ON THE FEATURE OF SCALES DEVELOPED IN THE REGENERATED SKIN OF THE GOLDFISH, WITH SPECIAL REFERENCE TO THE FORMATION OF THEIR CONCENTRIC RIDGES

Juro YAMADA

Faculty of Fisheries, Hokkaido University

Introduction

Since studies on the regenerated scales in teleost fish are valuable in elucidating the mechanism of the formation and growth of fish scales in general, a considerable number of reports on these scales have been published by many authors. Here, the regenerated scales mean the scales which were formed in the emptied scale pockets as a result of the removal of individual ontogenetic scales. In this paper, these regenerated scales are called 'replacement' scales.

In addition to these, when a patch of skin, both the epithelium and the corium containing several scales, is removed from the body, scales found newly in the regenerated skin area may also be called regenerated scales, but they differ from the above in their way of formation, since there is no scale pocket prepared in advance: their development is considered to be similar rather to that of the ontogenetic scales. These are called here 'regenerated skin' scales.

Early histogenetic observations of such scales were made in several cyprinid fish by Sauter (1934) and Nardi (1935), but no description of the changes of their superficial pattern was presented because the main purpose of their studies was directed to the analysis of factors which played in the determination and differentiation of cells or tissues concerned.

In fact, the scales developed in the regenerated skin of the goldfish show characteristic features differing from those of the 'replacement' and of the 'ontogenetic' scales. Therefore, it is of interest to compare such a peculiar appearance of their scale patterns with that of other kinds of scales in reference to the peculiar ways of their formation. Particularly, the comparison on spacing of concentric ridges, the most important constituent of the superficial pattern as an indicator of fish ages, is considered to be valuable in finding a clue to the mechanism of ridge formation.

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Material and Method

Twelve one-year-old goldfish ranging from 4.2 to 5.0cm in body length underwent an operation. The mean values of their body length and weight were 4.5cm and 3.6gr respectively. Before the operation, each fish had an intraperitoneal injection of 0.4mg chlortetracycline (110mg per kilogram of body weight; cf. Weber and Ridgway, 1962) in an attempt to mark the time of operation on its whole scales.

The operation was made on a part of the left side of the body of each fish. After removing several scales including some lateral line scales as indicated in Text-fig. 1, a part of the skin just inside of that de-scaled area was cut off in square so that the muscle became exposed. At the same time, five scales of the opposite side of the body, corresponding to the center of the operated area, were removed in order to obtain 'replacement' scales.

The operation was carried out on May 6. Then, the fish were kept in a rearing pond for 85 days ending July 30; on that day the scales grown in the regenerated skin were collected together with the 'replacement' and 'ontogenetic' scales.

The number of fish was reduced to ten at the end of the rearing, and they had grown to 5.6cm in length and 7.7gr in weight on average.

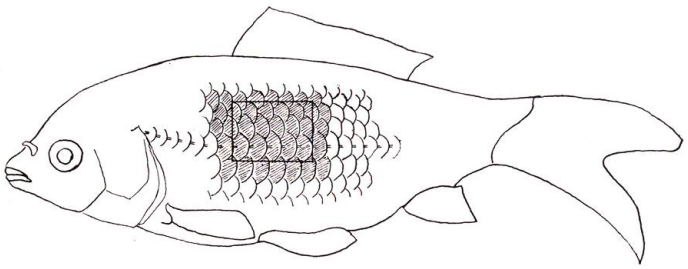
Four or five 'ontogenetic' scales were taken from the neighbouring sites of the 'replacement' scales as near as possible. By fluorescence microscopy, they showed a brilliant circular line of the deposit of tetracycline which enabled the distinguishing of the area grown after the operation.

The three kinds of scales obtained in this way were measured for their anterior radius or width of growth and number of ridges arising during the experimental period.

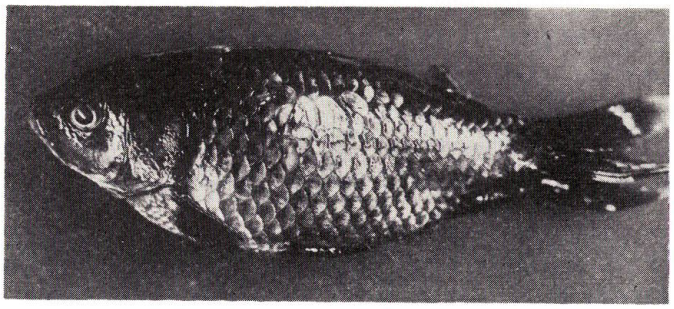
Observations

For a few days after the operation, the wound was being filled with hyaline plasma. An external examination of that area after a month, however, showed the complete recovery of the skin in which pigmentation had not yet reappeared. (Even by the end of the rearing period, 85 days after the operation, the regenerated skin still remained unpigmented in three fish.) The appearance of scales in the regenerated skin varied according to the individual; in some fish the scales were faintly recognizable, whereas in others not at all.

The scale formation began at the wound margin and proceeded to its center, and in all fish the 'regenerated skin' scales were observed apparently after two



Text-fig. 1. Showing the operated area of the skin which was cut off in square. The shaded scales indicate those removed before the operation.



Text-fig. 2. An abnormal scaling pattern appeared in the area of the regenerated skin of one of the experimental fish

months. Their arrangement was quite irregular and arbitrary, and the normal scaling pattern has never reappeared (Text-fig. 2); there arose some extremely large and small and sometimes even duplicated scales. Generally, the scales found in the regenerated skin area were smaller in number than those which occupied the same area before operation. These observations are well in accord with descriptions reported by Sauter (1934) and Nardi (1935).

The 'regenerated skin' scales can be grouped broadly into four types primarily according to their superficial pattern and secondarily to their size and shape.

The type-I scales (Fig. 1) have more or less a central field which lacks concentric ridges, the intervals of which show gradual decrease towards the scale margin. Accordingly, the scales of this type bear some resemblance to the 'replacement' scales (Fig. 2) as to the superficial pattern. But there are some differences between the two; there is no grooved network in the central field of the scales of type-I and the radial grooves are not so many, whereas the 'replacements' are characterized by the presence of the network and numerous grooves. Most of the type-I scales are very large and highly irregular in shape. The so-called 'giant' scales ('Riesenschuppen') noted by Sauter and Nardi belong to the type-I without exception.

The type-II scales (Figs. 3a, b, c) show evenly spread concentric ridges from the focus to the margin, and the radial grooves are very few. It may be said, therefore, that the pattern of these scales is similar to that of the 'ontogenetic' ones (Fig. 4) except that they have rather widely arranged ridges and a comparatively smaller number of grooves. Moreover, they frequently show two or more foci in one scale, a pattern never found in the 'ontogenetic' scales. The scales belonging to this type are nearly the same size as the 'ontogenetic' or the 'replacement'; their shapes are not rectangular as normal but triangular or circular.

A few scales which represent the superficial pattern of the above type are grouped separately as type-III according to their exceptionally small size (Fig. 5). These supplementary scales were noted also by Sauter as 'Ersatzbildungen,' and by Nardi as 'nachträgliche Schuppen.'

The type-IV scales (Fig. 6) are agglutinated forms between two scales of type-I and type-I or of type-I and type-II. The agglutination always occurs along the longitudinal axis of the scales with two neighbouring scale rows, so that the scales of this type show the largest transverse diameter among the scales described so far.

In addition to these, a few duplicated scales, each made up of two scales lying one upon another, were obtained. Each constituent of the duplicated scales can be regarded, however, as belonging to one of the above stated four types.

Frequency of the 'regenerated skin' scale of every type with ten experimental fish is shown in Table 1. It is apparent that most of the scales belong to type-I or type-II. In many cases, the scales of type-I and type-IV are found in the marginal area of the wound while those of type-II are in the central area. As previously stated, the arrangement of these scales is so irregular that small gaps would have been left at places unscaled. Thus the type-III scales are considered to have been supplemented later in order to fill them up.

Since the 'ontogenetic' scales in contiguity overlap each other all around the scale margin, it is impossible, at the time of operation, to cut off the skin corresponding strictly to a certain number of scales. As a matter of fact, the excision of the skin in the intended area was made after the removal of scales standing beyond that area. Therefore, the margin of the wound should have been bordered by the emptied scale pockets a part of which had been excised.

In healing the wound, regenerative cells flow into it all over the surface from the marginal connective tissue to form a loose layer of cutis. At the same time, the scale pockets left at the wound margin are supposed to extend their half-destroyed tissue into the wound area. This would explain the fact that the

Table 1. Number of the 'regenerated skin' scales grouped into four types with ten experimental fish

Fish	Scale types				Total
	I	II	III	IV	
1	5	7	2	0	14
2	5	5	0	0	10
3	5	3	1	2	11
4	5	5	0	1	11
5	8	4	0	1	13
6	8	2	0	1	11
7	2	5	3	3	13
8	8	3	0	0	11
9	4	3	0	0	7
10	4	5	4	0	13
Total	54	42	10	8	114

type-I scales are mostly found at the margin of the wound; that is to say, the fact that the scales of this type are large and their superficial pattern is similar to that of the 'replacement' scales indicates that these scales have developed in the tissue which had already differentiated into the wall of the scale pocket. In the central area of the wound, on the contrary, the just regenerated young corium brings forth type-II scales which resemble the 'ontogenetic' as to their superficial pattern.

Among the 'regenerated skin' scales, the most interesting ones with regard to the superficial pattern are those of type-II (including the fractions of type-IV), because they are unique in view of the fact that they should have developed in a wide but scale pocket-less space without being restricted in their growth by the surrounding scales. In this sense, the scale pattern of this type may be regarded as the true representation of the 'regenerated skin' scales.

In order to analize factors exerting effects on the changes of the superficial pattern, it is most important to know how many concentric ridges there are in a definite width of growth of the scales which have grown in the histologically different environments. For this reason, comparisons were made between the type-II, the 'ontogenetic' and the 'replacement' scales with every fish on the width of growth, the number of ridges, and the ridge intervals. The growth area of the 'ontogenetic' scales during the rearing period (85 days) could be learnt by the examination of the fluorescent mark. Of the 'replacement' as well as the type-II scales, the whole area was assumed to be the growth area during

Table 2. Comparison of the scale pattern between three kinds of scales. Width of growth and mean ridge interval (W/R) are shown in arbitrary units (1 unit=0.02 mm). Width of growth and number of ridges are represented as average values of several scales.

Fish	B.L. (cm)	Ontogenetic scales				Replacement scales				Regenerated skin scales			
		Number of scales examined	Width of growth	Number of ridges	W/R	Number of scales examined	Width of growth	Number of ridges	W/R	Number of scales examined	Width of growth	Number of ridges	W/R
1	6.3	4	25.8	12.0	2.15	4	71.3	26.8	2.67	7	64.3	29.7	2.13
2	5.9	5	20.6	9.6	2.15	5	65.6	22.6	2.90	5	63.2	29.2	2.16
3	5.9	4	20.8	10.3	2.02	5	71.4	25.2	2.83	5	77.6	30.4	2.55
4	5.9	5	10.0	8.2	1.22	4	67.3	23.5	2.86	6	67.7	30.3	2.23
5	5.8	4	13.5	8.5	1.59	4	64.5	23.8	2.72	5	57.4	26.2	2.19
6	5.6	5	20.2	10.6	1.91	4	62.3	21.8	2.86	3	74.7	31.3	2.39
7	5.6	5	13.6	8.6	1.58	4	61.0	19.8	3.09	8	71.1	24.4	2.91
8	5.3	5	22.0	11.4	1.93	5	62.4	21.2	2.94	2	52.0	27.5	1.89
9	5.0	5	10.8	7.6	1.42	5	58.0	19.6	2.96	3	51.0	24.0	2.13
10	5.0	5	6.8	5.0	1.36	5	57.0	21.4	2.66	5	40.6	21.6	1.88

the experimental period, although the actual growth periods of these scales should have been shorter than that of the 'ontogenetic' (about two weeks in the former case and a month in the latter). The mean ridge interval of each scale is obtainable by dividing the width of growth (the distance between the focus and the anterior margin) by the number of ridges counted in that growth area. For convenience, the calculation was made on average width of growth and ridge number with several scales for every scale type.

The results are shown in Table 2. It is obvious that the mean ridge interval grows larger in the order of the 'ontogenetic,' the 'regenerated skin,' and the 'replacement' scales. It is a matter of course that the width of growth, the number of ridges, and the ridge intervals all showed lower values in the 'ontogenetic' scales than in the other rapidly grown regenerated ones. On the other hand, there is no distinct difference recognized in the width of growth between the scales of the 'replacement' and the 'regenerated skin,' but the latter scales have clearly a larger number of ridges than the former, despite their shorter growing period. This evidently reflects on the difference of the ridge interval, suggesting that the histological condition is an important factor affecting the formation or spacing of ridges.

Discussion

It is well known that the 'replacement' scales in general manifest a special superficial pattern characterized by a wide central field having a grooved network instead of concentric ridges, and by numerous radial grooves starting from that central network. Such a pattern is explained as being caused by the pre-existence of the emptied scale pocket; the central network has traces of the assemblage of many scalelets formed synchronously in the pocket, the numerous radial grooves are the boundaries extending among many outerly situated scalelets which are growing outerwards, and missing of the concentric ridges in the central field is due to no directional growth of the central scalelets (Neave, 1940; Yamada, 1961).

It is therefore natural that the type-I scales which developed in the tissue extended from the half-destroyed scale pockets of the wound margin showed a somewhat similar pattern to that of the 'replacement' scales. The absence of the network, however, suggests that their central field is not composed of many small scalelets. Causes of the difference can not be explained at present, but the matter may well have a connection with the fact that the tissue responsible for the formation of the type-I scales is not in the form of completed scale pockets.

Also in the case of type-II scales, it is supposed that they have arisen in fusion of two or three, sometimes even more, growth centers which can be seen

as the several scale foci. Their radial grooves are running between adjacent growth centers so that their number is almost in keeping with the number of foci. Viewed in the light that they have developed in a wide scale-less space, they can be regarded as being in common with the 'replacement' scales, but from another viewpoint, they are just a kind of 'ontogenetic' scale because they originated in the yound undifferentiated corium and their growth is necessarily accompanied by the enlargement of their scale pockets. Accordingly, the concentric ridges in these scales appear from the beginning of their growth, and the mean value of the ridge intervals lies between those of the 'ontogenetic' and the 'replacement' scales.

Contrary to the 'replacement' or the 'regenerated skin' scales, the growth of the 'ontogenetic' scales is under reciprocal restriction between the adjacent scales and only the enlargement of body surface due to the growth of the fish enables them to grow larger.

It would be understood, therefore, that the differences of the superficial pattern among the 'ontogenetic,' the 'replacement,' and the 'regenerated skin' scales, and also between the type-I and type-II scales, are due to the different states of tissues in which they are formed.

It has been suggested by Wallin (1957) and Yamada (1961) that the primary factor in the ridge formation would be a pressure which is formed between a growing scale and the surrounding tissues (epithelium, loose and dense corium, adjacent scales etc.). The results presented in this paper seem to support the above assumption. The pressure must be greatest in the 'ontogenetic' scales in which the least ridge intervals were obtained, because they can not grow larger without growth of the fish body. In comparison of the 'replacement' and the 'regenerated skin' scales, the pressure is supposed to be less in the former which displayed larger ridge intervals, since each of them grows in the already completed scale pocket contrary to the latter which grow in the just regenerated corium.

This interpretation would be applicable to the growth of normal scales; a rapid or retarded growth of the fish will be reflected in the ridge intervals of its scales through reducing or increasing the pressure.

Summary

1. Scales developed in the regenerated skin area of the goldfish were obtained experimentally.

2. The general features of these 'regenerated skin' scales were described,

grouping them into four types primarily according to their superficial pattern and secondarily to their size and shape.

3. In each type, the correlation between the scale feature and the histological environment for their growth was analyzed.

4. The most interesting scales are those of type-II because they showed a characteristic pattern in reference to the fact that they have been formed in the entirely new regenerated corium.

5. The superficial pattern, particularly the mean interval of concentric ridges, of the type-II scales was compared with that of the 'ontogenetic' and the 'replacement' (customarily called 'regenerated') scales both taken from the same fish.

6. It was ascertained that the mean ridge interval in these three kinds of scales grows larger in the order of the 'ontogenetic,' the 'regenerated skin' (type-II), and the 'replacement' scales.

7. The result seems to support the assumption that a pressure formed between a growing scale and the surrounding tissues would be the primary factor which causes the ridges to form.

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Explanation of Plate

PLATE I

Fig. 1. Large type-I scale

Fig. 2. Typical ' replacement ' scale

Fig. 3. Three varieties of type-II scales

a Having one focus

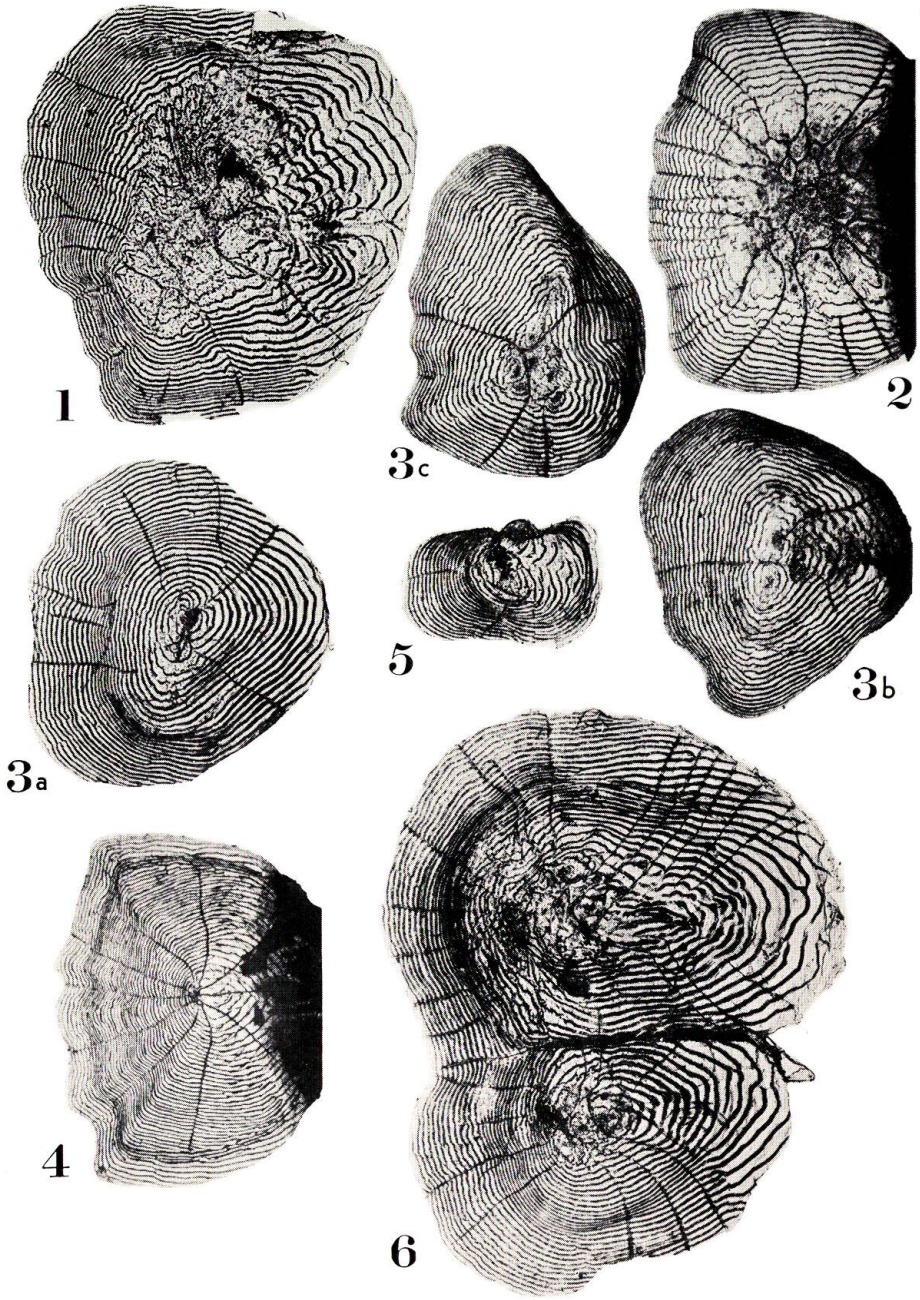
b Having two foci

c Having three foci

Fig. 4. Typical ' ontogenetic ' scale

Fig. 5. Small supplementary scale belonging to type-III

Fig. 6. Type-IV scale formed in agglutination of two scales of type-I and type-II



J. Yamada: Scales developed in the regenerated skin of the goldfish