the development of colonies of Hydroids *. Can it be a matter for surprise that the latter, which are doubtless of very small size, have never been seen and may still long remain unnoticed, if we reflect that the statoblasts of which we have been speaking are quite a recent discovery, and, above all, that, in spite of continual watching, it has taken nearly *five years* to find in London itself, with all the resources of a perfectly equipped laboratory, a hydroid phase of *Limnocodium*? †

IX.—*On the Development of the Fins of Teleosts.*

By Ross Granville Harrison ‡.

Excepting the Elasmobranch Fishes, we have no complete knowledge of the development of the extremities of any group of vertebrates. The skeleton alone has received due attention. The muscular system of the limbs of the higher vertebrates has been supposed by recent writers who have touched upon the subject to take its origin from masses of cells derived from the myotomes. These myotomic cells are in a general way to be regarded as homologous with the cells of the muscle-buds ("Muskelknospen"), out of which the

* The oyster-beds at certain points of the coast are justly regarded by zoologists as exceptionally rich localities. I have mentioned a very typical example of this lying off Dunkirk. Sponges, Hydroids, various Annelids, Bryozoa, and Cirrhipedes, to speak of fixed animals alone, multiply upon the dead or living shells with singular vigour, and this in spite of the violence of the gyratory currents, which in these parts may attain a speed of two metres a second (J. de Guerne, "La rade de Dunkerque," Revue Scientifique, March 11, 1885).

† A. G. Bourne, "On the Occurrence of a Hydroid Phase of *Limnocodium Sowerbyi*, Allman and Ray Lankester," Proc. Roy. Soc. Lond. xxxviii. p. 9. It will be remarked that the basin in Regent's Park in which the Medusæ have appeared at intervals, and where the hydroid phase of *Limnocodium* has at last been discovered, has been emptied and left dry for a somewhat long period on several occasions. This appears to indicate on the part of this freshwater type a singular power of resistance to the most abrupt changes in the condition of the medium. *Vide* the plate, p. 12 *loc. cit.*

While correcting the proofs of the present note I have received no. 1258 of 'Nature' (Dec. 7, 1893), containing an article by Prof. Ray Lankester entitled "Reappearance of the Freshwater Medusa (*Limnocodium Sowerbyi*)." This organism, which had not been observed again in London since July 1890, has unexpectedly come to light at Sheffield in a tank containing aquatic plants sent from Regent's Park.

‡ From the 'Johns Hopkins University Circulars,' No. 111, May 1894, pp. 59–61: being a preliminary communication.

definitive muscles of the elasmobranch fins are known to develop. The following observations made upon the salmon (*Salmo salar*) render a modification of this view necessary.

The Unpaired Fins.—The unpaired fins arise as proliferations of the mesenchyme cells, which, in the form of a loose meshwork, fill the median fin-folds. The caudal fin is the first to appear, and is followed by the dorsal, the anal, and the adipose, in the order named.

Shortly after the dorsal thickening has appeared muscle-buds appear at the anterior dorsal angles of the myotomes of that region, and grow rapidly into the fin-rudiment, as has been described by Dohrn ("Studien IX.," 'Mittheilungen aus der Zool. Stat. zu Neapel,' Bd. vi.). These processes converge considerably towards one another, so that, while the middle ones project at right angles to the long axis of the body of the fish, those at each end of the fin cut the axis at an angle of about forty-five degrees. Cross sections show that the buds are solid; a few cells are enclosed by an epithelium of similar cells. The nuclei closely resemble those of the mesenchyme, and the cell boundaries are indistinct. The buds are continuous with the cells of the lateral layer of the myotomes (cutis-plate). Similar buds grow out from the anterior ventral angle of the myotomes in the region of the anal fin. The tail-fin also receives outgrowths from several of the terminal myotomes; the adipose fin never contains muscle-buds or muscle-tissue of any kind. As the buds grow further into the fin-rudiment their outer ends become enlarged and somewhat flattened against the epidermis, in which a considerable bulging is caused. The stalk now disintegrates, and its component cells can no longer be distinguished from the mesenchyme. In the meantime the nuclei which lie in the median half of each bud accumulate considerable cytoplasm as a first step towards differentiation into muscle-cells. These masses of embryonic muscle-cells now grow centripetally; they ultimately become the erector muscles of the fin-rays. The lateral half of the bud now loses its identity as a cell-mass, having become undistinguishable from mesenchyme; but very soon the cells which lie opposite the peripheral end of each bud accumulate more cytoplasm, and these masses also grow towards the body, remaining close to the epidermis. They ultimately become the superficial muscles of the rays, which in the adult take origin from the skin. By this time the mesenchyme has developed to such an extent that the muscle-masses are not at all clearly defined, so that it is impossible to draw a sharp dividing line between

nuclei which will ultimately belong to muscle and those of the connective tissue.

By this time cartilaginous rods, alternating with the muscle-pairs, have appeared in the middle plane of the fin. These become the interspinal bones which support the rays of the dorsal fin; in the anal fin they are the interhæmals. The chondrification takes place centripetally. I shall call them ray-supports.

Horizontal sections of this stage show clearly the serial arrangement of the various structures of the fin. Opposite the cartilaginous rods the ectoderm is constricted to a marked degree. At these constrictions mesenchyme cells have aggregated close to the ectoderm, forming loose strands of cells, one on each side of each ray-support. This is the beginning of the definitive depressor muscles of the fin-rays.

The dermal rays now begin to develop, in lines which are distally continuous with each erector muscle rudiment, the cells of which are not separated from those of the corresponding rays by any sharp dividing line. Considerably later a small nodule of cartilage is formed at the tip of each cartilaginous ray-support, which has in the meantime become considerably bent with its convexity forward. Each pair of dermal rays grasps with its basal end the corresponding cartilaginous ball, and a strong fibrous tissue binds them together. Muscles now become inserted into this mass, in such a manner that each ray receives one pair of each of the three muscles belonging to each segment of the fin. Anterior to the pivot on which the cartilaginous ball rests the erector is attached; posterior to the pivot the depressor and the muscle which takes origin from the skin. The depressor and the erector arise from the ray-supports. The fin has now practically reached its adult condition, except that the cartilaginous skeleton has not yet ossified.

The above account holds good only for those segments which do not lie at the ends of the fin. In the first two or three segments the course of development is considerably modified, although the same definitive arrangement is reached.

The number of myotomes which produce buds is variable, but, as a rule, ten or eleven reach the dorsal fin and eight the anal. Both anterior and posterior to these buds may be formed, but they do not reach more than rudimentary development. The number of these buds is very variable. When the cartilage has just begun to appear, and the muscle-masses are on their way to segregation from the surrounding mesenchyme, these rudimentary buds have disappeared, presumably having disintegrated into ordinary mesenchyme tissue. Both

anterior and posterior to the regularly formed muscles are paired masses of closely packed mesenchyme cells, which form a distinct layer under the epidermis. These masses are undoubtedly derived both from the original mesenchyme of the fin and from the breaking down of the rudimentary buds. The posterior mass of each side differentiates into two muscles, one corresponding to the erectors, the other to the skin-muscles. These become attached to the posterior ray of the fin. A depressor muscle is wanting in the case of this ray.

Anterior to the first of the regularly formed muscles is a cartilaginous ray-support, and anterior to this are the masses or laminæ of undifferentiated cells described above. In the middle plane, anterior to the first cartilage, at the regular distance existing between the other ray-supports, cells aggregate, and, later, chondrify, forming another ray-support. That portion of the undifferentiated cell-mass lying between this new cartilage and the one next succeeding it segregates from the rest, and from it muscles corresponding to those of the region of the muscle-buds are developed. This process continues until the usual number of rays and muscles found in the adult fin is reached. In *Salmo salar* this is fourteen in the dorsal and ten in the anal. The anterior ray-support and its muscles are not so fully developed as the others.

Each fin is innervated by a series of spinal nerves. The nerves of each fin are connected by a longitudinal commissure, which is a branch of the ramus lateralis vagi. I am unable at present to trace the development of this interesting plexus.

In late embryonic and in the adult stages the metamerism of the fin corresponds to that of the body of the fish. In earlier stages the fin is more concentrated, as exhibited by the strong convergence of the muscle-buds which enter into it. This varies, however, greatly in different species, and is a matter to which but little importance is to be attached.

The Ventral Fin.—The ventral fin appears considerably later than the median fins. The first traces of it to be seen are slight aggregations of cells in the body-wall just below the ventral edge of the myotomes which lie in the region of the dorsal fin. About the same time the epidermis covering these parts becomes considerably thickened through multiplication of its cells. Before the aggregations of mesenchyme cells become very conspicuous muscle-buds grow out from the anterior ventral angle of each myotome in this region: about six enter the fin-rudiment; those at each end of it projecting very obliquely to the axis of the body, converge towards the middle of the fin.

Dohrn ("Studien IX.," p. 401) draws a sharp distinction between the mode of origin of the musculature of the anal fin and that of the ventral. In this he is followed by Kaestner (Arch. f. Anat. u. Physiol., Anat. Abt. 1892, p. 200). Dohrn remarks that while the anal fin derives its muscles from muscle-buds the musculature of the ventral originates "ohne Vermittelung von Muskelknospen, direct durch Einwachsen der Musculatur vom Urwirbel aus, wie sich leicht an Lachs- und Forellenembryonen nachweisen lässt." I am unable to confirm this statement, as my preparations, both surface views and sections, show distinctly that the muscle-buds which grow into the ventral fins are similar to those which enter the anal, except that the latter are considerably larger.

At this stage nerve-fibres from the spinal nerves of corresponding segments which give off muscle-buds may be detected in the fin. This is a very much earlier stage than the earliest at which nerves could be seen in the median fins.

The mesenchyme proliferates rapidly, while the ectoderm is raised into a fold which projects from the ventro-lateral surface of the body, parallel to its long axis. The mesenchyme forms a compact mass lying under the epidermis; the region next to the somatopleure is filled with less densely packed tissue. The muscle-buds project far into the fin, but, unlike those in the paired fins, are separated from the epidermis by a layer of mesenchyme. Very soon the buds disintegrate.

The region in which the first steps towards differentiation of muscle first appear is the space previously occupied by the muscle-buds. From the very first there are no traces of metamerism in this muscle, although it is safe to assume that the cells from the buds take part in its formation. About the same time at a corresponding position on the opposite or inner side of the fin a similar differentiation takes place. It is not so likely that cells from the muscle-buds take part in this. Between these two muscle-layers the cartilaginous skeleton has by this time appeared. The development of the skeleton has received such thorough treatment at the hands of Wiedersheim and others as to render further mention of it here unnecessary.

The fin rotates so that its line of attachment to the body makes an angle of about forty-five degrees with the long axis of the body. The inner muscle, of which the beginning was described above, becomes the protractor or abductor profundus, and the outer the retractor or adductor profundus. The superficial muscles develop before the twisting of the fin takes place. They are formed through differentiation of

the mesenchyme cells which lie between the deeper muscles and the epidermis. It is extremely improbable that cells from the muscle-buds take any part in their make up. The muscles and skeleton grow forward in the body-wall between the ventral ends of the myotomes, so that eventually only a very small portion of each of the muscles lies in the free extremity. In embryonic stages these muscle-masses are continued distally, without sharp dividing line, into the mother-cells of the dermal rays.

The Pectoral Fin.—The pectoral fin diverges from the primitive type more than the other fins, both in its definitive structure and in its course of development. It develops considerably earlier than the others, and lack of histological differentiation of the tissues at that time renders its study more difficult.

The first trace of this fin is to be seen in a thickening of the somatopleure; the thickening of the ectoderm and its fold arise later. This is in accordance with Boyer's observations on *Fundulus* (Bull. Mus. Comp. Zoöl. vol. xxiii. no. 2). The thickened portion of the somatopleure is not confined to the "pectoral plate," but extends to the portions of the splanchnopleure, on the same level, and through the nephrostome to the Wolffian duct. This thickened portion of the peritoneum is due to the cuboidal or columnar character of the epithelium composing it. Anteriorly, laterally, and posteriorly to it the cells flatten out. There is, just anterior to it, a portion of the body-wall in which are numerous mesenchyme cells derived from the head mesoderm. Ziegler (Arch. f. mikr. Anat. Bd. xxx.) has regarded this as the rudiment of the fin. Study of the later stages shows, however, that this region lies completely anterior to that in which the fin develops.

At a somewhat later stage the cells belonging to the pectoral plate become much more distinctly columnar than the others, and, multiplying rapidly, soon become several layers thick, and are much more densely packed than those lying anterior to them. A thickening of the epidermis now takes place, which, unlike that of the ventral fin, consists in an increase in size of the individual cells, and not in a multiplication of the same. At the crest of the prominence which the proliferation of the mesoderm has caused, the ectoderm is thrown into a fold parallel to the axis of the body and extending through three somites. In cross section the structure is triangular; the somatopleure, which extends out over the yolk-sac, is its base, and is nearly horizontal. In profile the crest is semicircular. Through rapid proliferation of the

mesoderm-cells the prominence becomes much more pronounced, and soon the height greatly exceeds the breadth. The cells which lie near the base are not so densely packed as those lining the ectodermal walls.

In the meantime the myotomes have sent out processes from their ventral growing edges; but instead of entering into the fin-rudiment, as given by Kaestner (Arch. f. Anat. u. Phys., Anat. Abth. Jahrg. 1892, p. 200) for *Salmo* and Boyer for *Fundulus*, they become greatly elongated and, growing forward, give rise to the coraco-hyoid muscle, as has been described by van Wijhe (Verh. d. Konink. Akad. van Wetenschappen, Amsterdam, Deel 22) for *Pristiurus* and by van Bemmelen (Anat. Anzeiger, Bd. iv.) for *Lacerta*. The first myotome is at this stage quite rudimentary; the second and third lie entirely anterior to the fin, which is on a level with the fourth, fifth, and sixth. The anterior end of the Wolffian duct is opposite the middle or posterior portion of the fifth segment. The first myotome has no ventral process; the second, third, and fourth send out long strands consisting entirely of cells from the cutis plate. These grow ventrally in the somatopleuric wall of the pericardial cavity. After a certain time the first one atrophies, the second and third bend forwards and are followed by the fourth (from the fifth myotome), which grows straight forwards and slightly ventrally. The foremost one becomes attached to the base of the hyoid arch by means of a tendon, the stalks connecting the buds with the myotomes atrophy, and the three buds unite to form a muscle which takes origin from the membranous shoulder-girdle, and is attached to the urohyal. This muscle is still divided into three segments, at least in young fish, in which the yolk-sac is entirely absorbed. The last of these buds extends for one whole segment under the pectoral fin, with the cells of which it is in close contact. Sections through this, a little anterior to the point of origin of the outgrowth, give such a figure as has been drawn by Kaestner (fig. 32). This outgrowth is less well-defined than the others, and in cross section it can often scarcely be distinguished from the mesoderm of the fin. It is not at all unlikely that individual cells may detach themselves from it and remain in the fin; but it is certain that *as a mass it takes no part in the formation of the fin-muscles.*

The sixth myotome has a ventral process, which, however, does not grow forward as the others do. It ultimately pinches itself off from the whole length of the myotome, and becomes an independent longitudinal strand of muscle-fibres which runs dorsal to the attachment to the fin, but which is

afterwards probably incorporated into the lateral muscle-masses. The seventh and succeeding myotomes grow ventrally and are concerned in forming the ventral muscles of the fish.

The changes that the fin has undergone are now considerable. The attachment has constricted considerably, at least in comparison with the free portion, which has become a fan-like expansion. With the absorption of the yolk the fin is brought to the ventro-lateral surface of the body, and, rotating on its axis, so that the line of attachment instead of being parallel to the axis of the body now makes an angle of about forty-five degrees with it, the anterior extremity is thus brought into a corresponding position with that described in the posterior. The internal changes that have taken place during this time are the differentiation of the central core of cells into cartilage, and of the proximal portions of the superficial mesenchyme layer into muscle. *It may be regarded as certain that the cells which give rise to these muscles originate from the somatopleuric thickening, and, as is the case with most of the muscles of the ventral fin, are in no way connected with the myotomes.* At first there are but two muscle-masses, a primitive abductor or protractor lying on the outer side of the cartilaginous skeleton and an adductor or retractor on the inner side. A superficial muscle is developed later from a mass of cells lying just within the fin between the deeper abductor and the inner epidermic wall. The superficial protractor or abductor does not appear till much later, and probably arises through delamination from the primitive muscle, though I am not perfectly convinced of this.

The nerves of this fin are distinguished very early in its development, just as in the ventral, *i. e.* before any differentiation of the tissue has taken place. They arise from the first four spinal roots. The first root corresponds to the second myotome, and its ramus ventralis unites soon with the second nerve to form the hypoglossal. This gives off a branch to the fin-plexus and one to the coraco-hyoid muscle. The arrangement is completed very early in the life-history of the individual, and seems to be quite typical for the Teleosts.

Recapitulation.

The mesodermic structures of the median fins are derived from mesenchyme cells derived from the sklerotome and from muscle-buds, which are outgrowths of either the dorsal or the ventral edge of the myotomes. To a certain extent these fins retain their primitive metamerism, in that each muscle-

bud may be traced directly into a certain muscle of each segment of the fin. Other muscles are derived from cells which are indistinguishable from mesenchyme cells, and which are in all probability to some extent derived from the same. The segmentation of the extreme anterior portion of the fins is secondary, although in the adult no difference can be seen between the two portions.

The ventral fins show in the early stages of development traces of a similar metamerism. The buds in this case soon disintegrate, and in the space occupied by them a single muscle-mass develops—the adductor or retractor profundus. The other three muscles of this fin are developed from cells which have arisen from the somatopleure, and perhaps also from the sklerotome. This condition in the Teleosts seems to be a step between the Elasmobranchs and the Amphibia. In *Triton* a few isolated cells break off from the ventral edge of several myotomes and mingle with the cells of the posterior extremity, which are, however, mostly derived from the somatopleure.

According to Paterson (Quart. Journ. Micr. Sci. vol. xxviii.) the myotomes in the chick take no part in the formation of the muscles of the limbs. Kaestner has cast doubt upon this statement, but it is doubtful whether his grounds for so doing are sufficient.

The pectoral fin is derived entirely from somatopleuric cells. The muscle-buds of this region are greatly modified and take part in the formation of the coraco-hyoid muscle.

I wish to postpone the full discussion of the meaning of this diversity in the origin of the muscles until some observations on other forms are completed. It is in all probability to be referred to delay in the differentiation of the component parts of the fin until they take up their position within it. In other words, instead of so much connective and skeletal tissue and so much muscle being contributed to the fin, it receives cells which still retain the potentiality to become any of these, and their position with regard to surrounding cells rather than their origin determines their ultimate fate.

This work, undertaken at the suggestion of Prof. M. Nussbaum, was carried on partly in the Anatomical Institute in Bonn and partly in the Biological Laboratory of the Johns Hopkins University.