

**DEVELOPMENT OF THE SILVER GAR (BELONE LONGIROSTRIS),
WITH OBSERVATIONS ON THE GENESIS OF THE BLOOD IN EM-
BRYO FISHES, AND A COMPARISON OF FISH OVA WITH THOSE OF
OTHER VERTEBRATES.**

By **JOHN A. RYDER.**

The development of the fish we are about to describe is in itself of little practical importance, but because it serves to illustrate in a very remarkable way the manner in which the blood is originated in an embryo teleostean, it may serve to teach us a useful lesson as to the origin of the elements of the blood in other forms, such as the salmon, whitefish, &c., a complete knowledge of which it is desirable that we should possess, in order that we may more fully comprehend the evolution of their structures in the egg. Another matter of peculiar interest is the presence of numerous filaments which are distributed over the whole surface of the very thick egg-membrane, and which are at first tightly coiled around the latter, but which afterwards uncoil, when they twist together into strands, and also become entangled with the filaments arising from other eggs so as to bind large numbers together into large clusters. Not only are large masses of one brood thus joined together, but it is also found that if the recently spawned ova come into contact with slender objects in the sea they immediately wind their filaments about the latter, and are by this means suspended very securely, so that with the ebb and flow of the tides they are constantly bathed by different water. It appears that in this way their incubation would be favored, for after their fixation by the filaments or threads, the ebb and flux of the tide sweeping through and by the clusters of eggs would in effect very closely resemble the conditions to which fish ova are exposed in the process of artificial incubation. It would seem that in this case nature had anticipated the protective designs of man in developing a means by which the survival of a species might be insured. How the filaments have been evolved it appears impossible to the writer to explain; he can think of no rational hypothesis of evolution by which it would be possible to account for their development. While the ova of a comparatively useless fish are thus provided with a means of protection and suspension, not only to favor their incubation, but also to keep them from being overwhelmed with the ooze and mud of the sea-bottom, there are other species of considerable value, the eggs of which are probably provided with similar thread-like appendages. I allude to the so-called "jumping-mullet," *Mugil albula*, a fish much esteemed for the table in some places along our eastern coast. It therefore becomes a matter of some importance to know how many of our native species have their ova provided with filaments for the purpose of attaching them to each other and to foreign objects.

Professor Haeckel appears to have been the first to describe fish ova

with filaments, but he thought the fibers were inside the egg-membrane instead of outside of it, as may be learned by reference to his paper on the subject in *Müller's Archiv* for 1855. Professor Kölliker, in the *Verhandl. d. physik. u. med. Gesellschaft in Würzburg*, eighth volume, for 1858, rectifies Haeckel's observations, and shows that the fibers or filaments are external, but it does not appear that he ever understood their real function, viz, to provide a means of fixation and support while the ova were undergoing incubation. Haeckel unfortunately observed only unripe ova, contrary to what he supposed, as is clearly shown by his figures, but he found the fibers present in the eggs of *Belone*, *Scomberesox*, *Hemirhamphus*, and *Exocoetus*. The writer has observed them in the unripe eggs of *Hemirhamphus unifasciatus*, but has not had any opportunity to observe them in the eggs of the flying-fish, *Exocoetus*. In all of these the fibers are distributed and attached at intervals over the whole surface of the egg, but in the *Atherinida*, as shown by my observations on the eggs of *Chirostoma notata* in Mobjack Bay, Virginia, in 1880, there are only four filaments, which are attached to the vitelline membrane at one pole of the egg and quite close together. These are at first coiled around the vitelline membrane in one plane quite closely, as in *Belone*, but they unwind when the eggs are discharged into the water, when the threads of adjacent eggs become entangled so as to form clusters of considerable size. In this genus the filaments are nearly half an inch long, without a swollen base, attached to an egg one-sixteenth of an inch in diameter. We saw that all of the genera of *Scomberesocidae* were found to have their ova provided with filaments. The genus *Arrhamphus* is the only one the eggs of which have not been observed. It is very probable that all of the genera of *Atherinida* have ova with filamentous processes; at any rate it is desirable that they should be looked for in *Atherina*, *Atherinichthys*, *Tetragonurus*, and *Labidesthes*. This supposition also raises the question whether the *Mugilida* do not have eggs of the same kind. A confirmation of this hypothesis would be desirable in that the large number of species in the family, their wide distribution and considerable size, conspire to render them of value as food-fishes over a large area of the earth's surface. It is quite as important for us to know what natural means exist to favor the survival of the germs of species as it is for us to know what artificial means to provide for their increase and protection. In fact the latter kind of knowledge ought to be based upon and supplement the former, since it is by a combination of natural and artificial protective agencies that much more can be done to increase the number of food-fishes than by the latter alone. It may indeed happen that we will yet learn that certain species need no protection save that which would prevent their capture during the breeding season.

DEVELOPMENT.

The development of the germinal disk of the silver gar is essentially like that of the Spanish mackerel and the cod. The egg is quite large,

measuring when mature about one-seventh of an inch in diameter. After impregnation the egg does not increase much in size in consequence of the absorption of water from without, as in the case of the eggs of the shad and whitefish. The vitellus, in consequence of this, lies almost in contact with the egg-membrane, as shown in Fig. 1. The egg-membrane is without pore canals, and is therefore not a zona radiata like that of the shad and salmon. It is about $\frac{1}{60}$ of an inch in thickness, which is more than six times that of the zona radiata of the shad egg. The fibers or filaments which arise from the surface of the egg are cylindrical and taper towards their free extremities. The attached end of the fiber is swollen into a truncated cone, which is joined to the surface of the egg-membrane by its base. From the truncated apex of the cone the fiber arises, and a very distinct transverse line indicates the point where the former joins the latter. The fibers may be forcibly pulled off of the membrane; when this is done a slight concave depression remains on the surface of the latter, marking the point of attachment of the conical base of the filament or thread. The thickness of the threads is about the same as that of the egg-membrane, and they are apparently composed of the same material, as indicated by their color and behavior towards reagents. An examination of the ovaries of different females in various stages of maturity reveals the fact that the fibers are tightly coiled about the egg-membrane in the immature condition in the ovarian follicles, and that they are also wound round the globular egg in but one plane, which we may designate as the equatorial plane. This appears to be the tendency of the fibers on the eggs of other Scomberesocoids as well as in *Chirostoma*. After extrusion the fibers on the egg uncoil and stand out, looped and twisted together in all directions, as shown in Fig. 1. The length of the fibers varies, but it does not usually much exceed the diameter of the egg.

The ovary of the silver gar is a very long, simple cylindrical pouch, varying in size and length very greatly, according to the degree of maturity of its contents, which are discharged by way of a wide oviduct opening behind the vent. The ovary when quite mature is sometimes a foot in length and nearly an inch in diameter. As usual in fishes the male is notably smaller than the female, and the milt or spermary is a simple, elongate, somewhat peculiarly lobulated, three-sided organ, extending, like the ovary in the female, for the greater part of the length of the body cavity; it empties its products into the water through a wide sperm duct behind the vent. The genesis of the spermatozoa is effected in much the same way as in the Spanish mackerel, as is shown by sections of the organ in my possession.

As in many other teleostean fishes the germinal protoplasm of the mature egg covers the vitellus as a thin envelope; in the egg of *Belone* it is extremely thin, but there is a great number of very transparent, refringent, minute vesicles scattered through this germinal pellicle, which is of uniform thickness over the whole vitellus. It has occurred to me that

inasmuch as the vesicles in the germinal pellicle disappear when the latter has been aggregated into the germinal disk, may it not be that they represent the fragments of the disintegrated nucleus? This view, however, as already stated in my paper on the Spanish mackerel, is negatived by the results obtained in staining the germinal pellicle of the cod egg, where these vesicles remain untinged. No oil spherules are visible in the vitellus, the latter being optically homogeneous. The whole egg is heavier than the sea-water, and quickly sinks to the bottom; its specific gravity must therefore be much greater than that of the shad or salmon.

The germinal disk is developed in the usual way by the aggregation of the germinal protoplasm of the pellicle, which covers the vitellus at one pole of the latter. It does not appear that impregnation certainly takes place before the formation of the germinal disk. Observations on this point are, however, still too scanty and untrustworthy to be of much value, and until special attention is directed towards this point it will be most commendable to maintain a skeptical silence in regard to the views held on this subject. Special apparatus is needed to conduct researches on the phenomena of impregnation of fish ova, supplemented by reagents which will act quickly, so as to fix the nuclear changes which occur almost instantly. We may then study the conditions presented by different stages in dead preparations which have been properly stained so as to develop the appearance of the nucleus, as little of a trustworthy nature can be learned from any of the twenty species of living eggs which the writer has seen, for in almost all cases the nuclei of living fish eggs are not visible under the microscope, even though magnifying powers of two hundred and fifty diameters be applied. With the use of reagents the matter is much simplified, the nuclei at once become distinct, and their metamorphoses may be very distinctly shown under a power of sixty to seventy-five diameters.

In Fig. 1 the germinal disk three hours and twenty-three minutes after impregnation has been segmented into eight cells; at the end of four hours and forty-five minutes it has been segmented into sixteen cells, as shown in Fig. 2. The disk during this time has not increased in transverse diameter, and is relatively smaller, when compared with the vitellus, than the germinal disk of the salmon. It is very transparent, and is less different from the vitellus in color and optical properties than the disk of any fish egg known to me. It is this feature which makes it hard to find in the live egg, and when found difficult to study, unless the light is skillfully managed so as to bring out the contours of its component cells. The nuclei are still quite invisible in the latter while alive. The stages which immediately follow are still more difficult to study, because as the disk spreads to form the blastoderm it becomes relatively thinner and more inconspicuous than in any other form known to me, so that it is necessary to manipulate the light in the microscope with extreme caution.

By the tenth hour the segmentation of the disk has advanced very much, and the cleavage of the component cells has proceeded so as to have split them up into superimposed layers lying in the plane of the great diameter of the disk, as shown in Fig. 3. Besides the development of superimposed layers of cells by another process, which I do not clearly understand, a portion of the germinal matter of the disk has been segmented off at its margin to form a wreath, *w*, of much depressed cells, which seem to be severed from the edge of the disk proper by a slight interval all the way round. These appear to take an important share in the development of the thick rim of cells *r*, which limits the border of the blastoderm after it has spread out somewhat, as indicated in Figs. 4, 5, and 6. Up to the tenth hour of development the disk has expanded but slightly; it now measures about one twenty-fifth of an inch in transverse diameter, exclusive of the wreath of marginal cells, or about the same as in the stage represented in Fig. 1. In Fig. 3 it is, however, lenticular, convex above and below, and it is only during the next twelve hours that it begins to spread, become of almost uniform thickness, convex above and concave below. The singular changes undergone by the disk of the cod were not so narrowly observed in this species, although they probably occur. What is alluded to is the change from the biscuit-shape of the morula stage, with a thick margin and almost flat upper and lower surfaces, to the lenticular form of Fig. 3, which is viewed somewhat obliquely, to that of the concavo-convex form, which is already assumed somewhat earlier.

With the lateral expansion of the disk, the segmentation cavity *sc* is developed beneath the upper germinal layers, which constitute its roof. Here, as in other forms studied by the writer, this cavity does not disappear, but persists and expands laterally as the growth of the blastoderm proceeds. In *Coregonus albus* the cavity is principally roofed over by the epiblast, which is composed of flattened, juxtaposed cells, while smaller, rounded cells constitute its imperfect floor. The cells of the floor appear to have been budded off from the mesoblast near the edge of the blastoderm. A similar state of affairs probably exists here, for as yet I can find no evidence of a positive character to show that we have in *Belone* an exception to the mode of development generally exhibited by embryo fishes; but this structural feature will be further considered, in relation to the genesis of the blood, at another place.

In Fig. 4 the embryo-swelling, which extends from *e* to the edge of the blastoderm, is still in a very primitive condition. The cells, which are to develop into the body of the embryo, have not yet been arranged into tracts, and little more than the upper or epiblast layer, with the mesoblast lying below the latter, and above the hypoblast, can be said to be differentiated. There is still no indication of a neural or primitive groove; no differentiation of lateral mesoblastic plates, from which the muscular segments or somites are to be differentiated. Whether these are lateral outgrowths, or diverticula from the hypoblast of the primitive

enteron or gut, as the latter is pushed inwards from behind, we are not yet ready to assert, but such a mode of origin appears possible, if not probable. By the end of the first twenty-four hours of development the germinal disk measures almost a line across, as shown in Fig. 4, and the part of it from which the body of the embryo will be developed is the widened portion of the blastodermic rim *r*, just below *e*. The cells composing the disk at this stage are already too small to be successfully represented in figures of the size we have adopted, consequently the blastodermic rim and embryonic portion of it will hereafter be merely more densely dotted.

In Fig. 5 the disk or blastoderm is represented at thirty-one hours and twenty minutes after the commencement of development; it now measures about a tenth of an inch across, but is still extremely thin and has apparently added nothing to its substance by an incorporation of any of the underlying yelk. The blastoderm is here again viewed somewhat obliquely, in consequence of which the rudiment of the embryo *e* appears to have its head end inclined to the right hand. The embryonic rudiment is relatively small, much more so than in other forms in the same stage of development. When the blastoderm is viewed from the edge in the living state, as a transparent object lying at one side of the vitellus, the segmentation cavity *sc* is found to be exceedingly shallow vertically, but its lumen may still be distinguished. The embryo, however, is much more clearly marked than in Fig. 4; it is more prominent and is rapidly growing in length from the rim towards the center of the blastoderm. This brings us to the consideration of the growth in length of the embryo from the edge of the blastoderm. I am inclined to believe that the theory put forward by Balfour (*Comparative Embryology*, II, 254) must be accepted with considerable qualification, as stated by him in the following language: "The growth in length takes place by a process of intussusception, and, till there are formed the full number of mesoblastic somites, it is effected, as in *Chaetopods*, by the continual addition of fresh somites between the last-formed somite and the hind end of the body." The only apparent exception to this rule is in *Elecate canadus*, where it appears that the segmentation of the mesoblast on either side of the neurala or spinal nervous cord is continued backwards so as to involve the rim of the not yet closed blastoderm, and that the somites of the hind end of the body are formed by the coalescence of the blastodermic rim in the median line continuous anteriorly with the primitive groove. Should this be found to be the constant mode of development in *Elecate*, it will be necessary to accept in part the view urged by His and Rauber. It is to be observed, however, that the segmentation of the rim of the blastoderm in *Elecate* proceeds from before backwards, and that while it extends beyond the posterior extremity of the neural cord, and notochord, the unusual segmentation of the rim of the blastoderm behind the proper embryonic body into muscular half segments may be a mere acceleration or hastening of the

usual mode. Such unusual acceleration or retardation in the development of certain structures in various species of teleosts is not unusual, and would be as likely to affect the segmentation of the mesoblastic blastodermic rim into muscular segments as any other part of the embryo. There is another most serious objection to the unqualified acceptance of Balfour's theory of the growth of the embryo from the edge of the blastoderm without further addition from that source. If we do not admit that the blastodermic rim becomes transformed into the body of the embryo, what becomes of it? Nothing can be more certain than that, upon its closure, little or nothing is left of it; it has apparently been incorporated into the embryo's body.

This view appears to be well sustained by what may be observed in the development of *Belone*. Up to the time when the embryo may be said to be fairly outlined as in Fig. 6, forty-three hours and forty minutes after impregnation, the material of the blastoderm and embryo has acquired little or no increase of bulk in consequence of the incorporation of portions of the massive yelk. In Fig. 6 we see that the embryonic body occupies about a quarter of the circumference of the yelk. The blastoderm has grown down over and inclosed more than half the yelk globe, and its rim is contracting at the tail to complete the closure. When this is accomplished, the point where the closure takes place entirely disappears; the edges of the rim have been so perfectly fused together that the point of union, marked at first by a pore behind the end of the tail, with radiating wrinkles running out from it fifty-one hours after development began, as shown in Figs. 8 and 10, has soon after completely vanished. The material of the slowly contracting rim is finally fused into a solid flat plate of cells at the caudal end of the embryo, after the membranes of the latter—epiblast and hypoblast—have inclosed the yelk. The conversion of this caudal plate into the mesoblastic, epiblastic, and hypoblastic structures of the tail end of the embryo accordingly appears to me to be beyond question. But I would not commit myself to an adherence to the doctrine that the embryonic body was formed by a gradual coalescence of the thickened edge of the blastoderm from before backwards along the median line. If the reader will observe Fig. 9 he will see that the annular blastodermic rim r , as it approximates the closed condition at the fifty-first hour, is not circular, as in Fig. 5, but decidedly oval. The sides of the oval blastodermic annulus are now approximated more rapidly than the ends, as we see still further exemplified in the oval pore-like openings in Figs. 8 and 10. It is, therefore, probably nearest to the truth to say that the embryo grows in length both by intussusception from behind forwards of the blastodermic rim as well as by the coalescence of the latter, not along the median line, but by a gradual fusion as it is finally closed over the yelk.

The segmentation of the mesoblast proceeds in the usual way in *Belone* from before backwards, as shown in Fig. 6 at *so*, and there is no

reason to suppose that the somatic mesoblast extends much beyond the sides of the body at this stage, but it ends abruptly on either side between the epiblast and splanchnopleure the same as in *Alosa*, as shown by transverse sections. Fig. 6 shows the optic vesicles *o* developing at the head end of the neural or spinal cord, which is solid in this species at this time, as in embryo bony fishes generally. The notochord is also faintly indicated at this stage. The vesicle *k*, the nature of which is so puzzling, shown at the under side of the tail in Figs. 6, 8, 9, and 10, is well developed; it was originally described by Kupffer, and it has been supposed to be primitively joined to the posterior end of the intestine, but of this there is as yet no satisfactory proof. It disappears entirely at a later stage of the evolution of *Belone*, and appears to play only a transient and comparatively inconsequential part in the process of development. The usual lateral flattening of the anterior end of the neural or spinal cord takes place, as shown in Fig. 9, in optic section through it and the optic vesicle on either side. Further stages in the development of the optic vesicles are shown in Figs. 7 and 10, in which the rudiments of the auditory invaginations are also represented. In Fig. 10 the embryonic body only is represented, as in Fig. 9; the mesoblast has been segmented into a greater number of muscular somites, and the point of closure of the blastoderm is shown at the tail, where it forms the caudal plate already alluded to.

DEVELOPMENT OF THE HEART AND BLOOD.

As stated at the beginning of this paper, the genesis of the blood of *Belone* is perhaps the most interesting part of its history. The heart develops in the usual way in the segmentation cavity below the head; at first an annular mass of cells, it soon becomes tubular, and is prolonged forwards until its venous end *a* extends to the front end of the head. An arterial channel is at once developed from its hinder end through the body of the embryo between the notochord and intestine, and just below the tail at *z* it widens into a capacious vessel of very uneven caliber and passes entirely around over the yolk between the epiblast and the hypoblast to again empty its contents into the anterior venous end of the heart at *a*. The vitelline blood-system is at this stage, seventy-two hours after impregnation, as simple as it can possibly be. It is a mere channel which is as yet hardly provided with proper walls, except in the region of the heart and body, running the whole length of the body of the young fish and continued around the yolk back to the heart. On either side of the body of the embryo a small vessel also makes its way outwards from the aortic channel or vessel, but suddenly returns again to empty its fluid contents into the heart at *a*. This vessel, or rather the one on the right side, is shown at *v''* in Fig. 11. With the progress of development the most noteworthy change which takes place in the arrangement of the two vessels on either side of the body is their rapid extension and growth outwards over the yolk, as shown

at v' , v'' , in Fig. 12, ninety-four and a half hours after development had begun. The blood corpuscles or disks at once begin to be formed, but they do not appear to be uniformly oval at first, but very soon acquire the red color characteristic of this tissue. The corpuscles have a tendency to adhere together in clusters or clumps, and circulate in this way in masses through the blood channels of the embryo, as shown in Fig. 11 at v . The blood channels soon develop communicating branches, and these are formed in a very interesting way, as represented in Fig. 13. Narrow blind prolongations of the hollow vessels are formed at their sides and at each pulsation of the heart these are lengthened; several of them are shown at c, c, c , Fig. 13. Frequently two such blind prolongations meet and join, so that a communication is established between the larger channels. In this way the vascular network is developed over the yolk, as shown in Figs. 14 and 16. In Fig. 14, one hundred and sixteen hours after impregnation, the vitelline vascular system is moderately complex; there are three vascular channels, the right one, v' , the left one, v'' , and the median one, v , all of which join and pour their contents into the venous end of the heart at a . It may also be observed that where the vessels cross the semi-diameter of the egg at the side and where their cavities are seen in optic section, the epiblast is lifted up to give them passage. Between the vessels at this stage it was possible to observe in optic section here and there at the sides of the vitellus the space between the epiblast and hypoblast, which we have regarded as the remains of the persistent segmentation cavity. The heart space p , which in this as in other cases appears to be derived from the segmentation cavity, becomes progressively more and more spacious in the successive stages represented in Figs. 11, 12, 14, 15, until it attains a most extraordinary development in Fig. 16, one hundred and sixty-five and a half hours after impregnation. In Fig. 11 the heart is tubular and not differentiated into regions; in Fig. 12 the ventricle and venous sinus are beginning to be marked off from each other; in Figs. 14 and 15 the bulbus aortæ may be for the first time distinguished, but in all of these phases the whole organ is dragged forward in the median line far beyond the front of the head. In Fig. 16 the venous end of the heart begins to be inclined downward, but is at the same time very remarkably elongated; the bulbus aortæ ba is almost tubular and the ventricle ve , almost globular, is held in position to the floor of the heart cavity by a muscular or fibrous band, s . Below the ventricle the greatly elongated tubular venous sinus appears to be fastened by diverging muscular bands to the lower part of the enormous heart space p . The point of attachment of the venous end of the heart in the lower portion of the heart space is the scene of the very remarkable mode of genesis of blood corpuscles of this species. Where the vessels v, v', v'' , in Fig. 14, converge, it is already apparent that an active metamorphosis of the yolk substance into blood disks is in progress. The first sign of this has, however, already made its appearance in Fig. 11, where the

blood corpuscles are clearly derived by budding off from the inferior hypoblastic walls of the vitelline blood channels. Clusters of adherent, not fully formed, blood disks are circulating *en masse* through the vessels. Some of them appeared to be amœboid in character. But the process of blood formation is in its most active phase in Figs. 15 and 16, where the vitelline vessels converge to join the heart. Here it was observed that the vitellus was breaking up into clear globular corpuscles from $\frac{1}{1500}$ to $\frac{1}{400}$ of an inch in diameter; the largest corpuscles were always observed to be most deeply imbedded in the yelk, or most remote from the vascular channel. A progressive segmentation of these corpuscles was also observed, from which it was concluded that they were directly concerned in the formation of the nucleated oval blood disks. The rapid formation of blood disks in this region had the effect of piling them up into great adherent masses about the venous end of the heart, which was also more distinctly marked as the red color of the ovoidal corpuscles became developed as hæmoglobin was formed. The pulsation of the heart would for a long time sway these masses of corpuscles back and forth, until finally one after the other would be detached from the mass and carried along in the current of blood. Not only were the corpuscles budded off in this way into the blood channel itself, but they were also found to be held in suspension in great numbers in the great heart space *p*, where every pulsation of the heart would cause them to vibrate in the surrounding serous fluid. At the upper part of the heart chamber great numbers of blood disks were found to be collected together below and in front of the origin of the breast fin *f*. The hypoblastic origin of the blood in this species is therefore undoubtedly a fact, as was learned from repeated observation; whether the hypoblast was more than the intermediary parent of the blood disks I am not in a position to state, but this was probably the case, for as the hypoblastic structures were broken down into corpuscles in the blood-forming region at the venous end of the heart, there appeared to be a constant renewal of germinating cells from below which were clearly derived from the yelk. The actual phenomenon of cleavage of the cells was not observed since the nuclei were relatively indistinct, and their genesis at this point was assumed to be undoubted from the constantly augmenting numbers which were developed independently of any which might accumulate in consequence of eddies in the blood current. The blood disks themselves were not measured, but as compared with the size of the corpuscles from which they were derived they were estimated to measure somewhat less than $\frac{1}{2000}$ of an inch in their greatest diameter.

What may lie beyond the stage represented in Fig. 16 I am not able to say, as we were unable to keep the eggs in a healthy state after this period. The species was found in abundance, in spawning condition, at Cherrystone during July and August last, and I take this occasion to express my appreciation of the assistance of Colonel McDonald and

Messrs. Sauerhoff and Walke, who were instrumental in obtaining the eggs which were the subjects of the foregoing study.

But a few more points in relation to the development of other portions of the embryo may, perhaps, profitably engage our attention. It will be noticed that there are over eighty muscular segments or somites represented in the body of the embryo shown in the egg in Fig. 11. This very large number is unusual in bony fishes at this stage of development; less than half as many are to be observed in the young shad, cod, or mackerel at the same stage. In explanation of this difference we can only suggest that, since the muscular somites of the adult silver gar are vastly more numerous than those of the three aforementioned species, we should expect the number in the embryo *Belone* to exceed those of the other species at a very early period, which is found to be the fact.

The breast fins *f* are developed early; the first rudiment appears in Fig. 11, and they increase in size progressively from that stage onwards; at the same time they are gradually pushed farther forwards, and their bases assume a vertical position as in Fig. 16.

The vent *x*, with the growth and prolongation of the tail backwards, communicates with the exterior of the body, as shown in Figs. 14 and 16. The intestine extends forwards from it, but the mouth will apparently not be developed until considerable progress has been made beyond the stage represented in Fig. 16. There is an embryonic urinary vesicle or bladder, *b*, behind and above the vent, which is connected with the segmental ducts anteriorly. The liver is still but slightly developed.

The mid-brain is the most massively developed portion of the neurula, and consists of a pair of large, flattened, saccular outgrowths, which are developed from the upper wall of the second cerebral vesicle, which partly cover the cerebellum behind and the lower part of the brain at the sides. The cavity inside the brain is spacious in the embryos of *Belone*, as is indicated in Figs. 12 and 14; the primary vesicles are as yet but little modified in our latest stage.

Of the history of the development of the unpaired fins, these stages tell us but very little, but there was a slight dorsal and ventral natory fold developed on the tails of the oldest embryos.

Of the relations of mesoblast to that of the hypoblast and epiblast, we clearly know that the mesoblast of the muscular somites ends abruptly on either side of the body between the upper and lower embryonic layers. In Fig. 14 the epiblast and hypoblast are indicated by two diverging lines which end at the sides of the body just in front of the breast fins. This figure shows in optic section the space between epiblast and hypoblast which runs along the whole length on either side of the body of the embryo. The epiblast amounts up over and covers the embryonic body consisting of the muscular mesoblast, spinal cord and brain, or neurula, and the notochord, segmental tubes and intestine; the hypoblast on the other hand passes beneath all of these.

The great bulk of the body is therefore inclosed between the epiblast and hypoblast; the segmentation cavity extends in reality all round the embryo's body up to the point where the mesoblast ends, and from this point all round the yelk between the epiblast and hypoblast after the latter has been enveloped by the blastoderm. Usually the mesoblast is freed from contact with the hypoblast for some distance beneath the head; in the space which results the heart is developed as a ventral mesoblastic outgrowth of cells annular at first, tubular at last, and soon divided into three principal chambers separated by two constrictions, which are not at first truly valvular. The space around the yelk is now continuous with the heart space or pericardiac cavity; the latter is indeed a part of the segmentation cavity; into this space the blood corpuscles of *Belone* are budded from the yelk through the intermediation of the hypoblast inclosing the latter. The vessels themselves appear to be intimately related to the hypoblast, and appear indeed to be placed between it and the epiblast, but to make their progress mainly along the former, plowing channels through it and the adjacent yelk. The mode of forcing or breaking open channels from one vessel to another over the yelk of *Belone* is well shown in Fig. 13, where the blind beginnings of vessels are arising at *c, c, c*, and two such from the larger vessels have met and joined but a short time since so as to connect the larger channels together. The median vessel which traverses the yelk is fed by the caudal vein behind; the lateral venous arcs *v' v''*, on the other hand, are fed directly from the cardinal veins.

It is a very significant fact that the segmentation cavity plays a very important part in the process of the formation of the blood and the incorporation of the yelk into the body of the embryo. There is no more reason why the segmentation cavity should disappear in the germinal disk of the fish-egg than in the segmenting egg of the amphibian, where it actually is as intimately concerned in the formation of the heart as in the fish, according to the evidence of the plates of A. Goette's classical *Entwicklungsgeschichte der Unke*, but this, I am aware, is not that embryologist's view of the matter. Kupffer* has advanced another view which it is important to notice in this connection, as it is very different from the one advanced by the writer in the foregoing pages. He supposes that there is a mesoblastic layer surrounding the yelk besides the epiblast and hypoblast, and which lies between the two latter. The blood, according to him, originates by germination from the hypoblast between the latter and the mesoblast. The origin of the heart is described essentially in the same way as it has been observed by the writer. Kupffer in all his writings has, however, completely overlooked the fact that the segmentation cavity of the fish-egg persists, and he was not, therefore, in a position to estimate its importance in relation to the development of the blood. As to the mesoblastic layer said to

*Beobachtungen über die Entwicklung der Knochenfische. Arch. f. mik. Anat. IV, 1868.

intervene between the hypoblastic and epiblastic layers which cover the yelk, sections through whole ova in various stages of development have thus far failed to show its existence, except in the salmon, in which it is quite evident in sections of advanced embryos. Even in the latter I am not sure that it extends entirely over the yelk. Cellacher has apparently understood the relations of the segmentation cavity much in the same way as the writer, except as to the heart.

COMPARISON OF THE TELEOSTEAN OVUM WITH THAT OF OTHER VERTEBRATES.

A comparison of the different types of vertebrate ova will be useful as leading to a clearer comprehension of the true nature of the yelk in the teleostean egg. The eggs of the common frog (*Rana*) and *Bombinator* undergo total segmentation in the process of development. There is no distinct vitellus or yelk, and the yelk of the fish egg is apparently not homologous with any part of the amphibian ovum. There is, however, an almost complete homology between the germinal disk and blastoderm of the fish and the whole of the amphibian egg. The completeness of the homology is impaired only by the peculiar way in which the neurula or brain and spinal cord and the intestine are developed in the fish. The fish egg may be regarded as the frog's ovum plus a large store of food, which may be either homogeneous or heterogeneous, and which at first takes absolutely no share in the process of segmentation. If it were possible to place a frog's egg on a sphere of protoplasm several times its own size and cause it to spread out and gradually grow over the latter so as to completely inclose it and yet develop perfectly, the condition which obtains in the fish ovum would be very nearly attained for the amphibian. The segmentation cavity, which appears in the germinal disk at an early stage of development of the fish, is perfectly homologous with a similar cavity in the egg of the amphibian, except that in the fish, instead of remaining a simple cavity it has been so greatly modified by the peculiar way in which the disk of cells in which it is contained is obliged to spread and grow over and around the yelk that it is at first not easy to see a likeness between the two types. The development of the tailed Batrachians and of the Lampreys is very similar to that of the frog, and their ova undergo total segmentation. The development of the ova of the genus *Lepidosteus* is probably not essentially different from those of the typical teleostean. The process of spreading and inclosure of the yelk by the blastoderm has not been observed in the bony gar, but, as far as I am able to judge from the account given by Balfour (*Comp. Embryol.* ii, 91-98), a segmentation cavity is probably formed, and the differentiation of the embryonic layers is apparently not essentially different from the same processes as observed in teleosts by various persons besides the writer. Fig. 58 in the work just referred to, and relied upon by Balfour to show the segmentation of the egg of *Lepidosteus*, appears to me to be taken from

a specimen preserved in alcohol, in which that reagent has produced the appearance of partial segmentation of the yelk. The germinal disk is represented above with the surface of the cells flattened, probably by the contraction of the egg membrane in a preservative fluid. The yelk of teleosts, as in the egg of *Elecate*, for example, is sometimes apparently divided into large cells, but such they are really not; they are merely homogeneous masses of protoplasm involved in a different kind of yelk protoplasm. To sum up the matter, the comparatively full account of the later development of *Lepidosteus* given by Balfour, and our lack of knowledge in regard to the stages immediately following the segmentation of the germinal disk and attending the formation of the cleavage cavity and blastoderm, lead me to conclude that it is probable that it will be found upon further investigation that the development of that form is almost identical with that of the ordinary teleostean type.

The development of the sturgeon when compared with the teleostean differs from the latter mainly in the way in which the yelk is inclosed by the intestine. This is certainly anomalous and not a little puzzling, as it is the only vertebrate type yet known in which such an extraordinary state of affairs has been shown to exist, and it is desirable that this observation of Salensky's should be confirmed in our common American species. The development of the germinal disk and blastoderm, from the account given of it by Kowalewsky, Owsjannikow, and Wagner* does not differ essentially from that seen in the teleostean egg. There is the same gradual envelopment and inclosure of the yelk by a blastoderm with a thick rim, which makes the statements to the effect that the segmentation is total appear at first to be founded upon doubtful evidence; even Balfour admits that it "approaches the mesoblastic type more nearly than the segmentation of the frog's egg." The point where the blastopore closes appears to be the homologue of the anus of Rusconi in the frog's egg, which is not the case in either the teleostean or *Lepidosteus*. The three ichthyan types, however, appear to agree pretty closely in the formation of the segmental organs, muscle segments, notochord, heart, and brain. In the blastoderm of the sturgeon there is apparently a thick rim as in the teleostean, which is mainly mesoblastic, and which in all probability contributes towards the formation of the caudal plate, and the posterior muscular segments, as in the latter. The segmentation cavity, according to the figures of Kowalewsky, Owsjannikow, and Wagner, appears to be persistent as in the teleost, and, if Salensky's representations are to be trusted, it probably enters into the formation, not only of the body cavity, but also that of the heart.

The principal difference between the blastoderm of the typical teleostean ovum and that of the Elasmobranch appears to arise from the mode in which the germinal disk continues to spread over the yelk for some time after the embryo has been formed and raised above the lat-

* Bulletin de l'Acad. Imp. des Sci. de St. Petersburg, XIV. 318-325. 1870.

ter upon an umbilical stalk, so that the portion of the blastodermic rim which still remains, but is separated from the embryo, and which will finally coalesce some distance behind the umbilical stalk, is probably not homologous with any part of the rim of the blastoderm of the teleostean. It is clear, at any rate, that this part of the rim of the blastoderm of the Elasmobranch takes no share in the formation of the caudal plate, and indirectly of the tail end of the body, as happens in the teleost. To urge the example of the Elasmobranch blastoderm, as Balfour has done, in refutation of the arguments of His and Rauber in relation to the part taken by the blastodermic rim of the germinal membranes of the teleost in the formation of the body, is therefore hardly fair.

As already stated in my paper on the development of the Spanish mackerel, the teleostean ovum is remarkable for the way in which the superficial layer or pellicle of germinal protoplasm, destined to form the germinal disk, migrates towards one pole of the vitellus to aggregate into a biscuit-shaped germ-mass. The process has been studied by the writer in detail in the egg of the cod (*Gadus*), where, owing to the low temperature of the water in which the eggs develop, it requires some time for its completion, so that it may be studied very minutely. It appears that the nucleus undergoes disintegration or rearrangement in the fish ovum before it leaves the ovarian follicle in which it grew. The nucleus in young ova is observed to be embedded in the center of the ovum; as the latter acquires maturity it migrates toward the surface and its contents are apparently broken up to be involved partly or wholly in the peripheral germinal protoplasm. In some teleostean ova it appears that the germinal disk is formed at the time of oviposition, but this is not the case in any of the species studied by the writer. In the cod, for example, the germinal disk was not formed until about four hours after impregnation. In this species, as well as in *Belone* and *Cybium*, the germinal layer of protoplasm from which the germinal disk is developed is a distinct external layer enveloping the true vitelline protoplasm. It appears that in some species this peripheral layer of protoplasm is connected with the interior of the vitellus by strands or processes of itself which pass inwards between the vitelline corpuscles, often forming an intricate investing matrix in which the latter are embedded. Notwithstanding all these modifications, however, the portion of the ovum which is directly influenced by the act of impregnation is the germinal disk alone, which in turn has been derived from the external germinal pellicle. The vitellus is, throughout the whole of development, passive; as the embryo is developed, the heart, through the intermediation of the segmentation cavity and blood vessels, becomes, in part, the means by which it is absorbed, the process being assisted by the formation of free nuclei in its substance as well as by germination, and, perhaps, by intussusception or absorption by the overlying hypoblast itself. The theory of the *intermediary layer* proposed by the writer, in the essay on the develop-

ment of the Spanish mackerel, in which it was assumed to be derived from the germinal pellicle, simplifies our theory of the constitution of the teleostean ovum. But I find myself unable to clearly determine its presence, as understood by Van Bambeke, in some forms, as in *Belone* and *Alosa*, for example. This layer may retain in it some part of the original nuclear matter of the egg, which may be the effective agent in reducing and effecting the incorporation of the substance of the vitellus by the formation of free nuclei from part of the original nuclear substance which has remained in the *intermediary layer*, which is immediately in contact with the yelk. But I have shown good reasons, as they have appeared to me, for regarding the *intermediary layer* as really equivalent to the hypoblast. If this view be sustained, and no evidence to the contrary derived from sections made during the early stages has yet been brought to light, either by the researches of myself or others, it would appear that we may rightfully maintain that the blastoderm of the fish is the homologue of the whole of the amphibian or marsipobranch ovum, and that the yelk has been superadded and is not directly concerned in the process of development, at least not until about the time the tail of the embryo begins to be budded out, shortly after which the heart is developed and begins to pulsate. The migration of the nucleus of the teleostean egg towards the surface and apparently into the peripheral germinal matter is, I apprehend, a very different thing from what occurs in the ova of the lamprey and frog, though upon comparison they present a superficial resemblance. The behavior of the ovum of the sturgeon, according to Salensky, appears to be similar to that of the teleost in respect to the formation of the germinal disk; the nucleus, too, seems to undergo disintegration into fragments.

Summarizing the arguments presented in the foregoing pages the following conclusions appear to me to be warranted:

1. The germinal disk of the teleostean egg is homologous with the whole of the amphibian and marsipobranch ovum.
2. The yelk, while it is in intimate organic union with the blastoderm, may be regarded merely as a nutritive appendage to the teleostean egg from the center of which the nucleus has migrated at about the end of intraovarian development into the germinal pellicle or disk, leaving the yelk a passive structure, the presence of which has greatly modified the mode of development of the blastoderm.
3. The rim of the blastoderm is more or less extensively transformed into the body of the embryo as argued by His and Rauber.
4. The difference between the development of the ganoids and teleosts is much less than between the former and amphibians.
5. The blood in *Belone* is developed directly from the yelk through the intermediation of the hypoblast, quantities of its corpuscles being found in the heart or pericardiac chamber.
6. The intestine of the teleost embryo is formed from behind forwards by splitting of the hypoblast, and not by an invagination conterminous

behind with the neurula or spinal canal, as in the Amphibian and Marsipobranch, and there no evidence to show that the point where the rim of the blastoderm closes is comparable to a blastopore, or to the anus of Rusconi.

7. The gastrula of the teleost is extremely modified on account of the extreme flattening and epibolic mode of growth of the blastoderm over the yelk, but the type of development is, in reality, similar to that where there is a neurenteric canal developed as in embryo sharks, lampreys, and frogs, since the vent is always broken through long before the mouth, and there is a strand of cells representing the neurenteric canal.

8. The blastoderm of the teleost may be regarded as a very depressed concavo-convex hollow sack resting on the yelk, the hollow space beneath it representing the persistent cleavage cavity. One side of the blastodermic disk or sack is filled with mesoblast cells, from which the somatopleure and splanchnopleure are derived, where the embryo is formed; the intestinal lumen is, at first, a narrow transverse split in the hypoblast which extends forward, eventually prolonging the enteric cavity beneath the head.

9. The uppermost or epiblastic layer of the blastoderm, several cells deep, roofs over the cleavage cavity, the hypoblast forms its floor, the rim of the blastoderm contains mesoblastic cells, which, as the germinal membranes close over the yelk form the caudal-plate which is continuous on either side with the medullary or muscle-plates at the sides of the body of the embryo. The caudal-plate eventually enters into the formation of the tail and caudal muscular mesoblastic somites, its hypoblast into the formation of the anal end of the intestine.

10. The cause of the at first flattened lumen of the intestine is probably to be sought in the very depressed and modified type of blastoderm of the teleostean, which differs widely from that of all other vertebrates. The lumen of the intestine gradually becomes round.

The embryo develops at the edge of the blastoderm in Teleosts, Elasmobranchs, and Ganoids, but only a small portion of the blastodermic rim appears to be appropriated to form the embryo in the Elasmobranch. This eccentric development of the embryo is in strange contrast with that of the Amphibian and Lamprey, and not less so when compared with the mode of development of reptiles, birds, and mammals where the embryo develops in the center of the blastoderm, and where the yelk, when present, appears to be merely nutritive and accessory, as in the teleostean egg. Only in the case of *Zoarces* is there an approach toward the formation of an umbilical stalk, according to Rathke, but even there it is not developed until some time after the blastoderm has closed over the yelk.

EXPLANATION OF REFERENCE LETTERS USED IN THE PLATES.

- a.* Venous end or sinus of heart.
- b.* Urinary vesicle or bladder.
- ba.* Bulbus aortæ of heart.
- c.* Blind capillary prolongations from the larger blood-vessels on the surface of the yolk.
- e.* Head end of developing embryo.
- f.* Rudiment of breast fin.
- g.* Germinal disk.
- k.* Kupffer's vesicle.
- o.* Optic vesicles; rudiments of the eye-balls.
- p.* Pericardiac or heart space.
- r.* Thickened rim of blastoderm.
- s.* Muscular or elastic band binding the ventricle to the floor of the heart space.
- sc.* Segmentation cavity.
- v.* Median vitelline blood-vessel.
- v' v''.* Right and left vitelline blood-vessels.
- ve.* Ventricle of the heart.
- w.* Wreath of cells around the germinal disk which enter into the formation of part of the blastodermic rim *r.*
- x.* Vent or anus.
- z.* Point where the caudal vein passes into the median vitelline blood-vessel.

EXPLANATION OF PLATE XIX.

☞ All of the figures except 13 and 15 are enlarged twenty-one and a third times the natural size.

Fig. 1.—Egg of the silver gar in its membrane, with the tentacular filaments attached to its surface, 3 hours and 23 minutes after impregnation. The germinal disk *g* at its upper pole has been segmented into 8 cells.

Fig. 2.—Germinal disk, 4½ hours after impregnation, divided into 16 cells.

Fig. 3.—Germinal disk, 10 hours after impregnation, showing the formation of a wreath of cell, *w*, round its margin.

Fig. 4.—Blastoderm of silver gar, viewed from above, 24 hours after impregnation to show the form and extent of the segmentation cavity.

Fig. 5.—Blastoderm of silver gar, viewed from above and obliquely, 31 hours and 20 minutes after impregnation, showing the body of the embryo budding out from the edge of the blastodermic rim.

Fig. 6.—Blastoderm nearly inclosing the vitellus, 43 hours and 40 minutes after impregnation, eyes *o*, muscular segments *so* and Kupffer's vesicle *k* are developed.

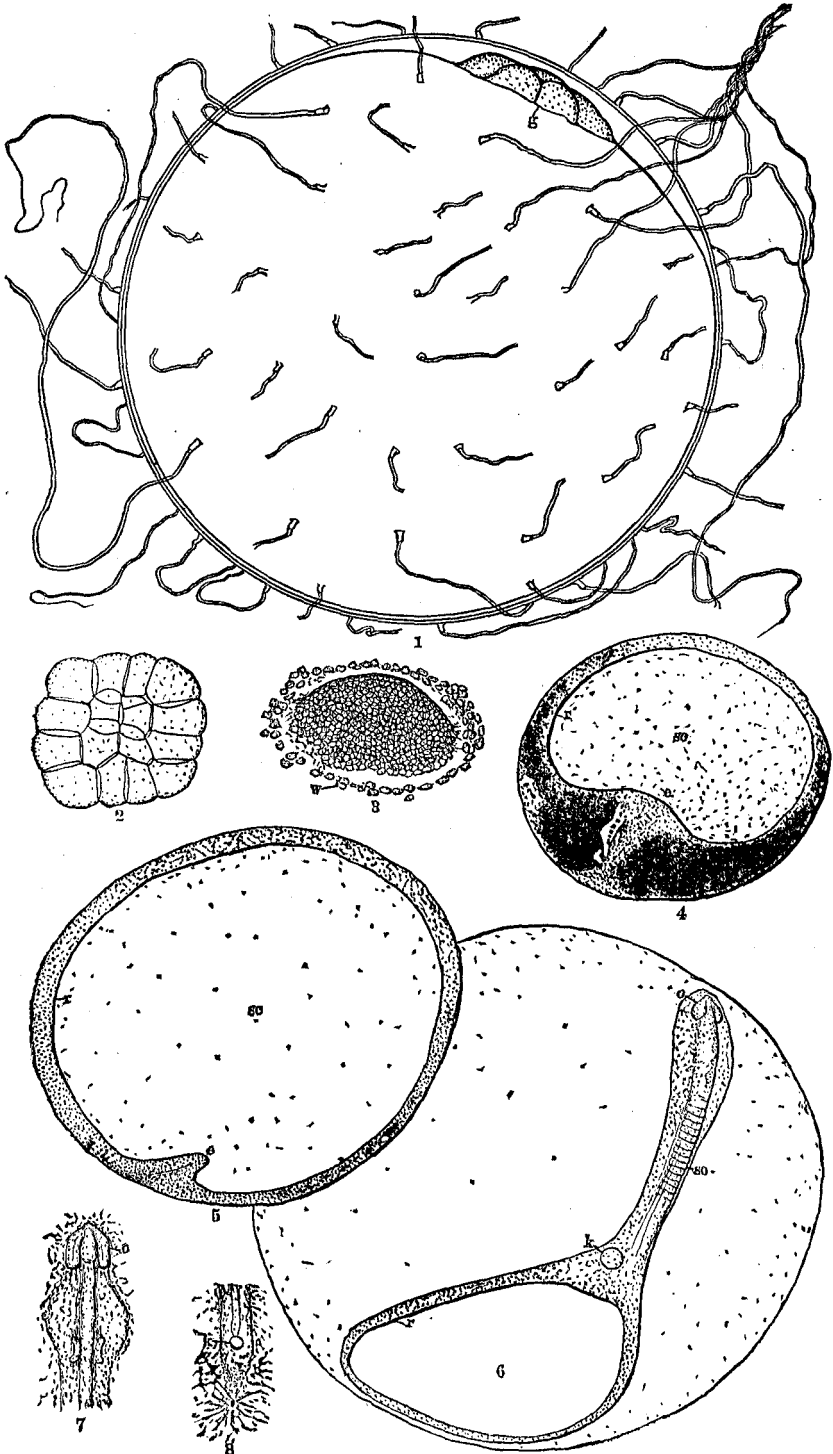
Figs. 7 and 8.—Head and tail ends of embryos, 51 hours after impregnation.

EXPLANATION OF PLATE XX.

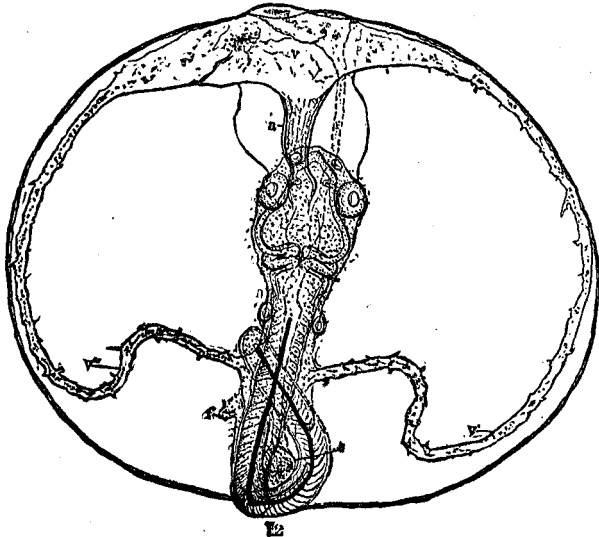
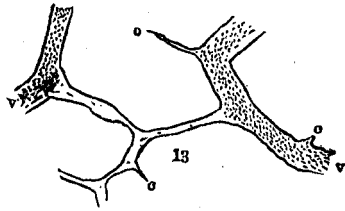
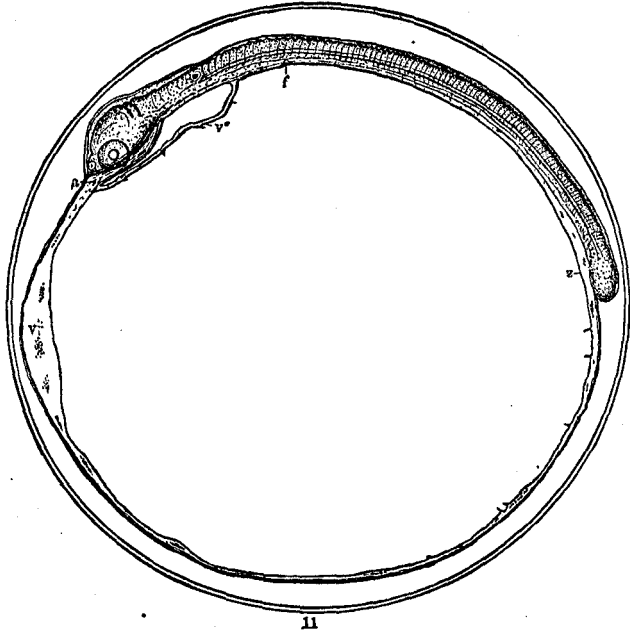
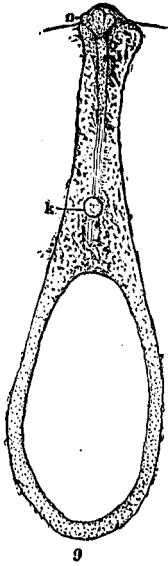
Fig. 9.—Embryo silver gar with the head seen in optic section, the tail end and the conjoined oval blastodermic rim seen through the transparent vitellus, which is not represented, 51 hours after impregnation.

Fig. 10.—Embryo one hour later, represented without the vitellus, the number of muscular segments has greatly increased in number, and the blastoderm has closed over the yolk.

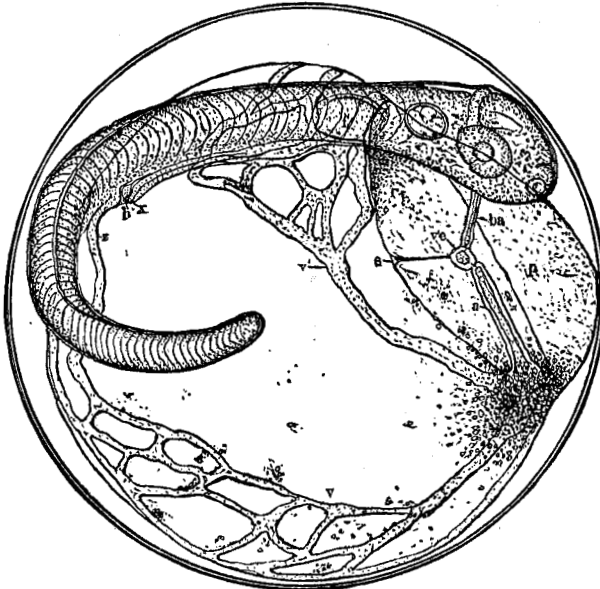
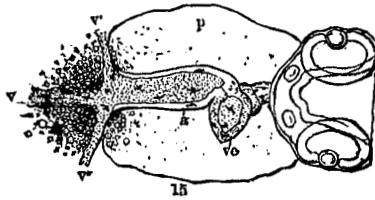
Fig. 11.—Embryo silver gar, seen from the side as a transparent object, 70 hours after impregnation. The tail is about to begin to bud out behind, the heart is formed but is still tubular, and a vessel passes forward around the yolk back to the tail and on forward through the body to the hind end of the heart. The direction of the blood



TYLOSURUS LONGIROSTRIS.



TYLOSURUS LONGIROSTRIS.



TYLOSURUS LONGIROSTRIS.

current is from the head to the tail. There are already over 80 muscular segments formed, and the breast-fin is developing at *f*.

Fig. 12.—Embryo, 94½ hours old, viewed as a transparent object. The notochord is shown as a broad black line, and the lateral yolk-vessels *v' v''* are much more developed than in Fig. 11.

Fig. 13.—Diagram to show the origin of the mode of anastomosis of the larger vessels, enlarged 52 times.

EXPLANATION OF PLATE XXI.

Fig. 14.—Embryo silver gar, 116 hours and 40 minutes after impregnation, showing the further development of the heart and blood-vessels traversing the surface of the yolk. Those on the opposite side of the yolk are indicated by the dotted lines. Pigment cells have made their appearance on the body beneath the superficial epiblast and on the yolk and the heart. The intestine and urinary vesicle *b* are well developed, as seen in the tail end of the embryo on the opposite of the egg through the vitellus.

Fig. 15.—Sketch of heart and vessels which empty into it in an embryo 140 hours old; the formation of the blood is in active progress where the vessels converge to join the heart, which is now blotched with pigment cells of two colors in life. Enlarged 26 times.

Fig. 16.—Embryo silver gar, viewed from the side as a transparent object 165½ hours after impregnation, to show the progress of development of the blood vessels over the yolk on the right side. The heart or pericardiac cavity *p* is now enormously developed, and the development of blood cells is going on with great activity in its lower part, where the venous end *a* of the heart is attached. The heart itself is now greatly elongated downwardly, and is one-third as long as the whole embryo.

ON THE REARING OF WHITEFISH IN SPRING-WATER AND ITS RELATION TO THEIR SUBSEQUENT DISTRIBUTION.

By FRANK N. CLARK.

[Letter to Prof. S. F. Baird.]

I am not prepared to say whether or not eggs of the whitefish are prematurely hatched in spring-water. I take it that the question is a scientific problem for scientists to solve; that it is a point on which even "doctors disagree."

If we could "reap what we sow" from our plants of fish in bodies of water like the great lakes we would soon have a practical test of the respective value of "premature" or "retarded" development of eggs or embryos; but this is impossible, and so if there is any difference we must detect it from evidence that is circumstantial or theoretical. It seems reasonable to assume that if the little fellows are vigorous when hatched, whether of three or five months' incubation, and are released when and where alimnt for their sustenance is abundant, a large percentage of those not destroyed by predaceous fishes ought to become adults. There is no difference in size and activity between fish brought out in three or six months, where the same water is used; neither are there points about the former that can be construed into evidence of abnormal de-