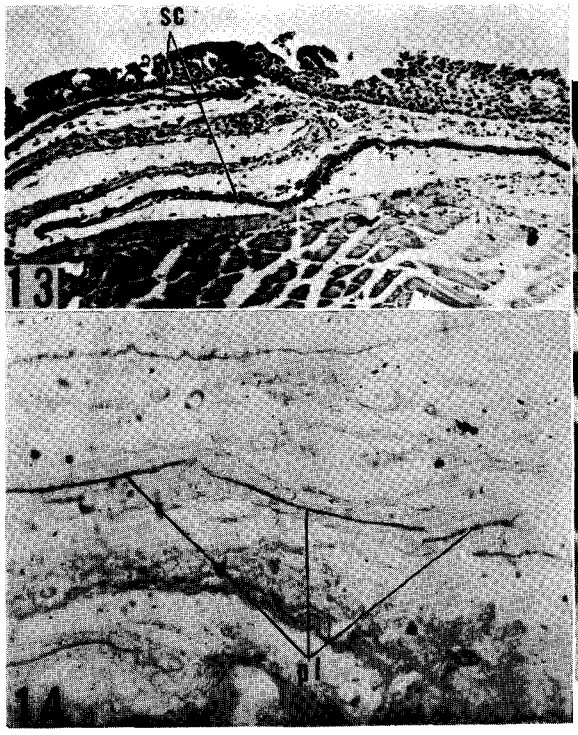
Fig. 15. Surface view of a part of the regenerated scale in flat mount. Mallory. ×400 er: erythrocytes; os: osteoblasts; rdc: cells remaining at the inside of ridges



12 d



D



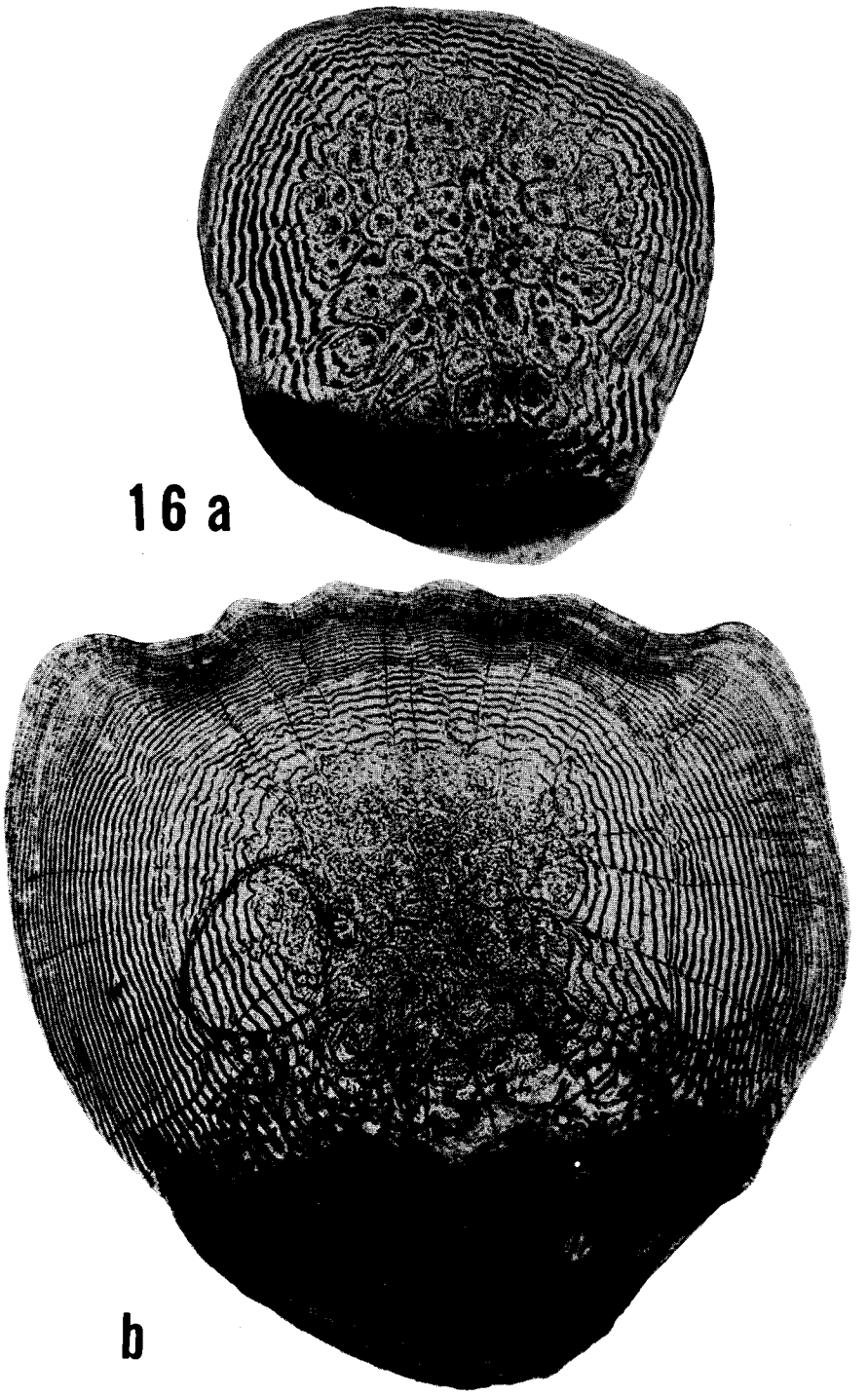
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE IV

Fig. 16. Two stages of the regenerated scale in fish No. 2, specifications shown in Table 3. ×18

a First examination at four weeks of regeneration.

b Re-examination 156 days later.



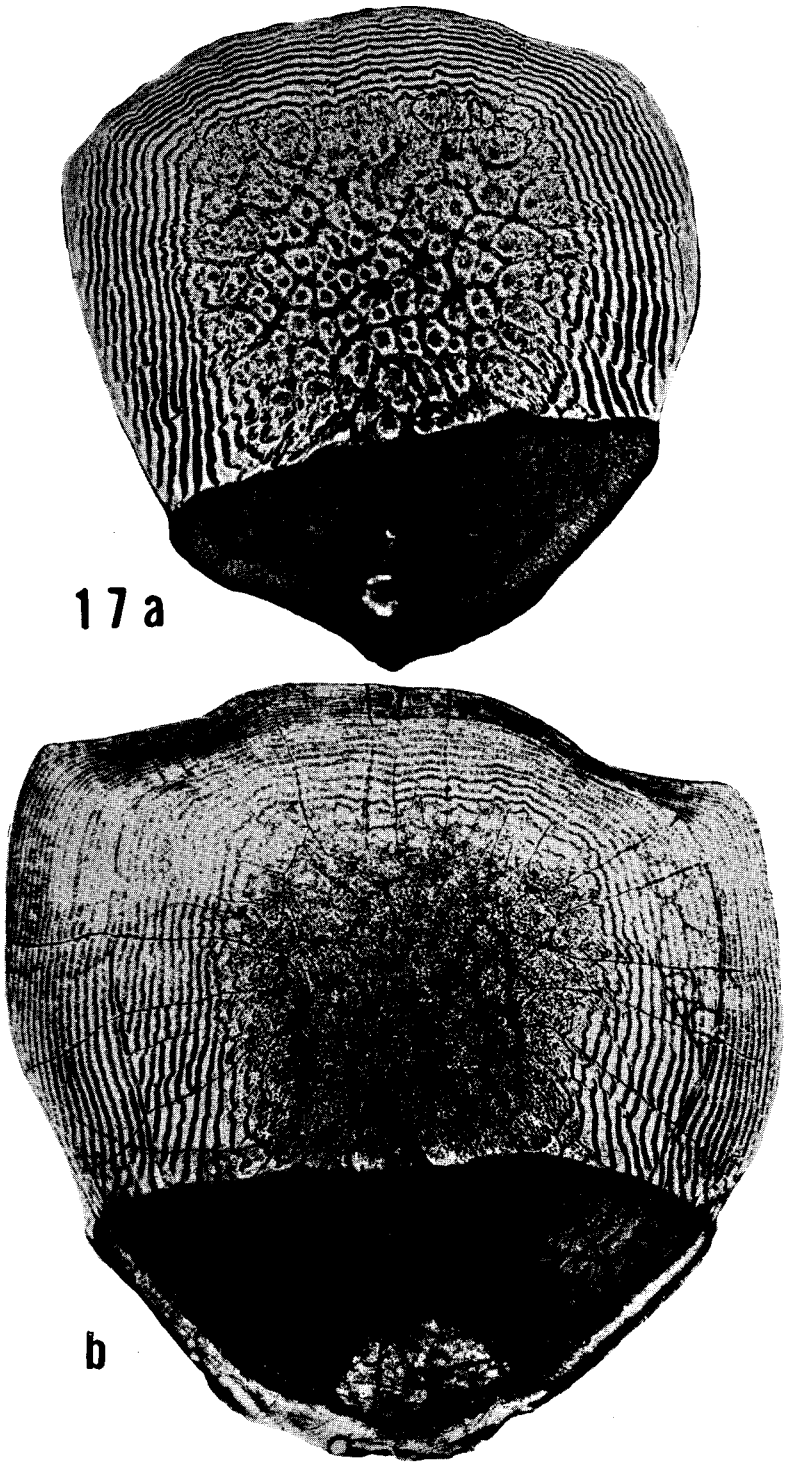
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE V

Fig. 17. Two stages of the regenerated scale in fish No. 4, specifications shown in Table 3. ×18

a First examination at six weeks of regeneration.

b Re-examination 149 days later.



J. Yamada: Studies on the Structure and Growth of the Scales

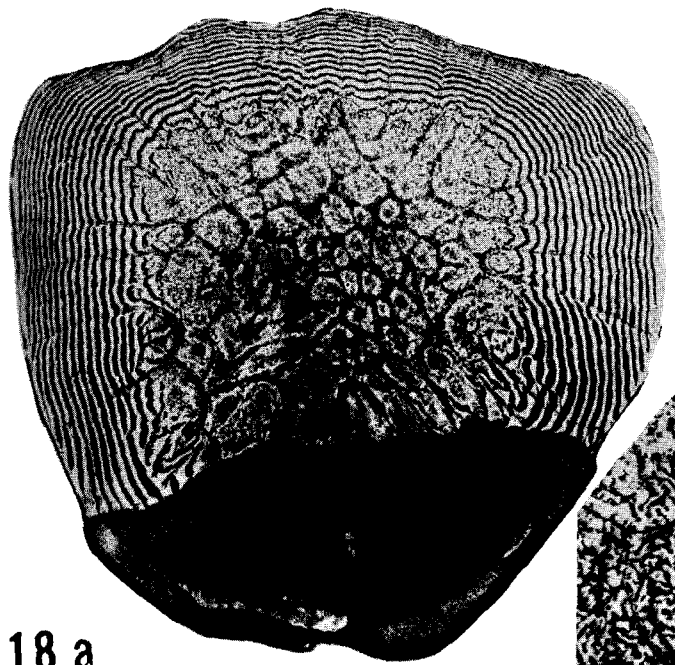
PLATE VI

Fig. 18. Two stages of the regenerated scale in fish No. 7, specifications shown in Table III. ×18

a First examination at eight weeks of regeneration.

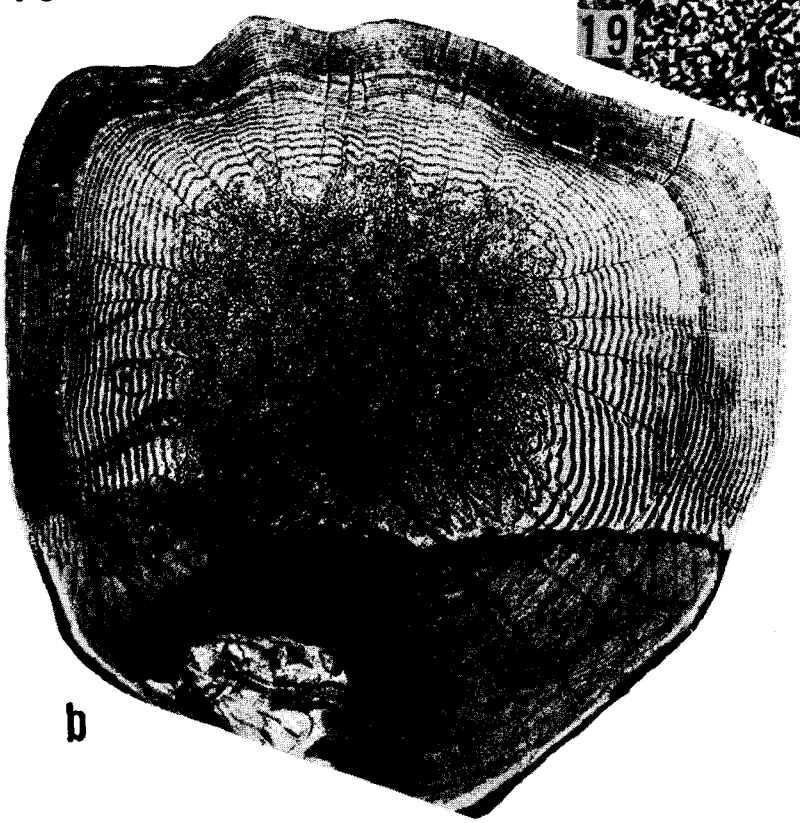
b Re-examination 149 days later.

Fig. 19. Magnification of the focal area of regenerated scale to show a plenty of irregular fragments which are gradually transformed to linear circular ridges. ×250



18 a

19



b

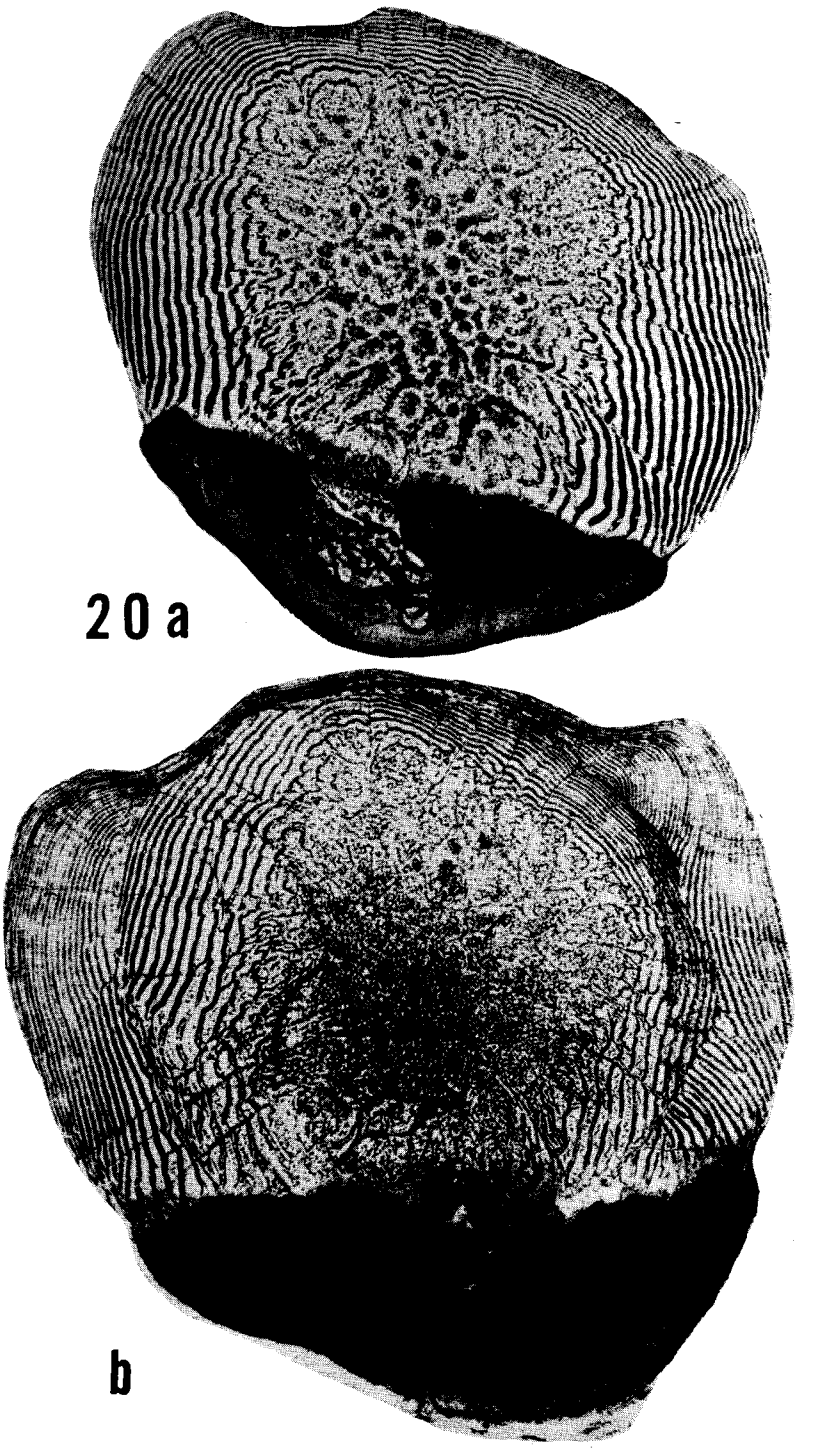
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE VII

Fig. 20. Two stages of the regenerated scale in fish No. 9, specifications shown in Table 3. ×18

a First examination at eight weeks of regeneration.

b Re-examination 135 days later.



J. Yamada: Studies on the Structure and Growth of the Scales

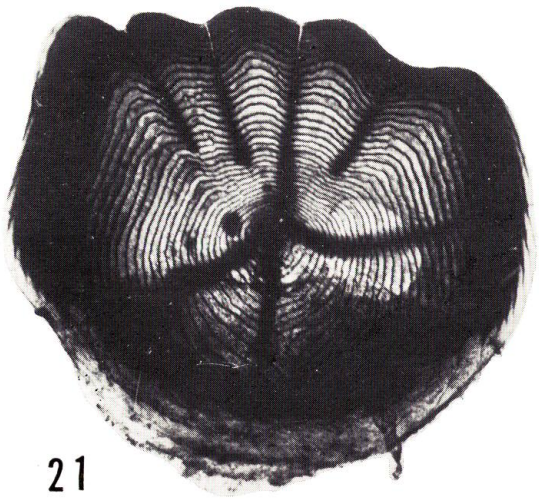
PLATE VIII

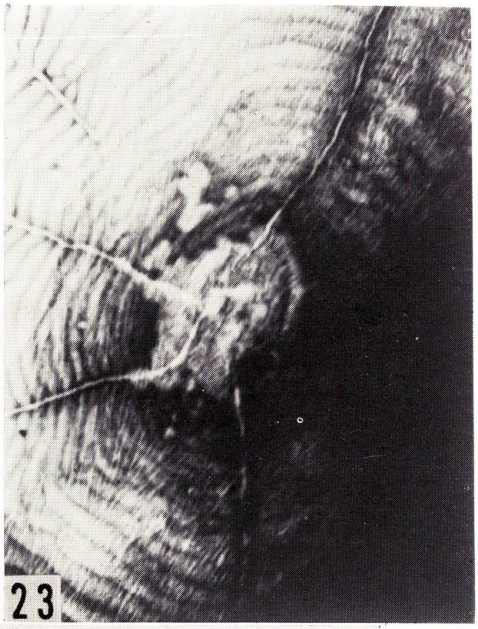
Fig. 21. Typical ontogenetic scale stained with Stoeltzner's method to show the deposit of calcium salts in the marginal area and along the grooves. ×40

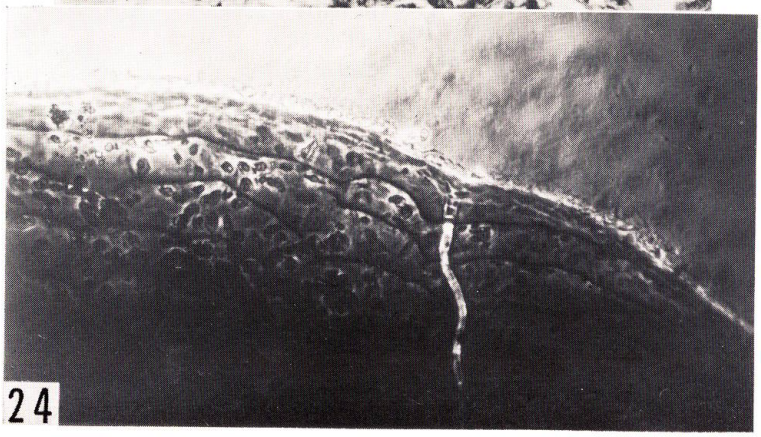
Fig. 22. Section of an absorbed scale margin. Stoeltzner. ×600

Fig. 23. Portion of ontogenetic scale showing the absorption which occurred in the focal area. Mallory. ×60

Fig. 24. Phase-contrast photomicrograph of the margin of a regenerated scale. Mallory. ×200







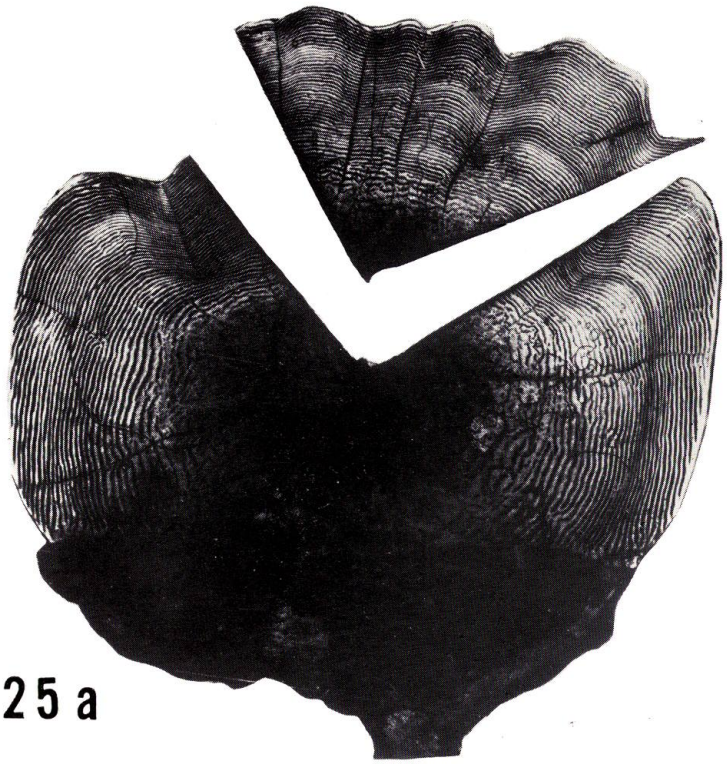
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE IX

Fig. 25. Two stages of the scale in which the anterior part was cut off in V-shape. ×18

a First examination when the operation was made.

b Re-examination 72 days later.



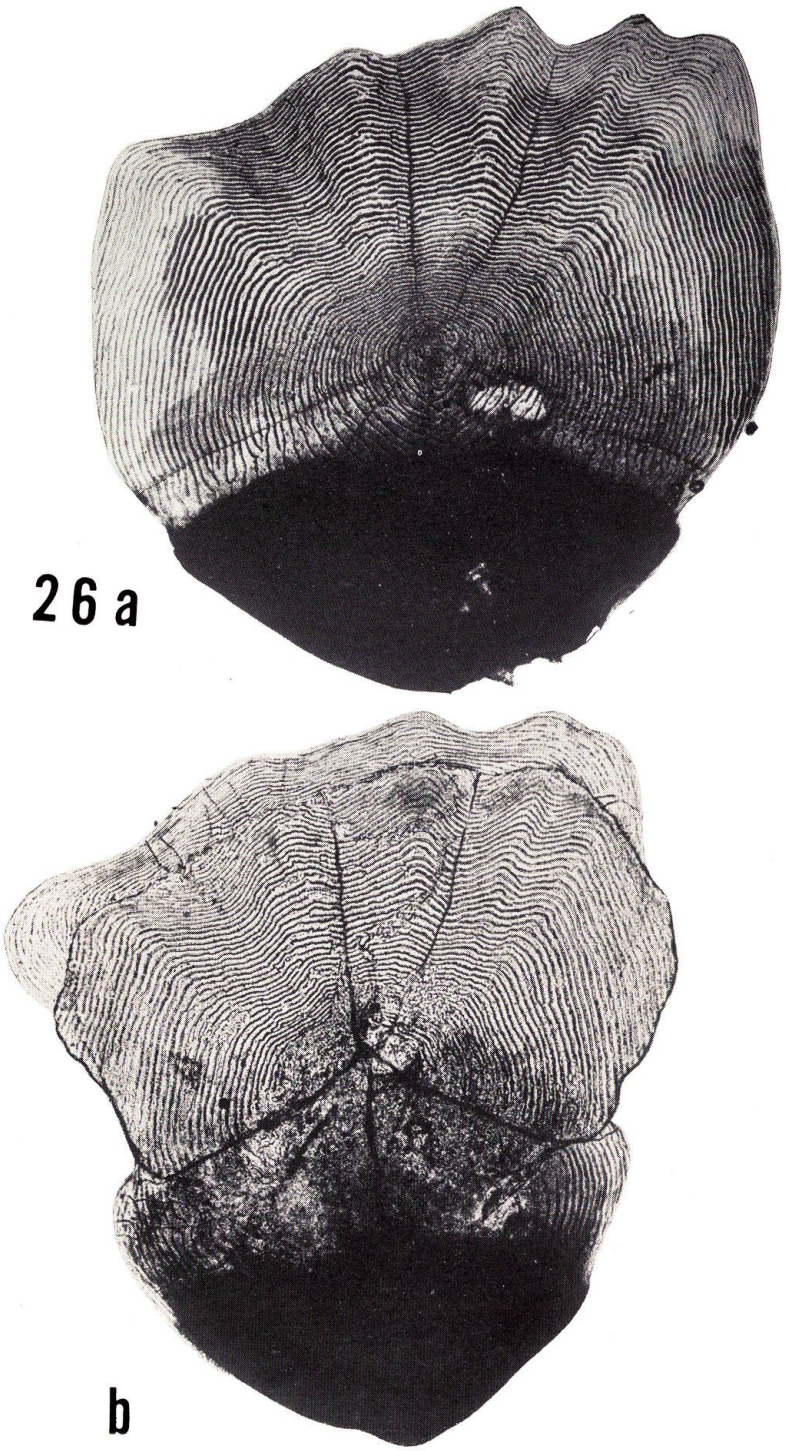
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE X

Fig. 26. Two stages of the scale in which the cellular elements were killed by rinsing in alcohol. ×18

a First examination just after the treatment.

b Re-examination 78 days later.



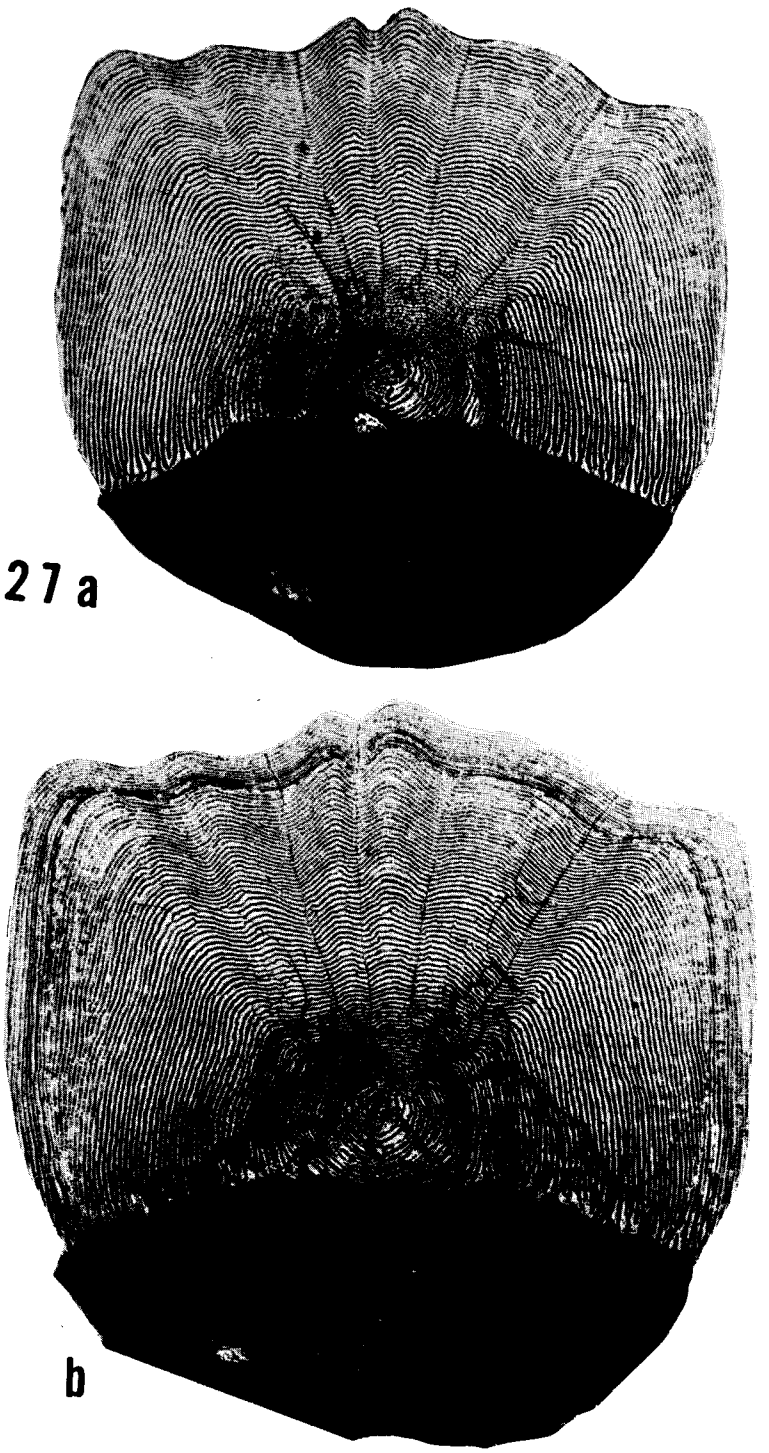
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE XI

Fig. 27. Two stages of the scale removed and inserted to the original place without any treatment. ×18

a First examination.

b Re-examination 188 days later.



J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XII

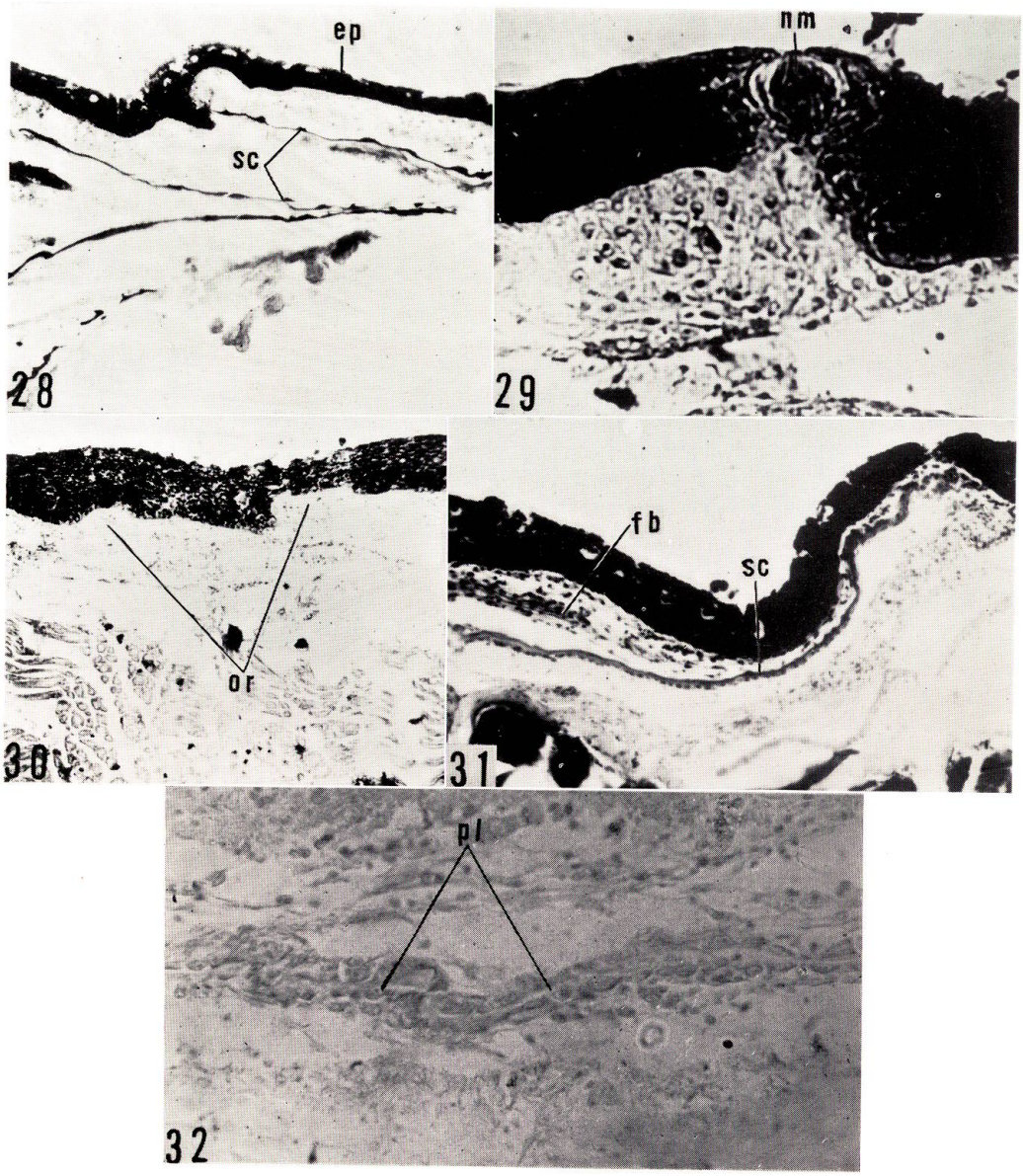
Fig. 28. Section through the ontogenetic scales. Positive alkaline phosphatase reaction is seen only in the epithelium. Gomori. ×80 ep: epithelium; sc: ontogenetic scales

Fig. 29. Section through the skin of a goldfish reared in CS, after 26 days of the removal of ontogenetic scale. Gomori. ×480 nm: neuromast

Fig. 30. Section through the skin of a goldfish reared in CF, after 78 days of the removal of ontogenetic scale. Gomori. ×100 cr: sites wherein original scales were situated

Fig. 31. Section through a regenerating scale of a goldfish, reared 12 days in WF after reared 86 days in CF. Gomori. ×150 fb: fibroblasts; sc: regenerating scale

Fig. 32. Section through the focal area of regenerating scale. Methyl green-pyronin. ×400 pl: platelets of regenerating scale



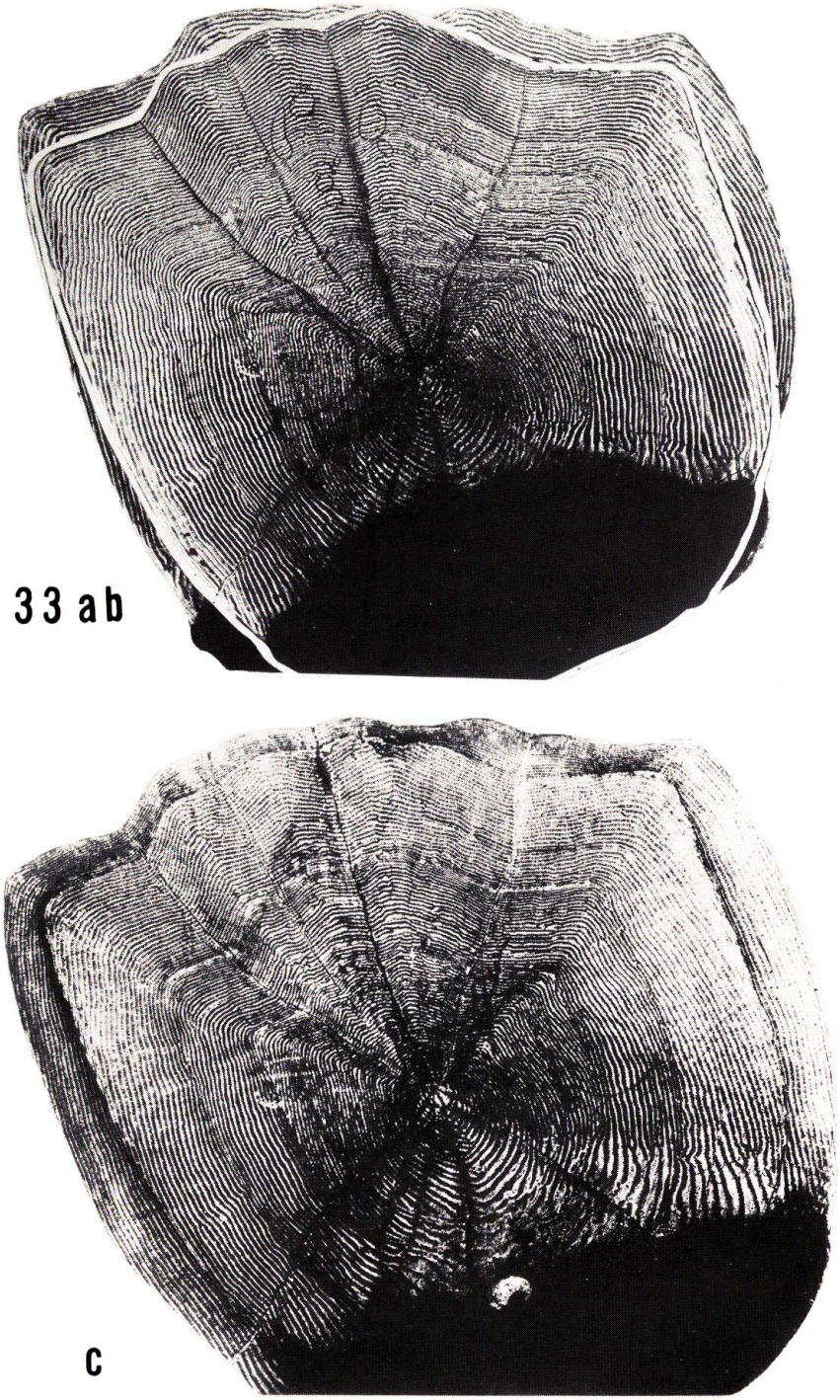
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE XIII

Fig. 33. The ordinary scale and two stages of the transplanted scale in fish No. 1 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 188 days after transplantation.



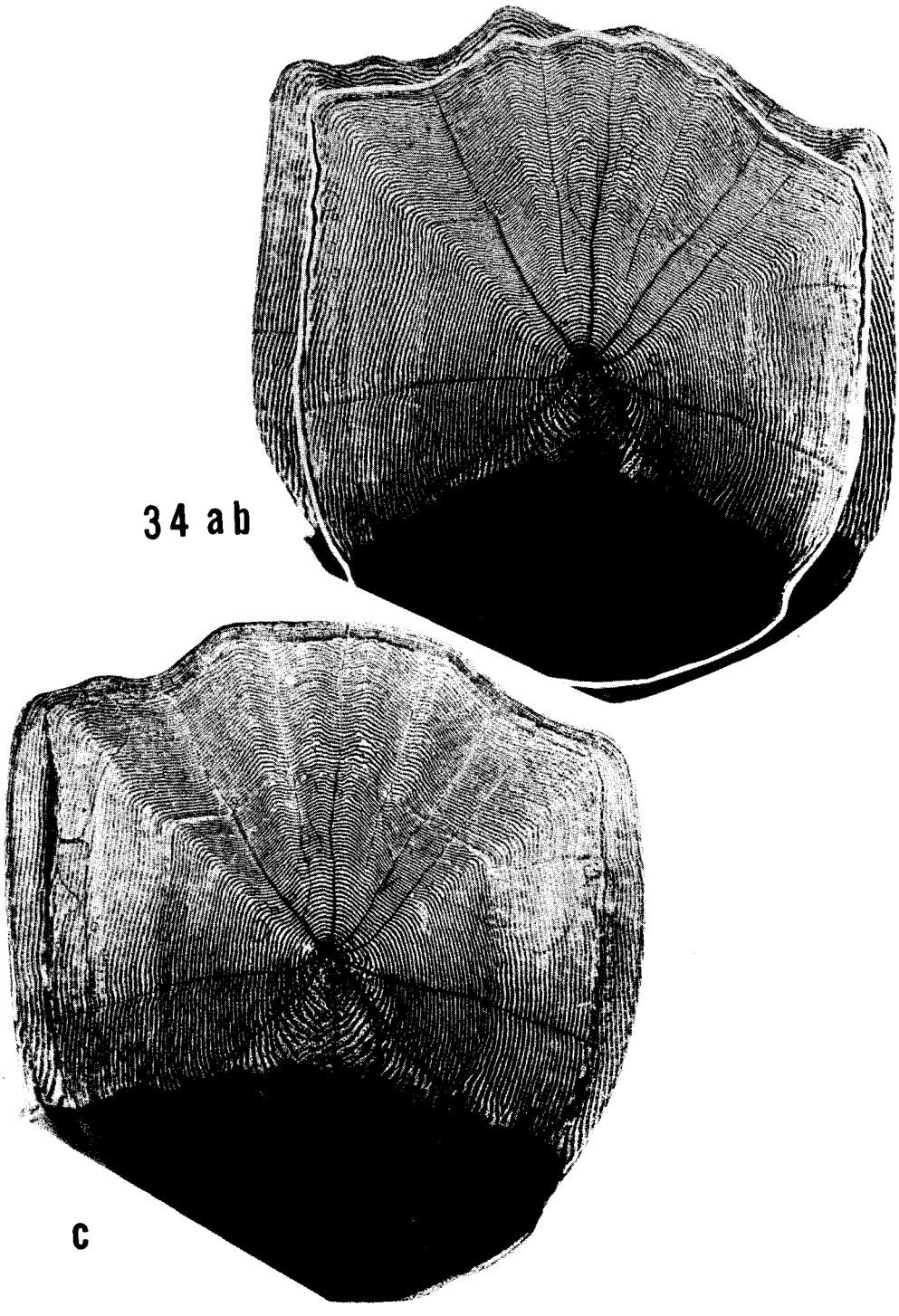
J. Yamada : Studies on the Structure and Growth of the Scales

PLATE XIV

Fig. 34. The ordinary scale and two stages of the transplanted scale in fish No. 2 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 188 days after transplantation.



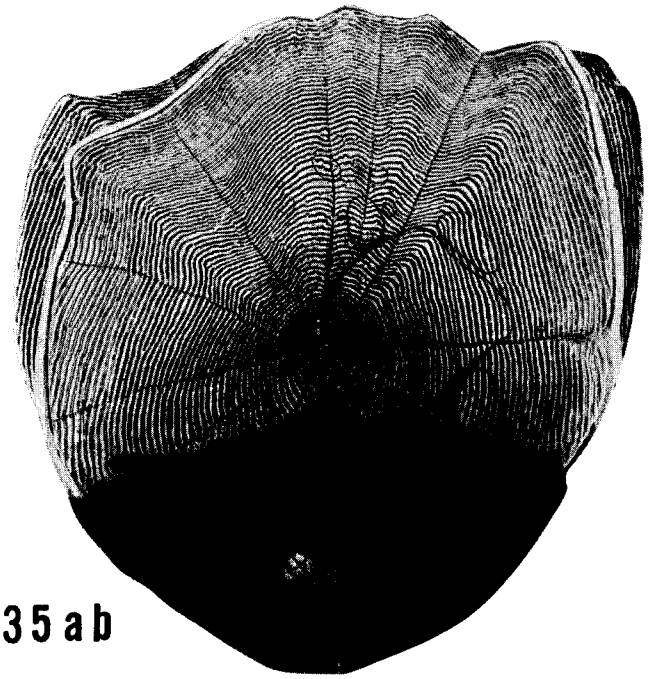
J. Yamada: Studies on the Structure and Growth of the Scales

PLATE XV

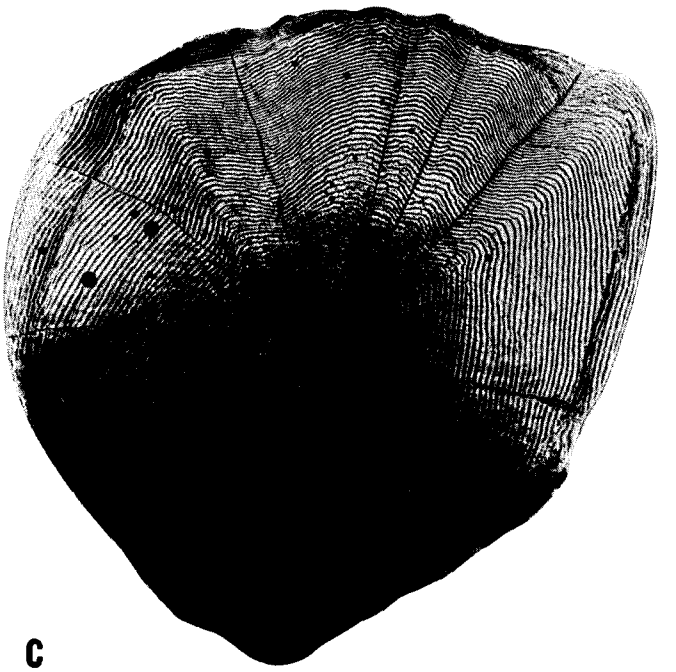
Fig. 35. The ordinary scale and two stages of the transplanted scale in fish No. 3 described in Table 7. ×18

ab Transplanted scale (b) placed upon the ordinary scale (a).

c Transplanted scale at 128 days after transplantation.



35 a b



c

J. Yamada: Studies on the Structure and Growth of the Scales

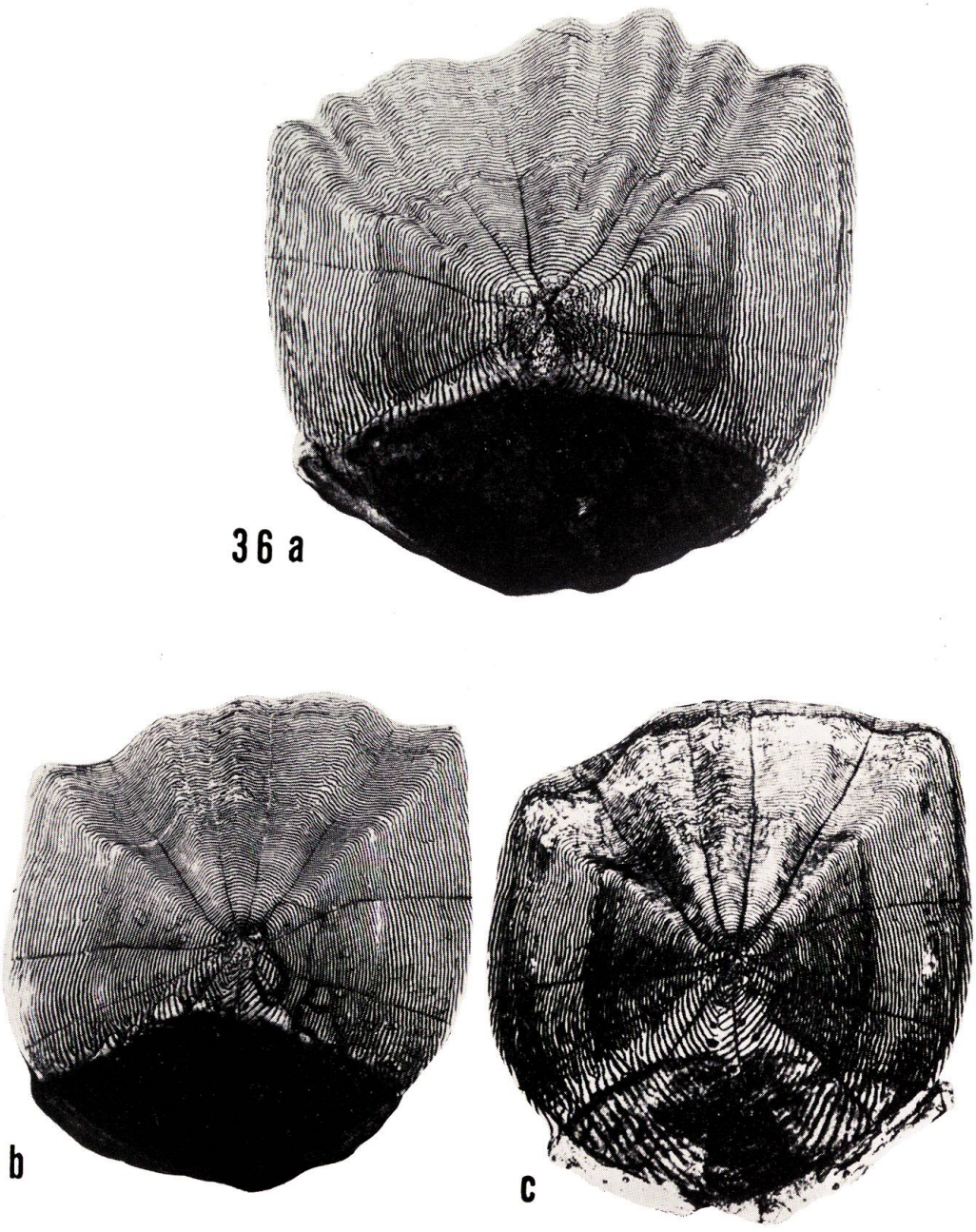
PLATE XVI

Fig. 36. The ordinary scale and two stages of the transplanted scale in fish No. 4 described in Table 7. ×13

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 73 days after transplantation.



J. Yamada : Studies on the Structure and Growth of the Scales

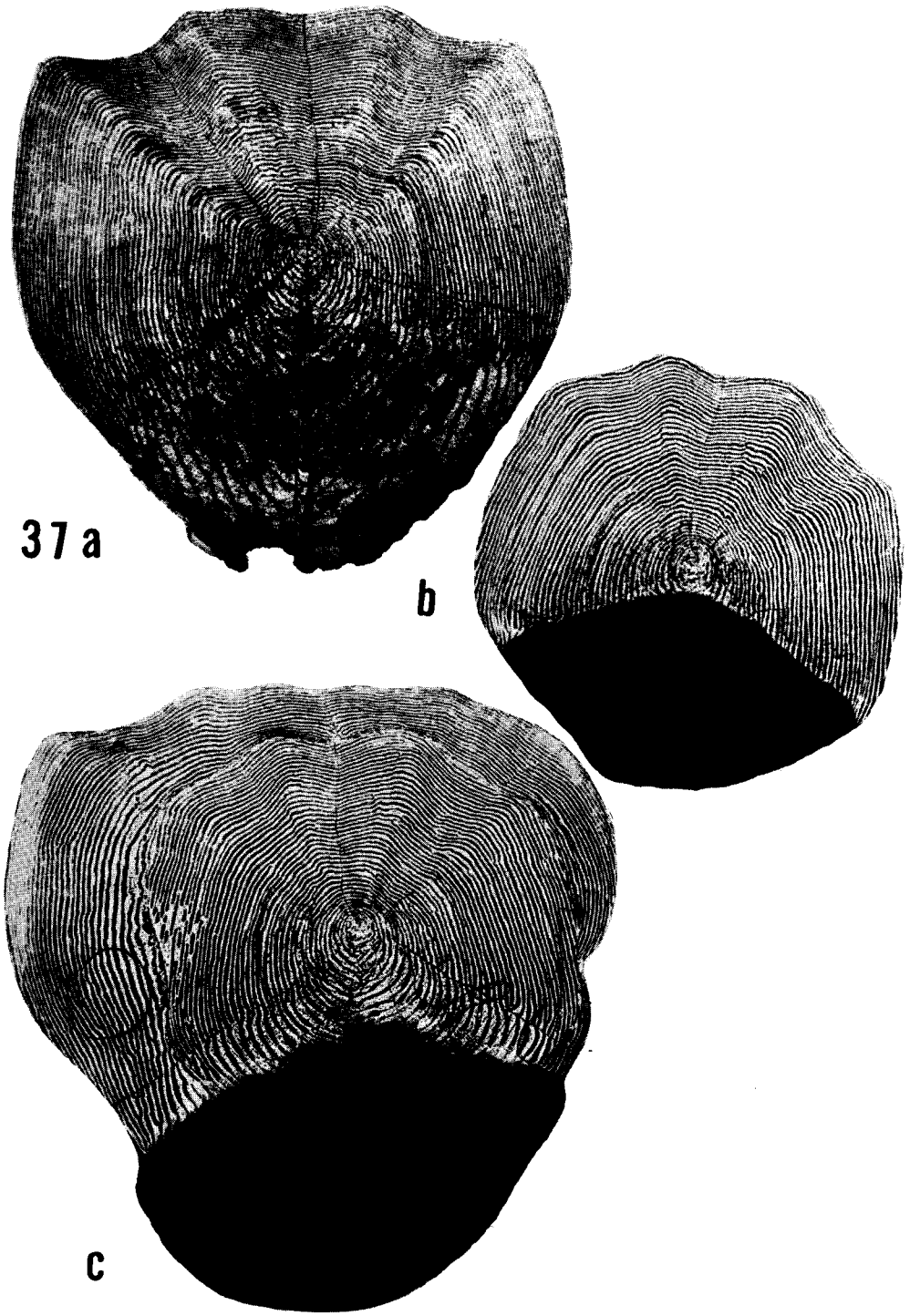
PLATE XVII

Fig. 37. The ordinary scale and two stages of the transplanted scale in fish No. 5 described in Table 7. ×18

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 187 days after transplantation.



J. Yamada: Studies on the Structure and Growth of the Scales

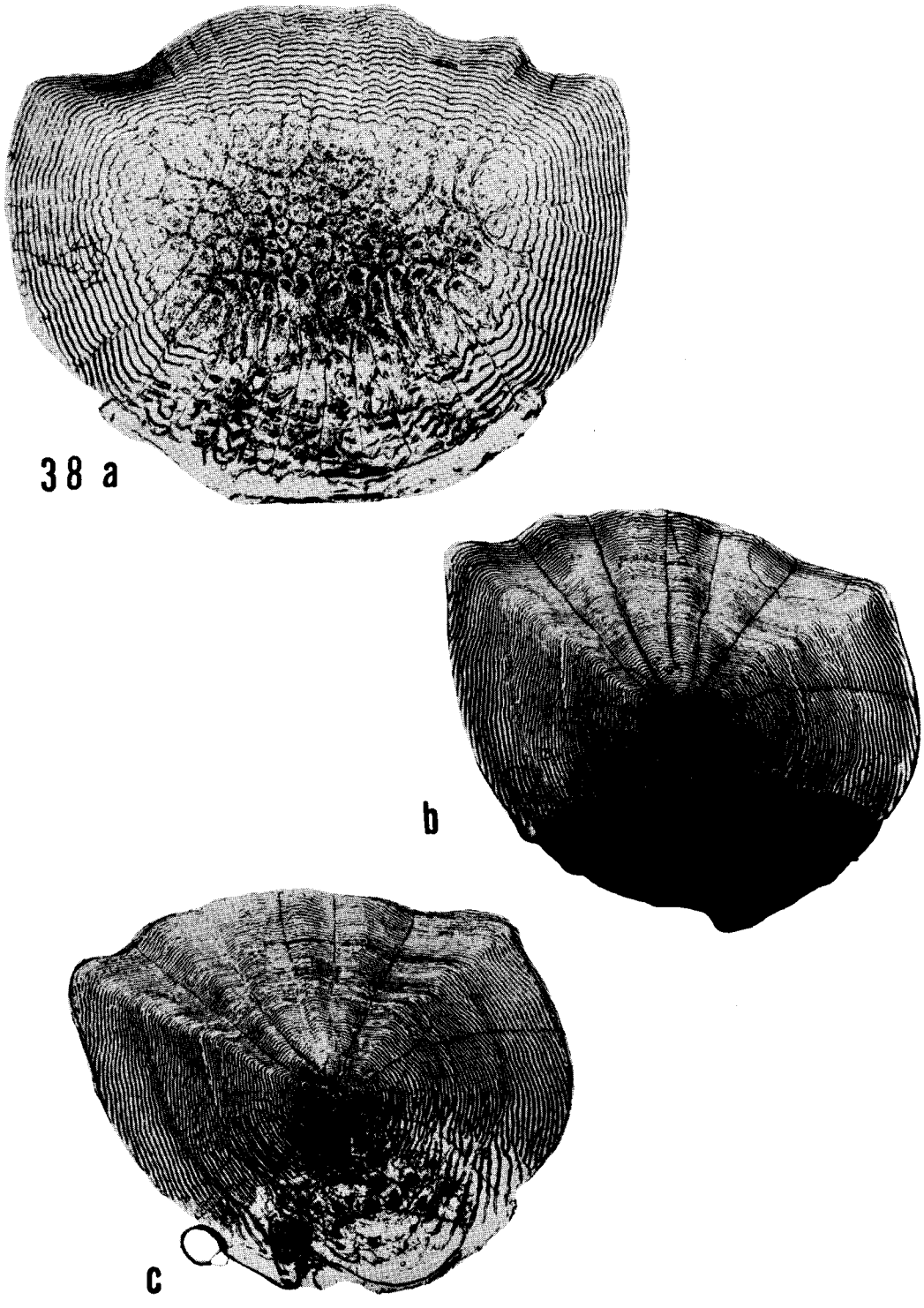
PLATE XVIII

Fig. 38. The ordinary scale and two stages of the transplanted scale in fish No. 6 described in Table 7. ×13

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 71 days after transplantation.



J. Yamada: Studies on the Structure and Growth of the Scales

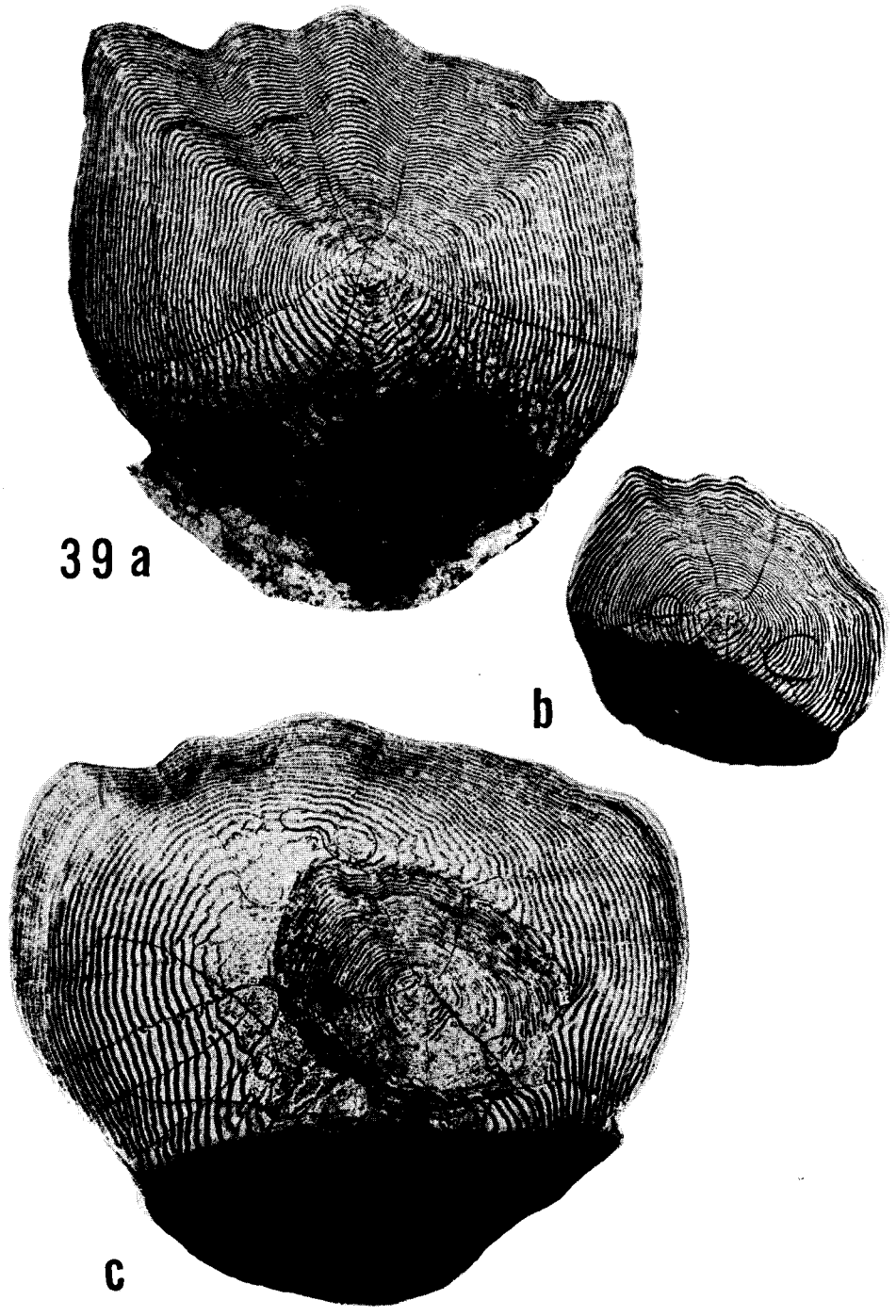
PLATE XIX

Fig. 39. The ordinary scale and two stages of the transplanted scale in fish No. 7 described in Table 7. ×18

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 138 days after transplantation.



J. Yamada: Studies on the Structure and Growth of the Scales

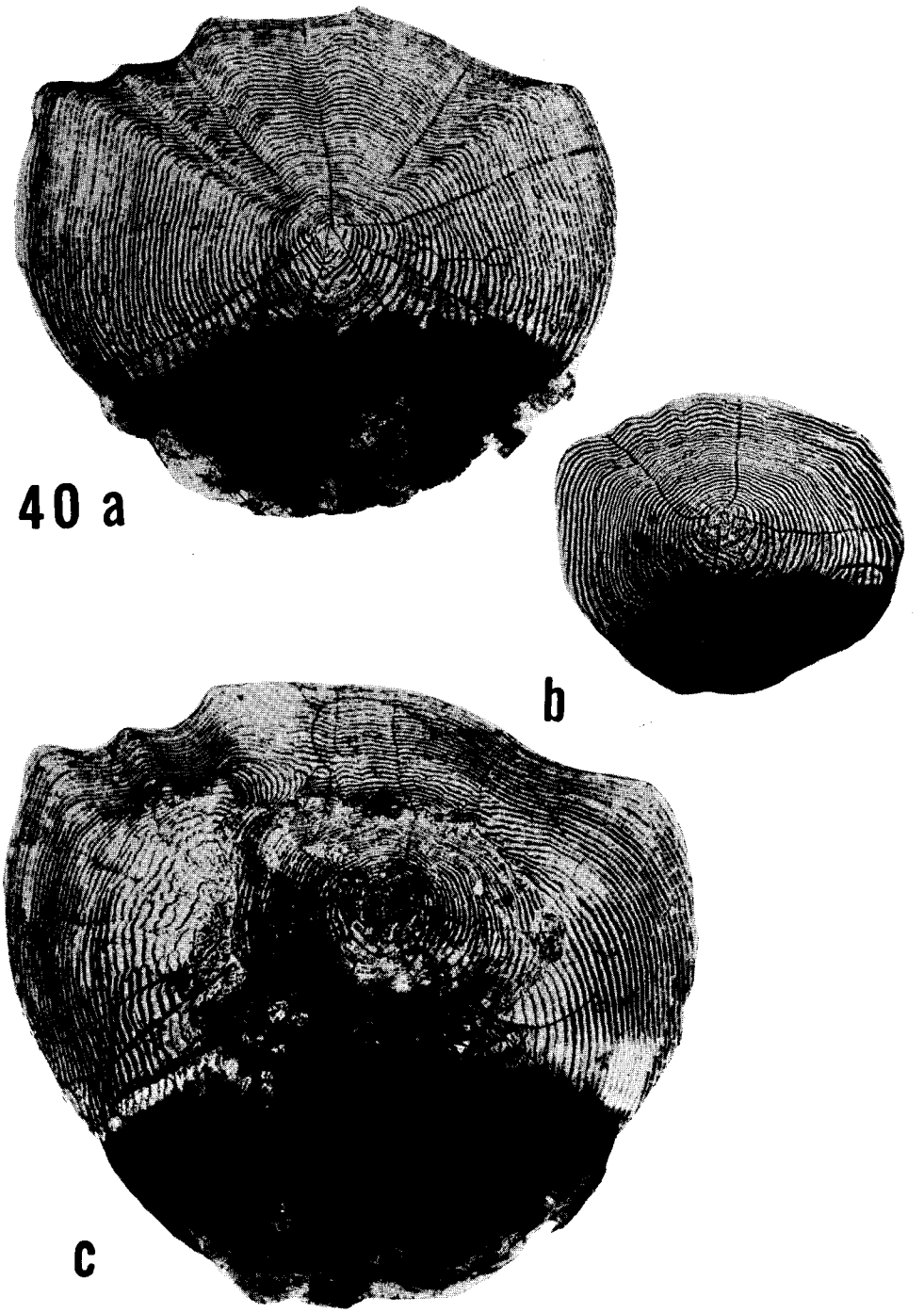
PLATE XX

Fig. 40. The ordinary scale and two stages of the transplanted scale in fish No. 8 described in Table 7. ×18

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 138 days after transplantation.



J. Yamada: Studies on the Structure and Growth of the Scales

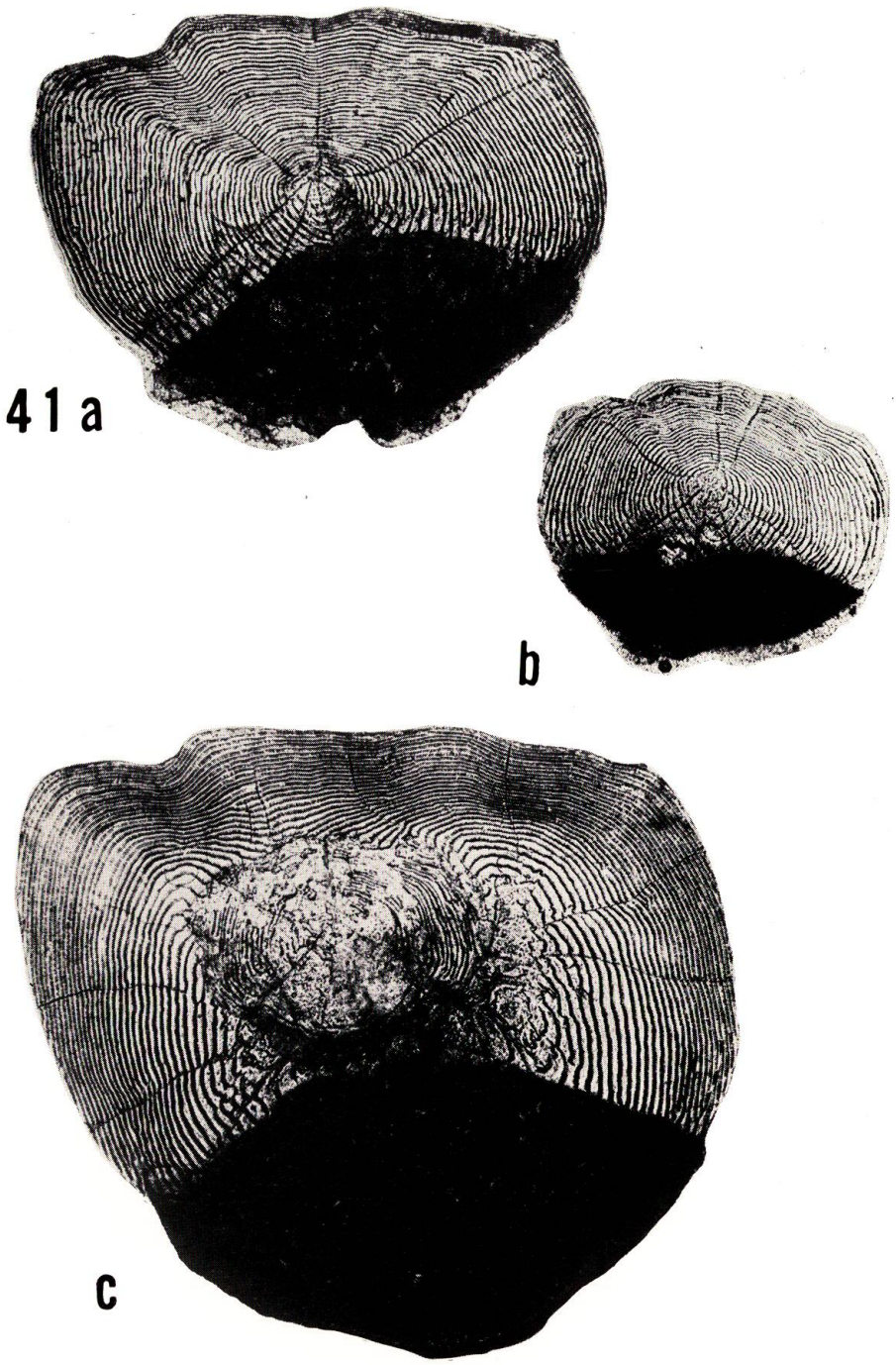
PLATE XXI

Fig. 41. The ordinary scale and two stages of the transplanted scale in fish No. 9 described in Table 7. ×18

a Ordinary scale.

b Transplanted scale.

c Transplanted scale at 138 days after transplantation.



J. Yamada : Studies on the Structure and Growth of the Scales