On the Histology and Regeneration of the Teleost Scale.

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With Plates 30-33

INTRODUCTION.

Despite the considerable number of published papers relating to the Teleost scale, a number of histological and physiological questions remain obscure or are the subject of conflicting views. This is especially the case with regard to the histology of the cells intimately associated with the scale, the manner of formation of the scale ridges, and the causes of the conspicuous differences in morphology and arrangement between ontogenetic and regenerate scales. In addition to the intrinsic interest of these questions, the continued importance of the Teleost scale in fisheries’ investigations and its more recent popularity in experimental studies have seemed to furnish good reason for further examination.

The work on which the present account is based was carried out in the laboratory of Professor Leigh Hoadley, Harvard University, to whom I am deeply grateful for facilities, material, and much kind assistance.

MATERIAL AND METHODS.

The investigation has been concerned mainly with the goldfish (Carassius auratus L.) and the guppy (Lebistes reticulatus Peters).

Histological methods included the use of a considerable number of fixing agents and stains. The former included Bouin, Helly, Zenker, and formalin (both neutral and commercial). Among the stains used were Harris’s, Delafield’s, and Mallory’s phosphotungstic acid haematoxylin, Mallory’s connective tissue
stain, basic fuchsin, methylene blue, eosin and acid fuchsin. Fresh material was stained with neutral red, methylene blue, and alizarin red S. Much use was made of silver nitrate impregnations both for the investigation of cellular details and for following the progress of calcification of the intercellular substance. These impregnations were made upon both fresh scales and scales which had been previously fixed in neutral formalin. This treatment was frequently followed by one or more of the above-mentioned stains. Sections were cut in hard paraffin or by the celloidin-paraffin method. The well-developed scale is notoriously hard to section by the ordinary paraffin process. In my experience, decalcification does little to assist the operation while having a decidedly harmful effect on the cellular elements. The celloidin-paraffin combination, without decalcification, gives better results. Many scales, of course, were studied in toto as flat mounts, either temporary or permanent.

HISTOLOGY OF THE GOLDFISH SCALE.

The ontogenetic scales of the goldfish are of typical cycloid type. They vary in shape in different regions of the body but in general are somewhat greater in width than in length, with prominent shoulders and a nearly central focus. Structurally they conform to the usual Teleost type, consisting of an outer bony\(^\text{1}\) layer and an inner fibrillary plate, the latter consisting of closely applied fibrous lamellae. The ridges ("striae", "circuli", "sclerites") of the bony layer are arranged in a concentric manner, but are less numerous in the posterior field. The bony tissue is interrupted by a number of grooves ("radii") which also tend to be more numerous in the anterior field.

In more or less close association with the scale are histological elements which may be placed in the following categories: epidermis; connective tissue; chromatophores; iridocytes; blood-vessels; osteogenic cells.

The epidermis is only in close topographical relationship with

\(^1\) The present writer uses the terms 'bone' and 'bony' for the calcified portion of the scales as he considers that this layer sufficiently resembles 'true' membrane bone to justify these designations as a matter of convenience, in the absence of a more specific term.
the scale in the posterior field. It shows no special character in this region but consists, as elsewhere, of about 6–8 layers of cells with scattered unicellular glands. The question as to whether it plays any direct part in scale formation will be considered later.

The connective tissue completely surrounds the scale and the osteogenic cells, forming the scale pocket. The walls of the pockets are continuous with the general corium at their deeper anterior ends. Throughout the greater part of the pocket the tissue is compact and so thin that capillaries frequently cause conspicuous bulges. Posteriorly, where the tissue is in close contact with the epidermis, the structure is looser and blood-vessels are more numerous, particularly on the upper surface of the scale. In the guppy blood-vessels actually pass through the scale and spread out on the upper surface. Lipophores, and in darkly pigmented regions melanophores, lie in the connective tissue layers. A reflecting layer of guanin crystals is present in the connective tissue below the scale.

The histology of the cells immediately investing the scale has been interpreted very differently by different investigators. Klaatsch (1890) in his classical study on the trout scale describes more than one cell-layer on the external surface during early development, the superficial cells lying on the external surface of the ridges. He says that in older stages there remain only nuclei and small masses of protoplasm. He describes an 'indifferent' layer of connective-tissue cells on the lower (inner) side of the scale. Ussow (1897) also describes cells on, or just behind, the surface elevations of the scale and implies that after the full development of the ridges little remains of these cells but their nuclei.

Paget (1920), also working mainly on trout, states that the scale is at first surrounded by a single epithelial layer of cells. As the striae are formed the epithelial nature of the overlying layer is lost and the cells are disposed without definite relation to one another except at points where striae are being laid down. 'When the stria is once formed ... the nuclei of the cells appear not to attach themselves so closely to it as at first. Nevertheless, nearly the whole of the plasma is used up in the process of stria
formation.' Between the most newly formed stria and the edge of the scale the cellular investment of the upper surface is several layers deep.

Nusbaum (1907) describes two cell-layers on both sides of the early trout scale. According to his account the deeper lying layer of the outer side is used up in the formation of the bony portion of the scale, the cells disappearing completely. The cells of the more superficial layer arrange themselves concentrically, secrete the ridges, and then degenerate to form a thin homogeneous cuticle over the scale, the nuclear remains being embedded near the ridges. He also describes certain osteolytic cells as assisting in the production of the surface sculpture. He gives a similar description of the cells beneath the scale, saying that the layer immediately against the fibrillar plate is used up in the formation of the latter, being represented in the later stages of degeneration by free, scattered nuclear bodies.

Pevsner (1926), dealing with Carassius, describes an external layer of cells the cytoplasm of which is used up in the production of the bony layer of the scale. Only nuclei remain in the areas between the ridges. Close alongside the latter the cytoplasm remains longer, so that each 'sclerite' is bordered by a row of cells connected by protoplasmic processes. At the periphery and beneath the scale the cellular investment consists of spindle-shaped connective-tissue cells.

Setna (1934) describes two layers on both surfaces of the developing trout scale. 'The nuclei of the dorsally placed cells become very much flattened and change gradually from a circular to a broadly conical form; the karyosomes break up into irregular fragments, giving a granular appearance to the nuclear contents very different from the first.' 'The nuclei in the dorsal scleroblasts show . . . whitish areas which are small in the beginning, but later become confluent to form one large area.' Similar areas develop in the ventral scleroblasts. He considers it probable that the cells from the ventral surface migrate around the edges to join the dorsal scleroblasts. (Creaser (1926), on the other hand, says that the upper cells tend to work around the edge and enclose the lower cells.) The proximal layer of ventral cells shows as an irregular network of large polygonal
cells. The nuclei of both ventral layers are smaller and rounded, while those in the two dorsal layers are larger and greatly flattened.

There is no doubt that these various interpretations of the cellular structure and arrangement are due largely to the inadequacy of such stains as alum haematoxylin in revealing the cell outlines. Much better pictures of some of the elements can be obtained by the use of silver nitrate followed by haematoxylin or by staining fresh scales with neutral red and alizarin red S. While the following account is based primarily upon Carassius the essential points appear to be entirely similar in such other species as I have examined.

**Cells of the External Surface of the Scale.**

As indicated above, scales which have been stained in alum haematoxylin and mounted whole usually show only nuclei over most of the external surface. Such scales ordinarily retain a patch of epidermis adhering to the posterior portion, which renders observation less easy in this region. Outside this area the nuclei usually appear most numerous along the proximal sides of the concentric ridges (the term ‘proximal’ is used here with reference to the growth centre or focus of the scale). Examination with a high power shows, however, that these nuclei lie at two different levels. Sections of scales embedded in celloidin-paraffin show these two cell-layers distinctly. While more conspicuous in the very young scale, they are present at all stages. At the extreme edge of the scale the cells may be more than two layers deep.

1. **The Superficial Cell-Layer.**—The boundaries of these cells can sometimes be seen well in flat mounts by the use of silver nitrate. In my experience, however, they are very capricious in this respect and usually remain invisible. They can frequently be seen in the freshly plucked scale with the help of a dilute solution of neutral red, particularly when continued immersion in a hypotonic medium has caused them to round up somewhat. A better method is to give fresh scales a preliminary staining with neutral red or methylene blue to show up the nuclei and then treat them with a weak aqueous solution of
alizarin red S. It can then be seen that these superficial cells form a continuous epithelium over the whole surface. Their disposition shows no relationship with the underlying scale ridges, across which they are perfectly continuous, though their nuclei, which are rather small and compact, frequently show some tendency to lie close to these ridges (fig. 1, Pl. 30). In many cases a clear sphere can be seen attached to the nucleus, which it approximately equals in size. This is probably the 'archoplasmic vacuole' which has been described in various types of cell, including osteoblasts, and which is said to contain the centriole and Golgi apparatus. The cells of this epithelium are extremely thin, the nuclei showing as conspicuous bulges in sections.

2. The Intimate Cell-Layer of the External Surface.—The outlines of these cells can be demonstrated very readily by immersing the entire scale in a weak (¼ per cent. or less) solution of silver nitrate for a few minutes. The silver nitrate can be applied to the fresh scale which may then be fixed in neutral formalin. Alternatively the scale may be impregnated during or after fixation in this medium. It may then be stained with haematoxylin, methylene blue, &c. If treatment with the silver nitrate is prolonged the whole of the underlying bony tissue becomes blackened and the cell picture is obliterated. Alizarin red also reveals the cell outlines by staining first the calcified tissue below the intercellular boundaries. In favourable silver nitrate preparations the cells appear as regular close-set structures filling the whole of the areas between the ridges. Over the greater part of the scale (in Carassius) each cell usually extends the whole distance between adjacent ridges. In the posterior field, however, where the ridges are more widely spaced, two or more rows of cells may lie between the elevations. In the regions where the individual cells extend from ridge to ridge the nuclei always lie close to the more distal ridge. These nuclei are usually elongated, sometimes very greatly, in the direction pursued by the ridge. Occupying a large part of the centre of each cell is a clear area containing, or surrounded by, black-stained granules. A small similar area is frequently present in close connexion with the nucleus (fig. 2, Pl. 30). The general
appearance of these spots suggests a Golgi apparatus. The cells of this layer, like those of the superficial epithelium, are greatly flattened.

At the growing margin of the scale, both above and below the formed substance, the cells are much less flattened and the double-layered condition is not adhered to so strictly. In the newly-formed scale, whether ontogenetic (trout) or regenerate (goldfish), the cells of the intimate layer are comparatively thick and unflattened over the whole scale. Flattening, however, takes place very rapidly, and in the well-developed scale only the cells of the extreme growing margin have not yet attained this condition.

The Cells of the Internal Side of the Scale.

In the very small ontogenetic scale, and during a considerable period of growth in the regenerating scale, a conspicuous layer of cells can be seen lying in contact with the inner surface of the scale. These are present in a well-developed condition during the laying down of the first bony tissue and for some time after the appearance of the fibrillary plate, which is formed between them and the bony layer. Their boundaries can be shown by impregnation with silver nitrate. The cells also stain well with alum haematoxylin, when they appear in surface view as polygonal, strongly basophilic cells. The nuclei are circular, sometimes chromatic, in other cases rather pale but with a large karyosome. Lying close to the nucleus is a pale area which has been interpreted as a scale-forming secretion but which in my opinion represents the Golgi apparatus. The cells are connected by slender cytoplasmic bridges. They are the 'polygonal cells' of Paget. (Klaatsch, followed by Nusbaum, used this term for the cells lying on the external side of the very young scale.) In section they appear as more or less oblong bodies. Despite the dissimilarity in appearance at most stages, these cells seem to be continuous with the intimate cell-layer of the external surface. At the extreme margin (and at the beginning of scale formation, throughout) the cells are similar in appearance on both sides of the scale. For reasons which will be given later, the marginal members of this cellular sheath may be properly described as
osteoblasts. Elsewhere they correspond rather to osteocytes (fig. 5, Pl. 31).

In more advanced stages the cells immediately beneath the scale are flattened and their cytoplasm is no longer basophil. Eventually this cell-layer disappears or is represented by greatly flattened nuclei lying against the much thickened fibrillary plate.

Sections of young regenerate scales show immediately below the still conspicuous osteocytes one or more thin sheets of tissue which rest on the corium. When the entire scale is examined from the internal surface this adventitious layer is revealed by numerous scattered nuclei, circular or somewhat crescentic in shape, the latter appearance being due to the presence of an attraction sphere similar to that of the superficial external cells of the scale. Cell boundaries are faint or indistinguishable, but fibres can be seen in the ground substance. As development proceeds and the intervening osteocytes disappear there appears to be a progressive application of similar thin connective tissue sheets which form a fibrillated tissue of appreciable thickness lying close against the scale. Nuclei occur in this tissue, chiefly on the lower surface, i.e. in the most newly added sheet. In whole scales which have been impregnated with silver nitrate or stained with alizarin red a conspicuous network is seen. This often gives an appearance of cell outlines and apparently has been so interpreted by Setna (l.c.). The network, however, appears to represent argyrophil threads or fibres. Many of these end freely in enlarged knobs which are sometimes in close relationship with nuclei. The network is incomplete and highly irregular towards the margin of a growing scale (figs. 3, 4, Pl. 30).

THE LAYING DOWN OF THE SCALE.

Investigators of scale structure have nearly always assumed that the scale is laid down as a direct secretion of certain cells. This view is implicit even in such recent publications as those of Setna (1934), Nardi (1935), Crichton (1935), and Fach (1936). In a previous paper (Neave, 1936) I pointed out that this idea is not in accordance with present-day histological conceptions of bone formation. Many of the writers cited in the present
report have identified certain intracellular areas as the first appearance of the scale substance. While it may be regarded as certain that the cells do liberate a secretion of some kind, this is not the definitive scale substance, and is probably not represented by the visibly differentiated areas. The constancy in presence and size of these intracellular appearances, even in areas on the upper surface of the scale where no scale growth is taking place, argues that they cannot be regarded as contributing directly to the scale. It has been suggested above that they possibly represent a Golgi apparatus.

In the paper referred to above, I stated that the bony portion of the scale is laid down before the fibrillary plate, being in this respect in agreement with Klaatsch (1890), Nusbaum (1907), and Crichton (1935). Paget (1920), Pevsner (1926), Petrov and Petruschevsky (1929), and Setna (1934) consider that the layers are laid down simultaneously or that the fibrillary plate is formed first. These authors and others, such as Goetsch (1920) and Peyer (1931), consider that both portions of the scale are laid down through the activity of the cells of the original papilla. If this view be correct the point as to which scale-layer is formed first is perhaps not of great importance. The present writer is of the opinion that the two formations are of different origin. The order of their appearance has a direct bearing on this point of view.

The developmental stages can be seen to advantage in the regenerating scale of Carassius, where large areas are undergoing rapid transformation.

After removal of an adult scale, the pocket appears to become filled with a gelatinous substance. The epidermis regenerates rapidly and, below this, the missing connective tissue is replaced by a loose cellular accumulation. The scale papillae are formed on the floor of the pocket from cells which appear to come from points at or near the periphery of the pocket and which arrange themselves in the layers previously described. The formation of osteoid tissue, i.e. the first appearance of the scale proper, always takes place within a close investment of cells. There is a continued addition of osteoblasts, these cells traversing the gelatinous substance and applying themselves to the extending margin of the osteoid tissue. In young and rapidly growing
scales the jelly (and its contained cells) forms a wide marginal area, and is sometimes sufficiently solid to withstand removal from the body (fig. 9, Pl. 31). It may be remarked that the above description is contrary to the views of Paget and Setna, who consider that all the cells of the scale throughout its development are derived from those which closely invest the original rudiment.

Sections which traverse the radial grooves (sulci) of newly formed portions of the scale show that the osteogenic cells of the external surface are continuous with the cells immediately underlying the scale. This shows that no formation of the fibrillary plate has yet taken place, since the grooves do not extend down into this part of the scale. After the commencement of formation of the fibrillary plate the continuity of the external and internal cells is broken at these points.

The osteoid tissue of course is collagenous. The process of calcification, i.e. conversion into the definitive bony tissue, can be followed very readily by immersing scales in a weak solution of silver nitrate, which rapidly blackens the calcified parts. In a rapidly growing scale a comparatively large osteoid plate is formed before any calcification occurs. The latter process begins in the oldest parts of the scale and spreads towards the periphery. As long as scale growth continues at all, however, there is always a recognizable osteoid margin. This is usually wider in the lateral and posterior regions of the scale. In the guppy most of the scales are present and largely calcified before birth. In the goldfish the newly formed ridges sometimes become calcified more rapidly than the intervening bony surface (figs. 11 to 15, Pl. 32).

The fibrillary plate appears first as a very thin layer in immediate contact with the bone, i.e. above the lower layer of osteogenic cells. As described previously, these lower osteogenic cells later become indistinguishable, and the plate increases in thickness under the influence of a fibrillated tissue which appears to separate from the underlying floor of the pocket. There appears to be no doubt that the fibrillary plate is also largely collagenous (see Green and Tower, 1902), though in the paper cited above I followed Pevsner, who has criticized this
view. In a well developed scale the fibrillar plate in general does not take the usual collagen stains, e.g. if Mallory's connective tissue stain be used (without the acid fuchsin) it reacts to the orange G, not the anilin blue. Presumably this irregularity is due to the presence of ichthylepidin, the other organic substance which has been recognized in Teleost scales. Close examination indicates, however, that when first laid down, the substance of the fibrillar plate does take the anilin blue stain, this colour showing in the innermost (newest) lamella. The yellow-staining property is gradually acquired. This leads to the conclusion that the collagen is laid down first and that it then becomes infiltrated with ichthylepidin. Further reference to the condition of the fibrillar plate will be made in considering the nature of the radial canals.

From the foregoing account it is evident that the cells of the papilla are (at least in the beginning) concerned only with the production of the bony part of the scale. Nevertheless the fibrillar plate when it first appears lies above the lower layer of osteoblastic cells in immediate contact with the bone. This has led to the currently accepted view that the intimate external and internal layers are physiologically quite distinct, the former laying down the bone ('hyalodentine'), the latter the fibrillar plate. Since all these cells are at first associated only with the bony tissue, which extends only at the margin, most of them can be regarded as osteocytes in the sense that they have been left behind by the process of osteogenesis. Although owing to the thinness of the formed bone they are not enclosed by the latter, many of them do become partly embedded between the ridges on the external side of the scale. Since the osteocytes of the lower side subsequently disappear (virtually or entirely) it is evident that they can at most only contribute to the initial layers of the fibrillar plate, whose later (and by far the greater) development takes place under the influence of the underlying connective tissue. Nusbaum (l.c.) admits the probability that the later development of the fibrillar plate is brought about in this way, but holds that the cells of the original papilla form the original part of the plate. In view of the previous association of these cells with the bony tissue and the probability that they
are of different origin from the connective tissue of the scale pocket, I am strongly of the opinion that the fibrillary plate is formed wholly from the latter. It seems to me that only in this way can the developmental differences between the bony and fibrous tissues be explained. The bone grows only at the margin, through the activity of cells from the periphery of the pocket; the fibrillary plate increases in thickness through addition of broad sheets from the floor of the pocket.

The conclusion that the two portions of the scale are of different histological origin accords well with the evidence presented in a previous paper (Neave 1936) that the papillae of ontogenetic scales (trout) do not arise from the underlying corium, as is commonly supposed, but are derived from cells which have migrated to their final positions from certain specific points in the body. Most of these points are located along the lateral line. When a centre of scale-formation is set up, the migrated cells form a ring the centre of which is occupied by a fluid or gelatinous intercellular substance. Cells pass into this, and form the papilla within which scale development begins. The cells which remain around the periphery I termed 'follicle cells'. As the scale increases in area these follicle cells provide new osteoblasts for the growing margin. In sections of goldfish skin from which adult scales have been removed, a small cluster of cells can frequently be seen at the point where the scale margin was in contact with the pocket wall. It seems probable that these are the descendants of the follicle cells and that they are responsible for the development of the bony layer of the regenerate scale, which, as already shown, grows by peripheral additions. General support for the view that the osteogenic cells do not arise from the underlying connective tissue is obtained from the results of experiments on scale regeneration reported on a later page. In order to maintain the correlative conclusion that the fibrillary plate is derived wholly from the connective tissue, it is necessary to suppose that there is an intercellular or transcellular passage of substances in the early stages of formation of the plate.

Nearly all recent investigators have accepted the view that the scale is mesodermal in origin. Creaser (1926) says that it is
'almost entirely of mesodermic origin'. Quite recently Fach (1936) has attempted to establish an epidermal origin for the bony layer of the scale. His paper is based on the European minnow (*Phoxinus*). He stresses the close association between the posterior region of the scale and the epidermis and endeavours to show that the scalelets of the outer surface of the scale originate as a secretion from certain unicellular glands, the 'cells of Leydig'. Fach considers that these cells secrete a calciferous substance ('Kalkmilch') which is deposited on the fibrillar plate of the scale. The mesodermal cells which are associated with the scale he regards as merely conductive elements for the 'kalkmilch', and he is at pains to demonstrate possible lines of communication between the Leydig cells and the anterior portion of the scale, which admittedly lies far removed from the epidermis.

While I have not examined the scales of *Phoxinus*, Fach's views may be criticized on general grounds. The distribution of the Leydig cells, both in the individual and through the various systematic groups of the fishes, in no way encourages the belief that they are concerned with scale formation. The conclusion that they secrete calcium, even if true, in no way improves their claims to be regarded as originators of the bony part of the scale, for this portion when first laid down is not calcified. Fach's suggestion that the fibrillar plate is formed first is certainly not correct in the species which I have examined. Fach emphasizes certain experiments of Wunder and Schimke (1935) who found that skin wounds in the leather carp (which normally develops no scales) sometimes induced the formation of scales in the epidermis. This he regards as a return to a primitive condition. There seems, however, to be no reason for supposing that the leather carp, which in its lack of scales is more highly specialized than its relatives, should show a more primitive method of scale formation under these circumstances. It may be pointed out that the experimenters themselves regard these 'aufliegende Schuppen' as derived from mesodermal materials which have been discharged into the epidermis in the wound area. Satisfactory sections of goldfish scales always show the entire scale, including the posterior margin, to be
completely enveloped in mesodermal elements. It may be stated confidently that the outer layer of the scale is in line with other bony structures in its tissue relationships, though this of course does not preclude the possibility of epidermal contributions of the general intercellular fluid.

THE RIDGES OF THE SCALE.

These are continuous and homogeneous with the general bony surface, and the chief reason for dealing with them separately is the interest which is attached to them because of their wide use as indicators of the age and growth of the individual. The ridges arise as elevations of the osteoid marginal area. They usually increase in height during the process of calcification. At their full development their free edges present a beaded appearance.

The ridges have usually been regarded as the direct secretion of certain cells—a conception which has been criticized on a previous page. Both Klaatsch and Ussow identified the superficial cells as the formative agents. Paget and Pevsner considered that each ridge was secreted by a row of cells lying close against it. This idea, as shown previously, is based on an erroneous conception of the form and arrangement of the cells lying between the ridges. Butcher (1936) in a brief report on scale development in Fundulus says that 'in the growth of the scale, the lower cellular end penetrates the connective tissue fibres which adhere in bundles on the upper surface and are incorporated into the homogeneous basic tip. ... As the scale grows, the bundles of fibres lose their attachment and ridges (circuli in surface view) mark their place of extension into the scale.' The present writer (1936) suggested the possibility that certain of the overlying cells might function as osteoclasts, the ridges being formed not only by a building up in front but also by a hollowing out behind. Since then I find that the same conclusion was reached many years ago by Nusbaum (1907) in a paper which has apparently been overlooked by English-speaking writers. Despite this added authority, a more careful investigation of the cellular elements of the scale has forced me to abandon the view that osteolytic activity of specific cells is of importance in the production of the scale sculpture. The figure which I gave
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(fig. 3, Pl. 1, l.c.) in illustration of this and other features of scale histology should be modified considerably. Although wandering cells, some of which might conceivably have osteolytic capacities, are sometimes seen above the scale, these are relatively scarce and do not seem to occur beneath the osteocytes which, as already shown, cover the formed bone with a close investment. Neither these osteocytes nor the cells of the superficial layer can be regarded as specific ridge-forming agents, either by osteogenic or osteolytic means, for these layers are equally well developed over large areas (e.g. in regenerate scales) where no ridges are formed and in other areas where ridges are short and irregular. Where ridges are present the superficial cells show no corresponding orientation. It is true that the osteocytes tend to show a very regular arrangement in relation to the ridges over a large part of the anterior field. In other parts of the scale, as indicated previously, there is no such regularity, the only apparent relationship being that the ridges always occur between the edges of the cells. In other words there is some tendency for some of the cells to become embedded in the bony tissue, as they do to a much greater extent in many other bones.

These facts point to the conclusion that there are no specific ridge-forming cells. The cells are concerned with the production of the bony layer in general and only contribute to the formation of ridges in so far as by their mere presence they limit the points at which these can be laid down. Ridge formation appears to depend on the presence of scale forming materials in the intercellular fluid in amounts greater than are utilized at the growing edge. These are then deposited at other points in the vicinity, between cells, increasing the thickness of the scale locally. (These statements of course are not made with the intention of throwing doubt on the well-established view that the osteoblasts in some manner, probably secretory, influence the deposition of material from the fluid.) That the margin of the scale has first claim on available material is indicated by a consideration of certain scales which differ from the ordinary ontogenetic lateral scales of the goldfish. As is well known, regenerate scales of many species (the goldfish is not a particularly good example,
for reasons which will be given later) usually show a large central area devoid of ridges. In other words, a young scale lying unconfined in a large scale pocket and receiving numerous reinforcements of osteoblasts at its margin can utilize all available supplies for increasing in area rather than in thickness. When marginal growth is slowed down, ridges are formed. Certain of the anterior ontogenetic scales of the goldfish abut against the supratemporal canal and are modified in shape accordingly. For example, the most anterior scale in the mid-dorsal line has a very small anterior field, most of the growth taking place caudad and laterad to the focus. On the anterior field, where marginal growth is restricted, ridges are crowded closely together, in other parts of the scale where there has been a greater increase in area the ridges are more widely spaced. Ordinary scales of *Brachydanio rerio* show the same condition in a more extreme form. In these, close-set ridges occur in the anterior field. The intervals between ridges widen out laterally, while the posterior field, which constitutes the main part of the scale, shows no ridges. In this region the whole of the available scale-forming material has been used for increasing the area without any superficial thickenings. The relationship between the spacing of the ridges and growth of the individual (and inferentially of the scale) has been recognized in a general way by various investigators (see Graham, 1929; Gray and Setna, 1931). That ridges increase in length after marginal growth has ceased has been shown by Lee (1920). In addition to these differences in the point of deposition, however, it is necessary to assume a differential distribution of scale-forming materials to various regions, since the total amount of formed scale, including ridges, often varies considerably in the respective fields.

There is therefore an element of truth in Setna’s suggestion that the ridges are formed by the restrictive action of the scale pocket, though his view that they represent an actual physical damming up of the margin is not literally correct. In regenerate scales, as pointed out elsewhere in the present paper, ridges are formed long before the growing margin is in contact with the wall of the scale pocket.

In general, the ridges, once fully formed, show no subsequent
changes except when they undergo absorption. At certain points in the posterior field of the goldfish scale, however, they acquire heavy pads or caps. These may be seen through the skin when they appear as local thickenings. Their appearance in section is shown in fig. 4, Pl. 30. They are mesodermal in position but in close contact with the basement membrane of the epidermis, which is deflected around them. They seem to be a fibrous product of the connective tissue. I am not clear as to their function.

**The Sulcari System of the Scale.**

In common with many other Teleosts, the scales of the goldfish show a varying number of sulci usually described as ‘radii’, though their direction is by no means always radial. They represent defections in the bony portion of the scale. In general they tend to increase slightly in number with age, at least up to a point. Their formation in the ontogenetic scale usually begins as a defection in the margin of the scale which is continued during subsequent growth. In some cases, at least, they also spread centripetally. Sulci are usually much more numerous in regenerate scales. In these, too, the central area (in the goldfish) is occupied by a network of connecting furrows. These regenerate scales will be considered separately. The grooves contain cellular elements of which only the nuclei are conspicuous in most preparations.

Taylor (1916) gave good reasons for considering that the ‘radii’ represent lines of flexibility permitting the scale to conform to the shape and movements of the body. He pointed out that radii are more numerous in scales covering the more flexible parts and that scales which overlie immovable parts, e.g. portions of the head, do not show these grooves. The goldfish does not bear scales on the head, but the condition described by Taylor can be seen to hold good in the guppy. Pevsner (1926) considers on the other hand that the radii are channels for the conduction of lymph. Certain previous writers had also adopted the view that they are canals for lymph or blood.

In the cases which I have examined, the sulci are not fitted for
the conduction of fluid since they are not enclosed, though their margins sometimes tend to approximate by undergoing a certain amount of growth. The contained cellular elements are not motile, but are interconnected and appear to be merely a part of the osteogenic cell layer (fig. 6, Pl. 31). Nor do they form a vessel as Pevsner apparently supposes, though they do become somewhat rolled up at their lateral edges. The numerous fine ‘vessels’ which she describes and figures as connecting with the radii in the scale of *Rutilus* are probably cracks in the bony layer produced by rough handling. At any rate similar appearances can be obtained in goldfish scales in this way.

The present writer had difficulty in accepting immediately Taylor’s view as to the function of the grooves, since these do not extend through the fibrillar plate. Taylor dismisses this point by saying that the fibrillar plate is sufficiently flexible in itself, a view which is also held by Peyer (1931). In point of fact, however, the fibrillar plate is very much more rigid than might be supposed from its high collagen content, as may be readily discovered by attempting to cut sections. This hardness is doubtless due to the presence of ichthylepidin. Moreover the bony layer in a well developed scale constitutes an insignificant proportion of its thickness. The difficulties appear to be reconciled by the discovery that the fibrillar plate, though continuous, is of different constitution below the canals. In sections stained with Mallory’s connective tissue stain a wedge-shaped area beneath the canal takes the anilin blue dye, whereas the plate in general takes the orange G (p. 551). If our previous inference is correct this indicates that ichthylepidin is not deposited in the scale immediately below the grooves. The pliable condition of the blue-stained tissue is sometimes shown by a bent position of the lamellae in sections (fig. 7, Pl. 31). In preparations of whole scales the different nature of the tissue underlying the canal is frequently indicated by a basic-staining area surrounding the furrow. This appearance is due to the increasing width of the soft tissue in the lower layers of the plate, a condition which of course gives a greater range of flexibility. The result of the juxtaposition of furrow and soft tissue is a simple form of ginglymoid arthrosis.
CHARACTERISTICS OF REGENERATE SCALES.

Regenerated scales have been recognized for many years by the 'deformation' of the central areas, though no satisfactory explanation of their peculiarities seems to have been given. The 'deformation' may take the form of a relatively large area devoid of ridges in contrast to the small focus of the ontogenetic scale. There has been some tendency to regard this area as representing the size of the lost scale (see Lea, 1919). Actually there is no sharp change in the sculpture of the scale such as would be demanded by this assumption. The first formed ridges are usually short, irregular and widely spaced and there is a gradual transition to the condition of fully formed, close-set ridges which is characteristic of the ontogenetic scale. By actually comparing ontogenetic scales with regenerates produced in the same scale pockets it is seen that ridge formation begins long before the regenerate approaches the original scale in size. On a previous page I have offered an explanation of the condition seen in regenerates on the assumption that ridge formation only takes place when the activity of the marginal cells becomes slowed down to a point at which they are unable to utilize all the scale-forming material available in the intercellular fluid.

In the goldfish, as previously stated, the central region of the bony layer is divided up into more or less numerous small areas by a network of grooves similar in structure to the sulci of the ontogenetic scale (figs. 11–15, 17, Pl. 32). Examination of early developmental stages shows that each of these areas is the product of a separate growth centre (papilla), which lays down its own bony plate and spreads out until its growth is inhibited by its neighbours. The network of sulci marks the boundaries between the individual growth centres. Such a scale is therefore (as regards the bony layer) a compound structure, consisting really of a number of small scales which become approximated to form the definitive organ. The process may be seen well in very young regenerates, where the incorporation of separate scalelets is quite evident (fig. 10, Pl. 31). After a time the production of new growth centres ceases and the peripheral members...
of the cluster continue to grow out towards the walls of the scale pocket. The central scalelets of course are unable to undergo much growth and their final shape conforms to their position in the group. Each one frequently forms a concentric ridge a short distance inside the margin—a further evidence of the relationship between ridge formation and restriction of marginal growth. The individual character of the scalelets is sometimes partly lost through irregular fusion between adjacent scalelets. The process of calcification begins in or near the centre of each ‘island’ and spreads to the margin. The marginal islets during their growth frequently develop sulci of the ordinary ontogenetic type. The two types of sulcus can usually be distinguished by their position and their relation to the focus and ridges of the scale. It may be noted that the ontogenetic scales of some species of fish (e.g. *Osteoglossum bicirrhosum*, figured by Peyer) show a pattern which closely resembles the central area of regenerate goldfish scales.

The total number of growth centres in a regenerate scale varies greatly in individual cases, but depends in a general way on the amount of space available. New scales developing inside large scale pockets generally show very numerous centres (up to 80 or 90 in the fish used). On the other hand the regenerating scales of small goldfish show very few. Guppy scales apparently never have more than one centre, except in rare cases where two scales fuse after a period of development. Similarly, in large goldfish, scales which have regenerated under restricting conditions of the surrounding tissue, as in wound areas, frequently show only one centre or a few which have become contiguous after considerable independent growth (fig. 8, Pl. 31).

When the fibrillary plate is formed, it becomes continuous beneath the whole group of bony scalelets—a further confirmation of the view that it is not laid down by the cells of the papilla but is a product of the floor of the scale pocket. It remains flexible under the interpapillary grooves as it does beneath the ontogenetic sulci. Apparently the failure of the fibrillary plate to become hardened is bound up in some way with the absence of the overlying bone and is not due to mechanical factors. If mechanical factors were responsible for deter-
mining the positions of the sutures there should be a detailed resemblance between the patterns of successive scales regenerated in the same pocket. Actually the pattern varies widely, the number and individual shape of the scalelets showing no such constancy.

EXPERIMENTS ON SCALE REGENERATION.

For this purpose approximately forty-five goldfish and fifty guppies were used. The process of regeneration was followed (a) after removal of scales without destruction of scale pockets, (b) after removal of scales and scale pockets.

1. Removal of Scales without Destruction of Scale Pockets.—The goldfish used for these experiments were mostly of large size, 6·9 to 9·0 cm., standard length. Scales were plucked individually either in isolation, in groups, or in rows or diagonals. This procedure involves the removal of (a) the epidermis covering the exposed portion of the scale, (b) the connective tissue underlying the epidermis and its contained blood-vessels, chromatophores, &c., (c) the osteogenic and fibrogenic cells which invest the scale, (d) iridocytes lying beneath these. The greater part of the scale pocket is left intact.

As is well known, scales removed in this manner are replaced in a comparatively short time under favourable conditions, the original arrangement of the scales being faithfully reproduced unless the pockets are badly damaged. Each pocket obviously functions as a unit for the production of one definitive scale, though as shown on previous pages such a scale may arise from numerous growth centres. The histological development of the individual scale has been outlined already.

2. Removal of Scales and Scale Pockets.—In the case of the goldfish used the procedure was to remove a patch of twelve scales and then scrape away the scale pockets with a sharp scalpel. The scraping was continued until all or most of the corium of the selected area had been removed. Guppies were treated similarly but no attempt was made to remove a specific number of scales. A relatively large area on one side of the body was excoriated. All the fish were anaesthetized with a weak solution of chloretone before the operation.
The re-scaling of an area from which the scale pockets have been removed takes place very slowly in comparison with the time required for the development of scales within existing pockets. In the goldfish the complete covering of the areas frequently took three months at a temperature of 20° C. In guppies the relatively larger but actually much smaller areas were usually covered with scales in from 25 to 50 days. At lower temperatures regeneration was much slower.

The resulting scale arrangement always differed noticeably, and sometimes very greatly, from the original condition. In some cases the original number of scales was reproduced, but the relative sizes of certain scales were changed. In other cases the ultimate number was either fewer or greater than the original. Figs. 18 to 21, Pl. 33, show representative types of regeneration patterns.

**Discussion.**

Sauter (1934) describes experiments on the European minnow (*Phoxinus*), in which portions of the body wall were removed down to the peritoneum. He observes that the regenerated scales almost never show the regular arrangement of the ontogenetic series. Sometimes giant scales are produced, sometimes small scales which may be more numerous than the original elements. The length of time involved in the process of regeneration varies greatly but is always very considerable. He considers that regeneration results from the activity of a class of cells occurring throughout the looser tissue of the dermis. These regeneration cells are derived from fibrocytes, histiocytes, macrophages, and lymphocytes and represent a provisional end-product. They are almost totipotent, and when stimulated are responsible for the production of corium, scales, bones, and muscle.

Experiments similar to those described in the present paper were made by Nardi (1935) on *Phoxinus* and other species. He stresses the fact that only the presence of the scale pockets can bring about the restoration of the original scale pattern. He considers that there are many important developmental differences between ontogenetic and regenerate scales, agree-
ment, indeed, being only found when the comparison is limited to the individual scale. In support of this statement he mentions the following points:

1. In ontogeny the scale pocket is formed after the young scale is laid down and consequently exerts no influence on its position. In regeneration, on the other hand, the original arrangement is only maintained when the scale pockets are present.

2. In ontogeny scale production begins at the lateral line and spreads therefrom. In regeneration following removal of scale pockets, scale development always proceeds from the margin of the wound and spreads inward, whether or not the lateral line is included in the wound area.

3. Ontogenetic scales arise at a time when the cutis is differentiated into a compact proximal and a looser distal part. Regenerate scales frequently arise when the cutis is in an entirely different condition from the above.

4. In regeneration ‘supplementary’ scale papillae may appear. On the other hand scales may fail to develop in certain areas, resulting in gaps in the final pattern.

Nardi finds no satisfactory explanation for these differences. In particular he is concerned by the inconstancy of the relationship between the formation of papillae and the condition of the corium and by the capricious manner in which, under similar conditions, an over-production of scales, an under-production of scales, or a nearly normal pattern may result.

In the fish used by the present writer three processes contribute to the re-scaling of the excoriated areas (figs. 18–21, Pl. 33).

1. Growth of scales which were left in position at the margin of the wound area. These scales tend to enlarge on the side towards the wound. In some cases a definite mark on the scale indicates the amount of the new growth (fig. 16, Pl. 32).

2. Owing to the contiguity and overlapping of the scale pockets, portions of some of these are always left at the margin of the excoriated area. Scales are regenerated in relation with these partly destroyed pockets, and tend to become much larger than the ordinary scales, growth taking place mainly
at the side which is not restricted by neighbouring scales and pockets (fig. 17, PI. 32).

3. New scales may arise within the wound area.

In the cases examined, the first of these factors contributes only slightly to the final result, growth of this kind being exceedingly slow. The second factor is always of importance, and is sometimes responsible for nearly all of the ultimate pattern (figs. 19, 20, PI. 33). The development of new scales in positions from which scale pockets have been entirely removed is a usual occurrence, and is often a major factor in the healing of such wounds in the guppy (figs. 18, 21, PI. 33).

The difficulties encountered by Nardi all appear to result from the commonly accepted view that scale papillae arise in situ through differentiation of the underlying corium. By accepting the present writer's contention that the osteogenic cells are of separate origin from the general corium, the difficulties disappear. On this view, the cells which are capable of giving rise to papillae are not generally distributed throughout the corium, but are confined to the scale pockets. When the scale pockets are removed from an area, such cells could only colonize the stripped region by migrating in from the wound margin. Moreover, they could only do this by emerging from lacerated scale pockets or by breaking through the walls of pockets which remain intact. The ultimate scale pattern probably depends mainly on the more or less accidental relation between the wound margin and the pockets and on the mechanical limitations imposed by regenerating corium and epidermis. The osteogenic cells of a lacerated pocket may confine themselves to the building up of a scale which begins within the remaining portion of the pocket but which grows extensively on the side that is not confined. This is the way in which the enlarged scales shown in fig. 17, PI. 32, figs. 19 and 20, PI. 33, are formed. On the other hand, certain of the released osteogenic cells may migrate further into the wound area and set up new papillae, whose number and position are probably determined by the resistance encountered from the surrounding tissues. These papillae are frequently crowded by their fellows or by the enlarged scales previously mentioned, in which case they pro-
duce only small scales. They may be very numerous (figs. 18, 21, Pl. 33).

The chief point that emerges from these considerations is the specificity of the osteogenic cells and their independence from the rest of the corium. The latter may be equally well regenerated over a large area, but in no case is there evidence that papillae arise de novo from the ordinary cells of the corium. Formation of papillae always takes place in the neighbourhood of pre-existing scales or papillae. It is true that the fibrillary plate of each scale is a product of the connective tissue flooring the pocket, but this structure apparently never arises in the absence of the osteogenic papilla.

**Summary.**

The Teleost scale, as typified in the goldfish, is covered intimately by two layers of cells on the external surface.

The internal surface of the young scale is invested with a cell layer which is continuous with the deeper external layer. These cells may be regarded as osteoblasts and osteocytes and are concerned with the formation of the outer bony portion of the scale.

In the development of regenerate scales, osteoblasts pass from the periphery of the scale pocket to the growing edge of the scale. The latter consists at first of osteoid tissue, which later becomes calcified.

The fibrillary plate is at first collagenous but apparently becomes infiltrated with ichthylepidin after being laid down.

The scale is wholly mesodermal in its tissue relationships.

The ridges of the scale are formed between cell rows of the deeper external cell layer. They are not the product of specific cells. Ridge formation probably depends on the presence of bone-forming materials in the intercellular fluid in amounts greater than can be utilized at the growing margin. These are deposited between cells in the vicinity.

The sulci of ontogenetic scales represent lines of flexibility. This condition is attained not only by the absence of the bony layer but by a special condition of the underlying fibrillary plate, which apparently does not become impregnated with ichthylepidin beneath the grooves.
Regenerate scales may be monocentric, in which case a relatively large central area is devoid of ridges. Absence of ridges is interpreted as due to the utilization of all available scale-forming material at the growing margin. The size of the central area does not correspond to the size of the original scale. In the goldfish the regenerate scale is commonly polycentric, the bony portion developing from more or less numerous growth centres whose ultimate margins are indicated by grooves similar to the sulci of ontogenetic scales. The number of growth centres depends in a general way on the space available. The fibrillar plate is continuous beneath the bony islets but remains flexible beneath the grooves.

Three processes contribute to the re-scaling of areas from which scales and pockets have been removed: (1) enlargement of ontogenetic scales at the margin of the wound area, (2) regeneration of scales in partially destroyed pockets at the margin of the wound area, (3) development of new scales within the wound area.

New and regenerate scales always develop in the vicinity of pre-existing scales or papillae, under the influence of specific osteogenic cells which do not arise de novo from the corium.

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TELEOST SCALE


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EXPLANATION OF PLATES 30-33.

PLATE 30.

Fig. 1.—Superficial cells of external side of goldfish scale. Alizarin red S.

Fig. 2.—Intimate cells of external side of goldfish scale. Silver nitrate and methylene blue.

Fig. 3.—Connective tissue associated with fibrillar plate of advanced goldfish scale. Nuclei and argyrophil fibres shown. Silver nitrate, alum haematoxylin, eosin.

Fig. 4.—Section through small portion of large goldfish scale. b., bony layer of scale; c.f., connective tissue associated with fibrillar plate;
ep., epidermis; f.p., fibrillar plate; p., fibrillated pad covering a ridge. Alum haematoxylin, eosin.

**Plate 31.**

Fig. 5.—Intimate cells of internal side of young regenerate scale of goldfish. Alum haematoxylin, eosin.

Fig. 6.—Section through portion of very young regenerate scale of goldfish, showing bony layer and cells of external and internal sides. Alum haematoxylin, eosin.

Fig. 7. Section through portion of adult scale of goldfish, showing radial furrow. Mallory's connective tissue stain.

Fig. 8.—Regenerate scale of goldfish developed in wound area.

Fig. 9.—Portion of margin of young regenerate scale of goldfish, showing osteocytes investing osteoid tissue and gelatinous area containing scattered cells. Alum haematoxylin, eosin.

Fig. 10.—Portion of very young regenerate scale of goldfish, showing incorporation of scalelets. Regions of active growth indicated by close-set basophilic cells.

Fig. 10a.—Section through marginal area of a growing scale, showing a ridge (r.) and cells investing the edge.

**Plate 32.**

Figs. 11—15.—Stages in calcification of regenerate scales from mid-lateral region of goldfish and effect of varying temperatures. Calcified areas black. In figs. 12—15 sulci in non-calcified areas not shown. Silver nitrate.

Fig. 11.—Six days. Temperature 20° C.

Fig. 12.—Forty-two days. Temperature 8.5° C.

Fig. 13.—Twenty-one days. Temperature 18° C.

Fig. 14.—Seven days. Temperature 32° C.

Fig. 15.—Ten days. Temperature 20° C.

Fig. 16.—Ontogenic scale of goldfish from margin of wound area, showing increased growth on side towards wound.

Fig. 17.—Regenerate scale of goldfish from lacerated scale pocket at margin of wound area.

**Plate 33.**

Fig. 18.—Scale pattern of guppy no. 6, 28 days after removal of scale pockets from large area.

Fig. 19.—Scale pattern of goldfish, 99 days after removal of scale pockets from stippled area.

Fig. 20.—Scale pattern of guppy no. 35, 69 days after removal of scale pockets from large area.

Fig. 21.—Scale pattern of guppy no. 34, 63 days after removal of scale pockets from large area.

F. Neave, del.