DEVELOPMENT OF THE SPANISH MACKEREL (CYBIUM MACULATUM).

By JOHN A. RYDER.

The mackerel-hatching operations at Mobjack Bay were conducted on board the steamer Lookout and at Bosman's Fish Guano Works, New Point Comfort, Va., from the 5th to the 13th of July, 1880. Spawn and milt were obtained in that vicinity from sixteen individuals; the number of males and females was about the same. These ova were subjected to as thorough and systematic an investigation as the limited time at our disposal permitted. Some time previously Mr. R. E. Earll had succeeded in taking some eggs in the vicinity of Crisfield which were successfully fertilized and which hatched out in the amazingly short period of twenty hours. In every case the eggs taken and cared for by my assistant, Mr. W. P. Sauerhoff, hatched in twenty-four hours; it is true that a few hatched somewhat sooner, but some left the egg-membrane even later. I attribute this difference in the times of hatching in the two cases to different methods of treatment of the ova or to a great difference in the temperature of the water. The eggs taken and fertilized at 4 o'clock p. m. were all hatched at the same hour the next day. This season's experience at Cherrystone showed that when the temperature of the water was unusually low it would require nearly thirty-six hours for the eggs to hatch, but the development was normal.

The hatching operations for the season of 1881 were conducted at Cherrystone Harbor, Northampton County, Virginia, in the earlier part of the season, by the crew of the steamer Fish Hawk, but were afterwards continued on Kimberley's wharf, under the direction of Col. Marshall McDonald, commissioner of fisheries of Virginia. It was not the good fortune, however, of the latter party, of which the writer was a member, to obtain as large a number of ova as they had been led to expect; this was in the main due to our inability to control the times of fishing with the pound nets, which were the sources whence our supplies of spawning fish were obtained; also in part to their distance from our hatching station, which, as our facilities for the prompt transportation of the crew of spawn-takers by water was inadequate during the latter part of the time we were engaged in our investigations, added not a little to the disadvantages under which the work was conducted. Add to this the fact that, although the number of ripe fish obtainable was probably sufficient for our experiments, it was learned that they seemed for the most part to discharge their spawn only in the evening or at night, the times when by far the larger proportion of ova were obtained. That this fish is nocturnal in its spawning habits was still further demonstrated by Colonel McDonald while on a visit to Tangier Sound, where the Spanish mackerel is taken at night in gill-nets, a mode of fishing not practiced at Cherrystone; an abundance of spawners, it was found, were taken under the above conditions at the former place, and it was believed, from observations made at the time, that many millions of eggs might be obtained there in a single night. From this it appears that what is now needed to make the artificial incubation of the mackerel a success is to choose some point for our operations where the fishing is carried on at night or in the evening. In relation to this part of the subject the writer will forbear to say anything further, as its discussion rightfully belongs to Colonel McDonald, whose observation it is; but I have been informed by Professor McCloskie, of Princeton, that while he was in company with Mr. J. S. Kingsley, during the present summer, on the Massachusetts coast, in the vicinity of Cape Ann, the latter gentleman conducted some observations on floating fish eggs which were taken at night in a tow-net and believed to have been laid after sundown; they were not identified, however. Ova which were found floating at the surface of the sea by Professor Haeckel,* at Ajaccio, off the island of Corsica, and afterward at Nice, were agglutinated together in clumps, but the species was not determined, and it was only supposed that they belonged to some gadoid, and are hence doubtfully referred to *Motella*. Edward Van Beneden † also, who describes at some length similar adhesive floating ova, which he had obtained in the same way, with the help of the tow-net, off Villafranca, does not identify them, nor does he state definitely at what time of day it was supposed they were spawned, but from the evidence afforded by his time record of the rate of their segmentation I am, nevertheless, prepared to believe that they were laid at night. The Cyprinodonts in the spawning season, as far as my observations go, are much more actively engaged in amatory play in the night than at other times, judging from the rapid motions and splashing noise which they make in the water during this part of the day. G. O. Sars, in his account of the development of the cod, says nothing in regard to the time of day at which this fish parts with its ova, but the writer believes that there are strong grounds for a belief that the bonito, or crab-eater (*Elacate canadus*), is a nocturnal spawner, the same as the mackerel, from the circumstance that it was only from individuals caught in the evening that ova were obtained, which appears to be the case also, judging from our experience, with the moon-fish (*Parephippus faber*). The foregoing data, although not all of them directly bearing upon the question of the time at which the Spanish mackerel discharges its spawn, are sufficiently within the scope of the evidence needed to help us to reach a conclusion in regard to the matter so that we will know how to proceed in the future. The artificial incubation does not appear to be the gravest part of the problem to be solved; the

* Die Gastrula und die Eifurchung der Thiere. Jenäische Zeitsch., IX, 402-508, 1875, 7 pls.

† A contribution to the history of the embryonic development of the Teleosteans. Quar. Journ. Mic. Sci., No. LXIX, 41-57, 1 pl., 1878.

question seems to be, under what conditions can the greatest number of eggs be obtained? Given a sufficient quantity of these, although the losses in hatching may be as much as 50 to 75 per cent., the number of young which it is possible to add to those hatched out naturally will still be prodigious.

OVARIES AND OVARIAN EGGS OF THE SPANISH MACKEREL.

The ovary of this fish is a paired organ composed of two nearly cylindrical sacks lying in the hinder upper portion of the abdominal cavity; both taper to blunt conical points anteriorly, and are joined posteriorly into a wide common ovarian duct, which opens just behind the vent. Attached to the walls of the ovarian sacks are a vast number of ovarian leaflets or folds placed transversely, and which depend directly into the space within the sacks. In these leaflets the ovarian eggs are developed, each one in a minute sack or follicle of its own, the walls of which are richly supplied with capillary blood-vessels joined to the blood system of the parent fish. At first the ova are very small, but as the season advances they, for the most part, increase in size, in consequence of which the entire ovary increases in bulk. At first, when they begin to grow larger, they are barely distinguishable from the ordinary cells which compose the great proportion of the tissue of the ovary; they are in fact nothing more than greatly enlarged cells when mature, in which we may distinguish an outer germinal layer or pellicle, *gp*, Fig. 2, covering a store of nutritive material known as the yelk, which is gradually absorbed as development progresses; besides, they are covered by an egg-membrane, *zr*, of extreme thinness, perforated at one point only by a minute pore known as the micropyle, which is shown in two positions in the same egg in Fig. 1, lying in the center of a circular area which has faint markings running out radially from the micropylar pore in its middle toward its margin. The micropyle is funnel-shaped and the radial markings and area around it seem to disappear almost entirely after impregnation. The egg-membrane may be regarded merely as a protective covering and the micropyle as a passage-way for the male element or spermatozoan to find its way through the egg-membrane and to the germ, in order that impregnation may take place, when the development or growth of the embryo fish will commence. The opening also connects the space inside the egg-membrane between the latter and the globular egg or germinal mass with the water outside in which the egg floats; the space here alluded to does not usually appear until immediately after impregnation, in consequence of which the egg-membrane at first lies laxly on the germ within, and in the eggs of some species, as in the shad, it is at first considerably wrinkled. It is only after impregnation that it normally absorbs water through the micropyle and becomes tense and perfectly globular. The history of the formation of the egg-membrane is not very clearly established, but it appears in the highest degree probable that it is secreted from the cellular walls of the sack or follicle in which the egg grows and is matured.

The youngest ova of the mackerel do not appear to be inclosed in independent follicles; these seem to be developed only after the egg has attained some dimensions. Very young ova are found to contain a relatively large nucleus or germinative vesicle inclosed in a thin layer of transparent homogeneous protoplasm, and for a considerable time this condition seems to be maintained, but as they increase in size it is found that, while the germinative vesicle increases in dimensions, the protoplasmic envelope also grows in thickness, and that there is a tendency to multiply the number of nucleoli or germinative spots included in the germinative vesicle. At a still later period the nucleus becomes apparently granular, and finally, when the egg is mature and ready to rupture the follicle in which it grew, the germinative vesicle, as well as the spots, seem to have disappeared; at any rate it is now generally held that when the egg has attained maturity the germinative vesicle undergoes disintegration, and perhaps a reorganization, by which a portion of it becomes what is known in recent years as the female pronucleus, which conjugates or becomes fused with a similar body called the male pronucleus, which results from the metamorphosis of the head of the male element, or spermatozoan, after its entry into the germinal matter of the egg. It is this body, made up, as it is, in part of male and in part of female or ovarian protoplasm, which constitutes the nucleus of the first segmentation furrow across the germinal disk in which it is embedded, and which must be regarded as the initial or starting point in the development of the young fish. It is also doubtless a fact that during the process of division of the germinal disk we would find the nuclei elongated with granular rays extending from their ends through the surrounding protoplasm, as well as bands or fibrils of denser protoplasm running from one end to the other of the nuclear figure. When the cleavage is completed the rays and bands appear to be withdrawn, and each pole of the formerly elongated nucleus becomes rounded independent of its fellow, and so two nuclei result, the dynamic or force centers of two cells, the products of this process of cleavage of the original cell. This process has been named *karyokinesis* by Prof. W. Flemming, of Kiel, in allusion to the apparent exhibition of the modes of motion of the matter of the life centers of cells. Nuclear figures of great complexity, but always bipolar during cleavage, have been described by this author and others in both animal and vegetable tissues. That they exist and will be found well developed in the very early stages of the cleavage of the germinal disk of the embryo fish, I have not the slightest doubt; it will depend upon the method of demonstration as to whether or not they will be made visible. My reason for this statement is the fact that the early stages of the segmentation of the germinal disk of all the species of fish ova which I have observed closely enough are essentially rhythmical; that is to say, between the periods when a given set of cells divide there is a longer interval of repose or rest, which may even in some cases be accompanied by a slight subsidence or depression of the cells which have just divided. Brooks speaks of it in the mollusks as a contraction of the resulting cells. It seems to me, from what I have seen in the segmenting eggs of *Mya* and *Alosa*, that the term "subsidence" is more directly applicable, since in the act of segmentation the protoplasm appears to have a tendency to become rounded or heaped up in the two new cells, and that afterwards or during the period of rest the protoplasm has a tendency to subside or spread out, as a result of which the segmentation furrows become much shallower. Brooks* has noticed these phenomena in ova supposed to be those of the toad fish (*Batrachus tau*), and alludes to observations in other forms by E. B. Wilson and S. F. Clarke. What first attracted my attention to the matter was the fact that, while ova in the early stages of development were under observation, I was frequently surprised to find, after having left the microscope for a few minutes, that a sudden and rapid change had taken place, while no change whatever was observed previously for the space of an hour or more. Brooks has kept a time record, which I neglected to do, but I can say, however, that in the shad egg, in which I have mostly observed this phenomenon, the intervals of rest are of much shorter duration than recorded by him, showing that he dealt with an egg which developed more slowly. In the mackerel ovum, in which I have had but little chance to observe these phenomena, the intervals of rest are of less duration still, but inasmuch as it develops with three times the rapidity of the shad egg, it is plain that it would be an admirable subject for investigation in respect to this point, and in which microphotography would be an invaluable aid.

The early stages of segmentation of the mackerel, studied by the writer, unless observed with the microscope, on an unsteady boat, where such observation is almost, if not altogether impossible, were usually too far advanced to keep track of the sets of dividing cells, which were already too numerous, so that they would confuse the student in his attempt to follow the changes marked by intervals of rest between periods of activity. The writer, however, must admit that he has never been able to distinguish the nuclei of the cells as clearly without reagents as figured by Brooks, although working with the most approved apparatus for obliquity of illumination, and with lenses of fine definition; but this may be due to the fact that different species were studied by us.

STRUCTURE OF THE TESTES AND GENESIS OF THE SPERMATOZOA.

The testes of the Spanish mackerel are paired organs, like the roe or ovaries of the female, and have much the same position in the abdom-

* Alternations of Periods of Rest with Periods of Activity in the segmenting eggs of Vertebrates. Studies Biolog. Laboratory, Johns Hopkins University, Vol. II, 117–118, 1 pl., June, 1881.

inal cavity, but are much flattened or compressed laterally, instead of cylindrical, as in the former, and both pour their secretion through a wide seminal duct which opens behind the vent. Their substance is composed almost entirely of a vast number of minute canals, which have a generally vertical or oblique direction, and which open into a wide sinus or space at the upper edge of the organs, and which empty their contents into the common seminal duct. They are essentially glandular in function, and secrete and pour out large quantities of milt or semen during the spawning season, which is developed in the tubules or canals already alluded to. These canals, or, more properly, seminiferous tubules, are lined with cells, which break up into bundles of spermatozoa, which fall directly into their cavities, and so find their way into the seminal sinus at the upper border of the testicle and out through the seminal duct into the water, where they are capable, if they come into contact with the ova discharged by the female, of effecting impregnation and of establishing the development of the embryos. The spermatic particles, or spermatozoa, are themselves very minute, and are composed, in the mackerel, of an oval head with a very fine, almost ultra-microscopic, tail or flagellum, which is incessantly lashed about in the living state, so that the spermatozoan has a distinctive wriggling, tadpole-like movement. As soon as the power to move the lash or flagellum ceases, they may be considered as dead, and no longer capable of effecting the impregnation of ova. They will not ordinarily live much over an hour after being taken from the fish, with which time their effectiveness ceases.

STRUCTURE AND PHYSIOLOGICAL CHARACTERISTICS OF THE UNFERTILIZED EGG OF THE SPANISH MACKEREL.

It is notorious that the egg-membranes of floating fish ova are extremely thin; moreover, they are not, as far as I have been able to make out with carefully conducted observations, perforated by pore-canals, as in the stickleback, salmon, and shad; the membrane of the ovum of the mackerel is no exception to this rule, and is consequently not a *zona radiata*, as defined by Balfour. It is a perfectly homogeneous, transparent film, less than half as thick as that covering the shad ovum, which measures approximately $\frac{1}{3200}$ of an inch in thickness, but differs from the latter in having minute papular prominences on the inner surface which project into the breathing chamber or water space around the germ, as shown in Figs. 2, 6, 9, and 12. These prominences usually seem to be confined to one pole or hemisphere of the membranous envelope.

The ova vary slightly in dimensions and range from $\frac{1}{25}$ to $\frac{1}{20}$ of an inch in diameter. This variation in size is a usual feature in the ova of fishes, but may be partly due to the unusual pressure exerted on the ovaries when the ova are removed artificially, so that some are squeezed from their follicles before they are quite fully mature, though it is to be remarked that the smaller ova develop just as readily as the larger ones, and every one is aware of the fact that the eggs of the same birds vary considerably in size, and that such variation does not interfere perceptibly with their capability to develop. Between the egg-membrane and the germ or vitellus there is always a more or less well marked space or cavity filled with water which has been absorbed from without, as is proved by the fact that when the ova are at first extruded the egg-membrane, in all the species studied by the writer, is more or less relaxed or even wrinkled, and that it is only after they have been in water for some time in the presence of spermatozoa that the membrane will distend so as to render it tense and spherical. Unimpregnated eggs which have absorbed water are said to be "water-swollen," and usually only a small percentage of them will become so in the absence of living spermatozoa, mixed with them in the water. Ova which have been impregnated and are water-swollen, that is, have developed the water space around the vitellus, are said to have "risen," which is probably in allusion to the fact that in some cases—minnows and shad—a lot of newly laid ova will by this process acquire several times their original bulk, so that if too large a quantity of freshly spawned eggs be put in a vessel the mass will swell in the presence of water so as to fill the receptacle or even run over its sides, somewhat in the same way as leavened dough would acquire increased bulk in a dough tray by the numerous vesicles of carbonic dioxide which are evolved by fermentation and held in confinement by the tough mass of gluten and starch. The water space may in some cases embrace more than two-thirds of the solid contents of the sphere included by the egg-membrane, but this space is smaller and is never at any time more than the $\frac{1}{250}$ of an inch in vertical thickness in the egg of the mackerel.

Its function is, doubtless, in the main, respiratory, as suggested by Ransom, who has actually named it the *breathing chamber*, and it would seem that there is very strong evidence in favor of such an opinion, in that most fish ova die if the water in which they are hatching is not frequently changed. The circumstance that some fish ova are fixed by filaments or by an adhesive material while the water moves over them on account of its gravity or in consequence of tidal action, would seem to indicate that these modifications were favorable to their aeration, or perhaps, more properly, their respiration, and exchange of salutary oxygen for hurtful gases through the membranous and fluid coverings of the egg. The habit which the male stickleback appears to have of pumping water through the nest with his mouth, so as to change the water surrounding the eggs in his charge, seems to be similarly significant.

This cavity serves another purpose. In it the young fish may develop immersed in fluid free from friction or hurtful knocks from without, if it is a free swimming egg, like that of the mackerel. Its function, aside from that of respiration, would then appear to be essentially that of an amnion, or "bag of waters," such as is developed from the edges of the germinal disk in reptiles, birds, and mammals, and which eventually incloses the embryos of these forms, and in which they undergo the largest part of their development. A similar spacious cavity appears to exist in the egg-cases of the oviparous sharks, and rays, filled with water or a thin serous fluid, in which the embryo develops as it absorbs the contents of the yolk sack. The same remark applies to the eggs of the Amphibia and *Amphioxus;* so it appears that all of the vertebrates below the reptiles have an egg-membrane, or what answers to it, and a water or respiratory space in which the germ or vitellus is included, and in which it undergoes a more or less complete development.

The vitellus or germ included by the egg-membrane of the mackerel is globular and very nearly fills up the cavity, bounded by the membrane, so that the water space or breathing chamber is small. The vitellus is made up of three principal portions: a thin outer germinal layer, as shown in Fig. 2, which incloses a globular yolk-mass, in which it is ordinarily difficult to distinguish the contour of the yolk spheres of which it is composed. Imbedded in this yolk-mass there is always a single oil sphere perfectly globular, highly refringent, and measuring about one one-hundredth of an inch in diameter, and which always occupies an eccentric position in the upper hemisphere of the living egg. It is the presence of this oil sphere which causes the egg to float; the drop of oil is always in such a position as to keep the developing embryo inverted or turned upon its back. This is probably due to a purely physical cause; the oil sphere, being the lightest part of the egg, will always be found in its upper hemisphere, while the germinal disk or embryo appears to be the heaviest part, and in consequence is always found in the lowermost hemisphere looking back downwards. It is difficult to trace any protoplasmic filaments passing off from the germinal layer of the mackerel's ovum down amongst the yolk spheres, in consequence of which it is difficult to demonstrate the latter. It has likewise not been my good fortune to trace and learn what is the fate of the germinative vesicle of the mackerel egg, but it will suffice to say that when the egg is mature it can no longer be distinguished; nothing whatever remains to indicate its former position, and the whole egg is now more transparent and presents the appearance shown in Fig. 2. The germinal layer or pellicle, however, is found to include a great number of very minute refringent corpuscles scattered through its substance; these disappear as the germinal disk is formed by the aggregation of the protoplasm of the germinal layer at one pole of the vitellus to form the germinal disk. I have frequently seen them apparently dissolve and disappear while I was observing them through the microscope. Whether these represent the remains of the more fluid and refringent germinative vesicles I am unable to say, but I am inclined to doubt it from the fact that if the germinal pellicle is removed and stained with haematoxylon these corpuscles do not tinge, while the protoplasm in which they are imbedded does, which appears to be a sufficient proof that they are not the remains of the germinative vesicle, but that they are merely vacuoles filled with fluid, such as are found in the Protozoa, but which, unlike the spaces found in those animals, are not rhythmically contractile or pulsatile.

ORIGIN AND FORMATION OF THE GERMINAL DISK.

The germinal disk of the egg of the mackerel measures one-fortieth to one-fiftieth of an inch in diameter, is biscuit-shaped, and is composed of a light, amber-tinted protoplasm several shades darker than the protoplasm which makes up the vitellus, which is remarkable for its glass-like transparency. Normally, the disk always lies directly at one side and apparently on the surface of the yelk, as indicated in Fig. 4; and when the egg is in the water it is always immediately and exactly below the latter. The disk may be developed independently of impregnation, but in that case an embryo is never formed, and the egg soon disorganizes, the vitellus collapses, and the whole protoplasmic mass, disk and all, acquires a brownish, granular appearance, indicative of death and disorganization. The disk takes its origin directly from the germinal pellicle, which incloses the vitellus just like the rind which covers the flesh of an orange. This layer at first thickens at one side, and its substance seems to flow gradually to the lower pole of the egg till the resulting disk acquires the shape of a concavo-convex lens, with a thin, sharp rim. Eventually the sharp rim disappears, it becomes smoothly rounded at the edge, and the whole disk biscuit-shaped.

It is probable that an extremely thin layer of protoplasm, which originally formed part of the germinal pellicle or layer, still covers the vitellus, and is continuous with the disk, and is synonymous with the intermediary layer described by Van Bambeke and E. Van Beneden. My reason for this statement is the fact that the disk is sometimes found to have a thin layer of protoplasm extended outwards below its thick rim over the vitellus, but which becomes so thin a little way out from the edge of the disk that it becomes impossible to demonstrate it without special methods. That the vitellus is covered by a structureless membrane is proved by the fact that in the event of its being ruptured its glairy contents will very rapidly escape, and its torn edges can be seen limiting the opening or rent in its walls. The segmentation of the thicker part of this membrane of protoplasm next the disk is shown in Fig. 4, and is also described at considerable length by Van Beneden in the paper already referred to in a foot-note. In the crab-eater (*Elacate*) a very peculiar wreath of flat cells is developed at the edge of the disk, which, as in the ova of the supposed gadoid studied by Van Beneden, appear to be continuous, with a layer of small cells below the thicker lens-shaped part of the germinal disk.

It may be well to retain the term *intermediary layer* for this structure, but if the distinguished Belgian embryologist had succeeded in following the development of the ova which he studied a little farther along, he would have learned, as he indeed surmises, that the wreath of cells becomes the thickened rim of the blastoderm, and that the portion of the intermediary layer beneath the disk becomes the hypoblast. I see no reason, however, for adopting the term *blastodisc* for the germinal disk of the fish egg, for the whole of the latter, as well as the intermediary layer, are both unquestionably derived from the primitive peripheral germinal layer, the existence of which has been fully illustrated and described in the herring ovum by Kupffer,* though Coste † describes the formation of the germ in language which shows that he had undoubtedly seen and watched the process, which differs in different species, as we shall learn when we come to discuss them. I reproduce Coste's words below.‡ Kupffer describes the process minutely in the herring, but states that the germ is never formed independently of the action of the spermatozoan in that species, which is practically the fact in the case of the shad and cod, but is not the case in *Parephippus*, *Chirostoma*, and *Ceratacanthus*, where a germinal disk is formed independently of impregnation, but not until some time after the egg is laid. Even in the three last-named species the process of the development of the disk is the same, viz, the peripheral layer of germinal protoplasm aggregates at one pole of the vitellus. In some species, as in the herring, shad, crab-eater, and stickleback, strands of protoplasm pass inwards from the germinal layer among the yelk spheres or corpuscles of the vitellus, so as to involve the latter in a sort of meshwork, which, after the disk is formed, trend toward the center of the latter, forming a protoplasmic mass below the disk, and continuous with it, which probably fills a space in the yelk below the disk, and which Kupffer calls the "latebra" and Van Beneden the "lentille." Upon close examination, however, these structures seem to me to be nothing but a portion of the germinal disk, in consequence of their connection with the intermediary layer lying between the latter and the vitelline globe or yelk. The failure of more observers to witness the mode of development of the germinal disk, and the fact that some have actually figured the segmentation cavity without knowing what it was, is only explicable from

---

* Die Entwickelung des Herings im Ei, in Jahresbericht der Commission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel. 4to, Berlin, 1878, p. 181.

† Origine de la cicatricule ou du germe des poissons osseux. Comptes rendus, tome 30, 1850, p. 692.

‡ "Ses éléments générateurs restent épars, disséminés dans tous les points de ce vitellus, jusqu'au moment où l'action du mâle les détermine à se précipiter vers une région de la surface où on les voit tous se réunir pour constituer le disque granuleux que la segmentation organise plus tard."

"Quand cette curieuse *émigration* des granules moléculaires que doivent former la cicatricule s'est opérée, l'œuf des poissons osseux ressemble alors, mais alors seulement, à celui des oiseaux."

The writer, in explaining the formation of the germinal disk, is in the habit of describing it as an *amœboid migration* of the germinal matter toward one point on the vitellus, which is essentially the meaning of this quotation; but the germinal matter is not always mixed among the vitelline corpuscles, as Coste describes in *Gasterosteus*. It is the case in *Clupea* and *Alosa*, but not in *Gadus*, *Cybium*, or *Belone*.

the fact that most of the naturalists who have made the study of fish embryos a specialty have had the ill fortune to have the chance to watch only a part of the developmental stages.

THE IMPREGNATION OF THE EGG.

Upon this subject there are very few reliable observations. As Axel Boeck truly remarks, the micropyle is often difficult to find; and what makes the matter still more troublesome is the size of the egg, which makes it necessary to use lenses of long working distance, and to amplify with high power eye-pieces. To get an egg into position is not unfrequently a difficult performance, and by the time everything is arranged for observation impregnation has been effected and your efforts are wasted. It is doubtless correct to say that a single spermatozoan is effective in the fertilization of an egg. I have frequently found a number of dead ones sticking fast by their heads to the egg-membrane near the micropyle, but I have never witnessed their actual entry, although I have frequently made attempts to see the phenomenon, but so far without success. From all that we can learn it is undoubtedly true that the presence of spermatozoa with freshly laid unimpregnated ova at once tends to cause them to absorb water, as is well known to every practical spawn-taker. That their presence in the egg exerts a great influence on the rapid formation of the germinal disk in the herring, shad, and cod is equally certain. What the exact nature of the changes may be that are first of all induced by the presence of the spermatozoan in the egg of the Teleostean fish, we are not yet prepared to say. Most if not all the most satisfactory observations upon the phenomena of impregnation have been conducted on the very much more minute ova of invertebrates, where it has evidently been much easier to see the process and follow it in detail. Its effects are soon visible, however, as the remarkable phenomenon of segmentation which begins soon after fertilization has been effected.

SEGMENTATION OF THE GERMINAL DISK.

The segmentation of the germinal disk of the mackerel is essentially similar to that of the cod. The first cleavage is transverse, resulting in two cells. The next segmentation is at right angles to the first, and, when completed, divides the two cells of the first cleavage into four; the next cleavage is in a direction at right angles to the last and results in the formation of eight cells. Beyond this point the cleavage becomes more or less irregular, except that the germinal disk remains for a considerable time composed of a single stratum of cells, as shown in Fig. 3, one hour and forty minutes after impregnation. The rhythmical nature of the process of segmentation up to this time has already been alluded to, and it no doubt continues, but the cells soon become too small to be followed up so as to observe the intervals of rest and activity. At the end of three hours the germinal disk, as shown in Fig. 4, has undergone profound changes; the cells are no longer arranged in a single stratum, but in several, superimposed upon each other, which has been the result of the segmentation of the cells of the morula stage of Fig. 3 in a plane parallel to that of the great diameter of the disk. At the same time there has been a wreath of flat cells segmented off from the edge of the disk, which would be considered by some as originating separately or independently of the disk, an opinion from which I dissent for reasons which I have already stated in dealing with the origin of the disk from a primitively homogeneous, external germinal layer of protoplasm.

THE BLASTODERM AND SEGMENTATION CAVITY.

In Figs. 5 and 6, a half hour later, we see that the fate of the marginal cells has been to form the rim of the incipient blastoderm, which is beginning to spread out, become thinner, and lose its distinctive features as a biscuit-shaped germinal disk. The inner edge of the rim of cells just alluded to limits the margin of the segmentation cavity of the mackerel egg, and I can see no reason why this space should not be considered homologous with the cavity which bears the same name in the blastoderm of the embryo elasmobranch and chick, in both of which it is probably of greater extent than it has been hitherto suspected. The roof of the cavity is at first two or three or even more cells deep, but as soon as the rudiment of the embryo fish is defined at the edge of the blastoderm its roof soon after is found to be composed of but a single layer of cells, corresponding to the epiblast or skin layer, while its floor is the *intermediary layer* of Van Bambeke, and corresponds to the hypoblast, mucous or deepest layer from which the intestine, the blood for the most part and perhaps the notochord is derived as development advances. With slight verbal alterations I will here quote from what the writer has published elsewhere*: "The disk begins to spread over the vitellus or yelk, and soon acquires the form of a watch glass, with its concave side lying next the surface of the yelk. Coincident with the lateral expansion of the disk, now become the blastoderm, a thickening appears at one point in its margin, which is the first sign of the appearance of the embryo fish. With its farther expansion the embryo is developed more from the margin of the disk towards it center; in this way it happens that the axis of the embryo lies in one of the radii of the blastoderm, its head directed towards the center, its tail lying at the margin" and continuous with the rim, which is soon two or three cells thick, and extends all the way round the edge of the blastoderm like a ring. "Before the embryo is fairly formed a space appears in the blastoderm, limited by the thickened rim of the latter, and the embryo at

* Structure and ovarian incubation of the Top-minnow (Zygonectes), "Forest and Stream," August 18, 1881.

one side. This space, *the segmentation cavity, is filled with fluid and grows with the growth of the germinal disk, as the latter becomes converted into the blastoderm, and does not disappear until some time after the embryo has left the egg as a young fish,* after remaining as a *space* around the yelk-sack as long as a vestige of the latter remains, as may be seen in the young of *Cybium, Gadus, Elecate, Syngnathus,* and *Alosa.*"

My observations have been conducted without hardening reagents, since it has been found that such methods abstract the water from the embryo, and cause the segmentation cavity to collapse and be obliterated, so that the only way in which the writer has been able to follow the history of this space was to study it in the living transparent eggs, which may be got into various positions so as to show all the phases of its development in the different stages of the evolution of the embryo. I believe it will be found to be present in the blastoderms of the ova of almost all teleostean fishes.

"Should this prove to be the fact [quoting from the same source] the teleostean egg will be as distinctly defined, in respect to the sum of the developmental characters which it presents, from the developing ova of other vertebrates, as the adult teleost is from the remaining classes of the subkingdom to which it belongs."

Later, as is shown in Fig. 7, or after seven hours, the blastoderm has grown so as to inclose nearly one-half of the vitelline globe or yelk, the rim is very distinct, and when viewed from above as a transparent object, the segmentation cavity is visible as a somewhat crescent-shaped area more transparent than the embryo or the rim. The embryo bounds the concave side of the crescent and lies in immediate contact with the yelk, except over a small space just under the fore part of the head, which is found to be continuous with the segmentation cavity beneath the latter; this space will be found to be very significant, and is the cavity in which the heart develops. In taking another look at Fig. 7, it will be noticed that the blastoderm is a hemispherical cap, and that on the left hand from its center to the edge of its rim there is a thicker portion shown; this is the embryo mackerel seen from the side with its head end lying in the middle of the disk and its tail at the edge. To the right hand and below a clear space is shown; this in like manner is the segmentation cavity seen from the side, and to the right of it the blastodermic walls are seen to be double, consisting internally of the hypoblast and externally of the epiblast, with a space, *sg*, between them; this is the segmentation cavity in optic section, which is seen to extend a little way under the head of the embryo at *crs*, to form the cavity in which the heart will be formed. To the left of *crs* the keel or carina, *cr*, of the embryo dips down into the vitellus; the carina is simply the fore part of the medullary canal, which for the most part becomes the great median nervous or spinal cord of the young fish; in all embryo teleost fishes it is much flattened laterally in its fore part, and in consequence it dips down far into the yelk as a flat tube, for such it is from the fact that a furrow appears on the outer surface of the blastoderm known as the medullary groove which extends from the head end of the embryonic area of the blastoderm to the rim and which causes the blastoderm to be pushed down before it. This groove first closes at the head of the embryo, while it remains open for a considerable time at the tail end. The cells of its walls form the embryonic spinal canal which afterwards becomes the spinal cord, brain, and retina of the more advanced embryo.

Fig. 8 represents an older embryo Spanish mackerel, eleven hours after development began. It is seen with the head towards the observer, and behind or beyond the head on the opposite side of the egg the rim *r* of the blastoderm is seen through the transparent vitellus, which will close over the latter entirely in the course of another hour. The segmentation cavity *sg* is shown in optic section between the epiblast *ep* and the hypoblast *hy*, extending all round the egg except the portion taken up by the embryo above, and that part not yet covered by the blastodermic rim *r* on the opposite side of the egg.

Fig. 9 represents an egg of the twelfth hour of development with the caudal pole turned towards the observer. The small area *y* is all of the vitellus which now remains uncovered, and the blastodermic rim is contracting and will soon close at the end of the tail of the embryo, when the formation of the blastoderm may be said to be completed. The segmentation cavity *sg* has of course been somewhat extended on account of the approximate closure of the blastodermic rim. In Fig. 11 a section is carried transversely from left to right through the opening *y*, which still remains behind the tail of the embryo represented in Fig. 9, to show how the segmentation cavity is finally limited at the caudal end of the embryo by the blastodermic rim, which after its closure takes a large share in the formation of the tail of the young fish, as pointed out by Kupffer and His. It may be considered the true tail-swelling, as it thickens into a round, knob-like prominence immediately after the inclusion of the yelk is accomplished. In consequence of the remarkable extent of the segmentation cavity in fish embryos, as amply proved by our studies upon a number of widely-separated genera, the yelk becomes inclosed in a shut sack derived from and including the greater part of the innermost embryonic layer or hypoblast, which is surrounded by a film of fluid, and which is again inclosed by an external epiblastic sack. Besides the demonstration of this structure in the living egg I have used the following method with success: Immerse a living embryo in a one-half per cent. solution of osmic acid for a few minutes, or till the outer sack becomes light brownish, then place it under a Fol's compressor and gradually bring pressure to bear on the vitellus while it is under observation in the microscope, when the outer covering, now made brittle by the osmic acid, will rupture and expose the vitelline or hypoblastic sack; the epiblastic covering will frequently open, wrinkle, and slide back off of the vitellus like a cowl when pushed back off of the head.

It is this structure which Cuvier and Valenciennes* allude to in the following words: "Le vitellus a deux tuniques, complètes l'une et l'autre, quoique très-fines. L'externe se continue par sa lame extérieure avec le peau et par l'intérieure avec le peritoine; l'interne, très-vasculeuse, se continue avec les membranes des intestins et leur tunique péritonéale; la cavité donne directement et visiblement dans celle de l'intestin, et la matière du jaune y afflue." This quotation shows that Cuvier, to whom it is in all probability to be ascribed, was aware of the existence of a double envelope over the yelk, but in no instance have I found what could be considered a communication between the cavity of the intestine and the vitellus. Von Baer is stated by Balfour† to speak of two types of yelk-sack, one inclosed within the body wall, and the other forming a distinct naked ventral appendage of the embryo, from which it is clear that the great German embryologist never clearly understood the manner in which the vitelline globe or yelk is inclosed by the blastoderm. Nor can I confirm Lereboullet's view that a connection of the vitellus and cavity of the intestine exists between the stomach and liver, because the stomach is not ordinarily differentiated in young fishes while the yelk persists. In the young California salmon (*Oncorhynchus*) the muscle plates grow down on either side between the epiblast externally and the splanchnopleure internally. The hypoblast covering the remains of the yelk is traversed externally by a network of blood vessels, as may be learned from an examination of transverse sections through advanced embryos. In this way it results that the segmentation cavity is obliterated or filled up during the latter part of embryonic life by the down growth of the somatopleure and splanchnopleure between the epiblast and hypoblast, both the splanchnopleure and somatopleure having originated from the mesoblast. This, I believe, is essentially the mode of development of the blastoderm in teleostean embryos and the way in which the segmentation cavity disappears. Any view, however, according to which the yelk is looked upon as a mere nutritive vesicle, and not at all times in intimate organic union with the embryo, betrays a want of comprehension of the way in which the teleostean ovum is developed in the ovary, the manner of the formation of the germinal disk, the development of the blastoderm and inclusion by it of the vitellus, and, finally, of the relation of the heart and blood system to the vitellus.

STRUCTURES DEVELOPED IN THE EMBRYO MACKEREL FROM THE ELEVENTH HOUR TO THE TIME OF HATCHING.

Starting with the stage represented in Fig. 9, when the medullary canal *m*, in section, the notochord or cartilaginous axial rod *ch*, and the somites or muscle plates on either side of the medullary canal are developed, it is apparent that the embryo by the fourteenth hour, repre-

---

* Histoire Naturelle des poissons, tome i, p. 399. Paris, 1828.

† Comparative Embryology, vol. ii, p. 65.

sented in Fig. 10, begins to exhibit some likeness to an animal organism, but as yet the species would not be recognizable were it not known from what parent form the egg had been obtained, though it could undoubtedly be referred to the class *Pisces*, subclass *Teleostei*. A careful study, however, of a number of forms will enable the student to distinguish them apart at a very early period of development. The number of somites or muscle plates increases from before backwards; they first appear some distance behind the rudiments of the ear, *au* Fig. 8, and by the regular successive segmentation of the upper mesoblastic plates of cells on either side of the medullary canal increase in number backwards toward the tail, and by the time the caudal swelling is developed there are about twenty muscular somites or segments of the body. As the tail grows and is extended backwards the segmentation of the muscular stratum of the mesoblast continues in the same way from before backwards, but does not for a considerable time involve all of the structure destined to become the lateral muscular masses of the young fish; the portion at the end of the tail remaining unsegmented for some time after hatching. The muscular segments, somites, or protovertebræ, as they have been variously named, originate from the two tracts of mesoblastic tissue on either side of the medullary canal, and are really the rudiments of the muscular masses, the edible flesh portions found on either side of the backbone of adult fishes. They appear at first in the embryo as quadrate masses lying on either side of the medullary canal, and in embryo sharks, according to Balfour, and in the lampreys, according to Scott, at first are said to be hollow; in our studies we have not succeeded in demonstrating this peculiarity in teleostean embryos. They are nearly or quite solid in the latter.

Immediately after the tail swelling has been formed, the caudal rudiment forms a blunt rounded point, which, as it is prolonged backwards, develops a continuous median dorsal and ventral ridge or fold of epiblast, as shown in Fig. 12, and which becomes the natatory fold *nf* of Fig. 13, from which all the unpaired fins are developed. Almost as soon as the tail begins to grow out a strand of hypoblastic cells, *v*, Fig. 12, is seen on the lower side of it, lying between the epiblastic layers of the natatory fold, and extending to the edge of the latter; this strand of cells appears to have been probably continuous with the medullary canal or cord on the dorsal side of the embryo before the tail swelling grew out, so as to break and obliterate its connection with the former. This strand of cells, which is seen to be apparently tubular, is the anal extremity of the gut, and seems to be closed posteriorly. It is found to extend forwards, as development proceeds, as a flattened tube, lying just below the notochord or cartilaginous axis of the embryo as shown at *i*, Figs. 12 and 13. The intestine was probably continuous with the medullary canal posteriorly, from the hinder extremity of which it has possibly been invaginated from above, in which case the *gastrula* stage of development of the teleostean fish embryo would be perfectly homologous with that of many other vertebrates. Our observations, it is admitted, do not rest upon the evidence presented by sections, but upon the appearances of the living transparent objects. The intestine is at first solid anteriorly; its lumen is a mere capillary tube on its first appearance behind. It does not appear to originate directly from longitudinal folds of the hypoblast which coalese in the middle line below, in some higher forms, but as a nearly solid band of cells just beneath the notochord.

It will be proper to discuss in this place the nature of the peculiar vesicle first described by Kupffer, and known as Kupffer's vesicle, but recently renamed the post-anal vesicle by Balfour.* It appears in all the cases which we have observed either some time before the closure of the blastoderm, or nearly at the time it closes. It is situated just beneath the tail, between the latter and the yelk. It appears in the Spanish mackerel before the blastoderm closes, and, as far as I can make out, is simply a vacuole filled with fluid, the direct connection of which with the posterior end of the rudimentary intestine, as has been held, has still to be satisfactorily demonstrated. In some forms it persists for a considerable time after the closure of the blastoderm, and is so far anterior to the point of closure that it is difficult to see how it can stand in a post-anal relation to the gut, the anal portion of which is developed almost at a point coincident with that where the closure of the blastoderm takes place, and behind the position of the vesicle. Moreover it is usually asymmetrical in position and finally disappears. Its form and position vary also in different eggs, so that I am at a loss to clearly understand its significance. That it cannot originate at the moment of the closure is proved by the fact that in some forms it is present when the blastoderm has covered but three-fourths of the yelk. I have never seen any communication between it and the medullary canal; however, its further discussion will be resumed when we come to consider forms in which it is more prominently developed. It clearly remains a fact, however, that the anal part of the gut is the first to be developed; that the œsophagus for a time appears to be a solid band of hypoblast cells below the head, while the point where the mouth will open is not indicated until twenty hours after the young fish has escaped from the egg; the vent therefore appears about thirty hours before the mouth.

The notochord *ch* appears in embryos eleven hours old as a rod of cells not different in character from those of the other portions of the blastoderm, but shortly afterwards in the region of the trunk of the embryo clearer cells make their appearance in the notochord, lenticular in form and arranged transversely to its axis. They may be seen to grow larger and larger until the primitive chorda cells form only thin transverse partitions, between which the large, clear cells are developed. Eventually the partitions entirely disappear, when the large, trans-

* Comp. Embryol., ii, p. 61.

parent cells become crowded upon each other, and now compose the entire medullary substance of the notochord, which is functionally the backbone or axis of the embryo fish. This metamorphosis of the primitive chorda cells begins about the twelfth hour in the embryo mackerel, and is completed by the time of hatching, and, like in the herring as described by Kupffer, the caudal end of the notochord is the last to undergo the change. The notochord for a considerable time after hatching does not become distinct at its caudal end from the cellular mass in which it terminates. The membranous sheath in which the notochord is inclosed seems to be differentiated when the metamorphosis of the primitive chorda cells into the clear axis rod or notochord has been completed. I have not succeeded in demonstrating from which one of the primary embryonic layers of the mackerel the primitive chorda and consequently the notochord are derived; the weight of the evidence afforded by the researches of others would appear, however, to indicate that it is split off from the lower edge of the keel or carina of the medullary canal just over the hypoblast.

The axis of the embryo is at first marked by a shallow groove which, by the time the blastoderm is closed, is completely obliterated, the last portion of it to disappear being the caudal. The blastoderm is pushed down before this groove, and when the latter closes dorsally and the medullary canal, neural canal, or neurula is formed, as it has been variously called. Certain anterior portions of it become differentiated into the various parts of the brain. Primitively the brain of the mackerel is much compressed laterally, as shown in Fig. 8. At the extreme anterior end a pair of lateral outgrowths, at first apparently solid, appear as the rudiments of the eyes, *opv*. The basal portions by which they are in communication with the fore part of the brain become partially the optic or second pair of nerves. With the more advanced development of the embryo these outgrowths become hollow and cup-like, the retina of the eye is developed on their inner surfaces, while a mesoblastic layer of pigment cells is developed on the outside to form the choroid coat. The cup has a cleft in its lower margin which closes later, and is known as the choroidal fissure. Covering the optic cups is the embryonic epithelial stratum of cells; from it an induplication is pushed into the cups, which is eventually constricted off from the parent layer, and becomes differentiated into a highly refringent spherical lens. Between the lens and the floor of the cup a space is formed very early which becomes the vitreous humor of the eye, and in front the lens is again roofed over by a very thin concavo-convex hyaline membrane, the cornea, likewise derived from the epidermis, between which and the lens the aqueous humor is confined. The iris appears to be developed from the extreme edges of the optic cup and becomes very brilliantly pigmented in a few days after the fish is hatched. The anterior part of the brain, from which the optic cups grow out, becomes the cerebrum or fore brain, in part, also, the optic chiasma. The spinal cord or medulla is at first nearly a perfectly solid cord or strand of cells; a canal makes its appearance in its center after the muscular somites have been differentiated.

The rudiments of the ears, or auditory organs, in the embryo mackerel make their appearance soon after the optic cups, as slight elevations or welts on each side of the region of the embryonic hind brain, *au*, Fig. 8. The ridge or welt is simply the lip or prominent border of the auditory pit, which is being pushed inward from the outside in a cup-like manner from the inner sensory layer of the epiblast. It soon, however, becomes a closed sack, Fig. 10, *au*, and by the eighteenth hour two calcareous otoliths are visible in it, as shown in Fig. 12. The complications of structure which develop in the ear beyond this point relate chiefly to the formation of the semicircular canals, and these are developed some time after hatching as ridges or folds on the inner surface of the auditory sack, the walls of which grow inward from above and laterally, joining each other in such a manner that the anterior and posterior vertical and horizontal semicircular canals are limited by them; the sacculus and the otoliths lodged in it, consisting of the asterisk and sagitta, finally occupy the lower anterior part of the sack, and the auditory, or seventh nerve, enters it in their vicinity. The auditory sacks, or vesicles, are now almost, or quite, as large as the eyes, and lie on either side of the cerebellum *cer*, and medulla oblongata *mo*, as shown in Fig. 17.

The nasal pits *na*, Figs. 10 and 12, are at first simple saccular depressions differentiated from the epiblast in front of the eyes, between the latter and the anterior end of the fore brain. At the age of one week, Fig. 17, *na*, they are neat, cup-like structures, situated some distance from the edge of the upper border of the mouth just in front of the eyes. At this time it is already possible to demonstrate special sensory cells in their walls. At a still later period the nasal pits are bridged over transversely by a coalescence of a part of their opposite edges, so that an anterior and a posterior opening is formed; these communicate with each other beneath the bridge of tissue, and constitute the external nares or olfactory organ of the type characteristic of the true fishes. At what period this last type of structure is developed in the mackerel has not been learned, as it was not formed in the oldest embryos studied by the writer.

The several portions of the brain begin to be clearly marked off from each other at the eighteenth hour, when the fore-brain or cerebrum, the mid-brain between the eyes, and the medulla oblongata behind the latter may be distinguished. When the young fish is hatched, Fig. 13, all of the divisions may be distinguished, as the cerebellum is now clearly marked off from the medulla. When the medullary groove closed in the region of the brain, a laterally flattened tube was the result, and there is no such extensive anterior downward flexure of the brain on itself as is observed in higher types. As the various constrictions appear in the walls of the brain tube, the cavity inside becomes divided into the so-called ventricles or cavities of the primitive cerebral vesicles. As development proceeds the cerebral vesicles rapidly dilate in a lateral direction, especially the mid-brain *mb*, in which a surprisingly spacious cavity is formed in some species, which answers to the passage-way from the third to the fourth ventricles of the higher forms. Between the fore-brain and mid-brain the pineal gland *pn* is developed; while the hypophysis cerebri or pituitary body depends from the floor of the brain down between the trabeculæ cranii. The fore-brain is at first not bifid or divided into hemispheres; its division occurs comparatively late in embryonic life. The mid-brain is the most conspicuous portion of the encephalon or entire brain of the young fish, and soon after hatching its lateral free lobes grow backwards and downwards somewhat at the sides, and more or less extensively cover the cerebellum.

At fourteen hours the embryos begin to show signs of the development of pigment just below the superficial layers of the epiblast; these cells are at first scattered irregularly over the body of the embryo and gradually grow darker; as they do this they also become irregular in form and flattened, with a number of points running out from them, as shown in Fig. 12. Later they tend to aggregate on certain parts of the body, as shown in Fig. 13, where they form a band on the tail and spots on the back; as the embryo becomes still older a band of them is formed behind the ear. They are now still more irregular in form and have evidently rearranged themselves very remarkably since the fourteenth hour; the rearrangement appears to be accomplished by their migration towards definite points by means of an amœboid movement of their entire substance. When fully developed the nucleus becomes very distinct, enveloped as it is in very dark protoplasm, and the prolongations of the latter look not unlike the pseudopods of those remarkably simple animals the *Amœbæ*.

By the eighteenth hour the oil sphere found embedded in the yelk of the Spanish mackerel was observed to be enveloped in a mantle of cells apparently of hypoblastic origin, which fastens it firmly to the wall of the yelk sack below and opposite the embryo, Fig. 12. By the time the young fish is ready to hatch, the covering of the oil sphere is found to be more or less covered with pigment, which seems to have in part developed in the cellular mantle, as indicated in Fig. 13. The fixation of the bouyant oil sphere to the ventral wall of the yelk sack makes the latter bouyant, so that when the young fish escapes from the egg-membrane it is turned wrong side up, and is not until some time after hatching that it has the power to right itself and counteract the bouyancy of the globe of oil.

The heart of the young mackerel, like that of the cod, originates in a mass of mesoblast cells, which are coarser in character than those in the immediate neighborhood; they appear to be budded off from the mesoblastic roof of the cardiac portion of the segmentation cavity lying beneath the head; at first there is no definite arrangement of the cells destined to become the heart, but they seem to be spread out in a loose mass between the hypoblast and the mesoblast at the point where the heart will appear. As soon as they have grown down and come into contact with the hypoblast a circular space or cavity is formed in their midst, which is the rudiment of the heart of the mackerel in its simplest possible form. It is now nothing more than a wide circle of coarse cells interposed between the mesoblast and hypoblast, so that one may look through the lumen or opening in the ring either from above or below. In the process of growth this ring of cells is drawn out into the primitively simple tubular heart, the hypoblastic or venous end being dragged forward while the branchial or aortic end is directed backwards. Thanks to the transparency of these embryos, every step of the process may be seen just as I have described it. By the eighteenth hour the heart *h*, Fig. 12, is fusiform and open at the venous end, and still bound to the hypoblast, and now begins to contract slowly and at long intervals, although there are still no blood corpuscles visible in the fluid held in its cavity. The next change observed is from this point onwards, when its anterior end is bent to the left and finally opens backward, and it is now clearly determined that the wide backwardly directed portion will become the venus sinus and Cuvierian ducts; the point where the bend is made will become the ventricle and the other narrow end the bulbus aortæ. At no time, nor in any form, have I seen any evidence of the origin of the cavity of the heart by the coalescence of two distinct spaces, as described in the development of other types of vertebrates.

The embryo on the eve of hatching has a relatively shorter tail than most other types of true fishes, and when just hatched measures a little more than one-eleventh of an inch in total length. It usually escapes head first from the egg, and manifests a singularly quiescent disposition, but as it grows older and begins to right itself, as its oil sphere becomes smaller, it will settle on the bottom of the vessel in which it is confined, but if disturbed it will dart off and out of the way with great quickness, and shows a disposition to avoid danger. The yelk has diminished in bulk before the egg-membrane is ruptured, because the embryo fish has grown at its expense, and a considerable quantity of protoplasmic matter has doubtless been budding off from it in consequence of the formation of free nuclei, which are found, in other species at least, in the superficial layers of the yelk just below the embryo. The diminution of the bulk of the yelk is not due to the development of the blood, which is not yet discoverable, nor will it appear until some time after the young fish has left the egg.

The rudiments of the breast fins appear just before hatching as a pair of delicate rounded folds, which, have a horizontal direction at the base, and which grow out on each side of the body in the vertical from the oil sphere. They may be regarded, therefore, as having very little genetic affinity to the gill arches, from which they are separated by an amazingly wide interval, as shown in Fig. 13, in which their position is indicated at *ff*.

STRUCTURES DEVELOPED IN THE YOUNG SPANISH MACKEREL AFTER HATCHING.

Twelve hours after the young fish has left the egg-membrane it has the appearance represented in Fig. 14; the yelk has diminished very perceptibly in size, while the anterior end of the head is considerably prolonged forwards as compared with its condition in the recently-hatched embryo shown in Fig. 13. The yelk in collapsing also leaves the hind portion of the intestine in an apparently more posterior position, while behind the latter the rudimentary urinary bladder *al* is very distinctly shown as a vesicle from which the simple tubular prolongation of the Wolffian duct *wd* passes up behind and above the intestine. The Wolffian duct appears to develop comparatively late in the mackerel, as it is difficult to make out anything corresponding to it before the young fish leaves the egg. It would appear to originate from the peritoneal (splanchnopleural) wall of the abdominal cavity, and to be continued on either side into the urinary vesicle or bladder *al*, which opens outward immediately behind the vent. During the latter stages of development, or from the fourth to the seventh day, the hind portion of the Wolffian duct acquires a decided flexure just before it enters the bladder, as indicated in Figs. 16 and 17. This hinder portion may be considered to probably represent the rudiments of the urinary ducts or ureters of still more advanced stages of development.

"The excretory system commences [in true fishes] with the formation of a [longitudinally disposed, paired] segmental duct, formed by a constriction of the parietal wall of the peritoneal cavity. The anterior end remains open to the body cavity, and forms a pronephros (head kidney). On the inner side of, and opposite this opening a glomerulus is developed, and the part of the body cavity containing both the glomerulus and the opening of the pronephros becomes shut off from the remainder of the body cavity, and forms a completely closed Malpighian capsule. The mesonephros (Wolffian body) is late in developing."*

I believe it possible, however, from my own studies on some forms, to show that a system of segmental tubes joins the segmental ducts at a late stage of development, which pass into glomeruli which lie for the most part along the middle line of the dorsal wall of the abdominal cavity, close to the median dorsal aortic vessel.

Upon the origin of the generative structures of the young mackerel I have made no observations, but their position in the adult would show that, as in other fish-like types, they must originate as specializations of tissue tracts on either side of the mesenteric suspensor of the intestine.

---

* Comp. Embryol. Balfour, ii, p. 63.

In Fig. 14 a large sinus, *ss*, is shown just over the brain, which is roofed over by the dermal and deeper layers of the epiblast; the cavity so formed is filled with fluid and persists for at least a week, as shown in Fig. 17. Below the hinder part of the head and at its sides, in Fig. 14, the branchial furrows *br* are visible; as these become deeper they are finally broken through into the wide branchial chamber of the fore-gut, as the gill-clefts. This takes place about twenty-four hours after hatching, and is on the eve of accomplishment in Fig. 15, in which the point where the mouth will appear is indicated at *m* on the lower side of the head; just behind the point where the mouth will soon break through, the rudiment of the lower jaw has made its appearance as Meckel's cartilage, *mk*. Above and behind it the cartilaginous rudiments of the branchial skeleton or framework of the gills have been in part developed. It will also be noticed that the head is higher and that the brain is bent downwards more than in Fig. 14, in consequence of the decrease in size of the yelk sack. The mandibular arch, the forepart of which is indicated at *mk*, does not yet reach forwards on a line with the end of the snout. While the hyoid arch, or that immediately following the mandibular, is still more or less obscured by the latter, and covered up by it externally, behind which the branchial arches or rudimentary gills are more crowded together from before backwards than in the stage shown in Fig. 14. The trabeculæ cranii have, however, been developed, but are still rudimentary, being present only as a pair of symmetrically disposed longitudinal rods beneath the brain. The eye, which was not heretofore completely pigmented, is now quite black and opaque, the choroid layer being developed. The ear and nasal pits have undergone farther development; the latter are present as distinct cups in front of the eyes. The heart has undergone considerable advance, in that its posterior end is now directed upwards, while the ventricle and bulbus aortæ are more fully developed and actively pulsating, although the blood system is still imperfect, no true aortic or venous channel having as yet been developed; however, there is already a partial branchial and cephalic circulation, but the blood channels are not yet supplied with a sufficient number of blood corpuscles to mark their courses distinctly by the color. The cavity in which the heart now lies is bounded in front by the branchial structures, below by what was formerly the anterior portion of the epiblastic sack covering the yelk, behind by the hypoblastic walls of the yelk sack, above by the intestine and body walls. In this and earlier stages of development I believe that I have seen blood corpuscles swimming freely in the cavity surrounding the heart, which would indicate that they had been derived by budding directly from the hypoblast, which is beyond the shadow of a doubt the way in which they are developed in the embryo silver-gar, or bill-fish (*Belone*). The yelk sack is shown much reduced and more strongly pigmented in Fig. 15, while the oil sphere has undergone considerable reduction in size. The widest portion of the young mack erel is now from side to side, through the eyes, the body seeming to be concentrated towards the head. The breast fin *bf*, Fig. 14, is still a semi-circular lobe, with its base nearly horizontal, but it has been advanced forwards somewhat twelve hours after hatching. It is merely a flattened epiblastic pouch into which has grown a tract of mesoblastic tissue. Twenty-one hours after hatching the breast fin has acquired a vertical position, as shown in Fig. 15, while the coraco-scapular cartilage *csc* is in its incipient stages of development in its base. Up to this time the intestine has maintained its primitive character as a horizontally flattened tube, which it will not begin to lose for twenty-four hours more, but below the breast fin a swelling has appeared in its lower wall, *lv*, which is the rudiment of the liver.

Embryos on the fourth day after development have the appearance shown in Fig. 16. The most marked advance which has made over the stage, shown in Fig. 15, is that the intestine has acquired a cylindrical form, and is hollow or tubular, while it also has been bent upon itself in its middle region. The liver is now a very prominent ventral sack-like outgrowth from the lower side of the intestine at *lv*, just in front of the bend in the alimentary canal. Its structure already shows a lobular character, its walls being subdivided into the rudiments of hepatic follicles. The epithelium over the rest of the inner surface of the intestine becomes differentiated into follicles at a very early period, which would indicate that the mucous membrane of the intestine had a specific, probably digestive function, as soon as the follicular structures were developed, which is accomplished about the time the young fish commences to feed. Outside of the epithelial layer the annular and longitudinal muscular layer of the intestine appears about the same time, and peristaltic movements of the intestinal walls begin to be manifested almost as soon as the intestine becomes tubular. Above the liver, and a little way behind it, a diverticulum appears on the fourth day, usually more or less obscured by pigment cells, which I regard as the rudiment of the air-bladder; by the seventh day it is much more plainly developed, as shown in Fig. 17, *ab*. On the fourth day the young fishes will begin to feed, as represented in Fig. 16, where the black mass *f* represents the remains of food in the hind gut which the animal had swallowed. From an examination of several specimens I am not able, however, to state what this food was, as it was in a much too disorganized condition to tell of what it originally consisted. The oil sphere *os* is now nearly absorbed as well as the yelk, which is entirely gone. A portion of the anterior wall of the yelk sack, however, has remained as a septum, partially shutting off the pericardiac cavity from the body cavity, in which the viscera are contained; it stretches across between the lower ends of the coraco-scapular cartilages *csc*. The circulation is now fully established, there being an aorta and cardinal veins, *cv*, which return below it to carry the blood back to the heart, a portion, however, first passing through a vascular network over the viscera and represent-ing essentially a portal system of vessels. The blood is forced through the branchial arches, and passes from them into the carotid arteries and aorta, to return to the heart again by way of the jugular and cardinal veins and visceral network. The venous or dorsal end of the heart is divided by the intestine, the Cuvierian ducts opening on either side of it upwards and backwards.

It will be worth while to here notice the fact that in the mackerel there is nothing which is comparable with a system of vitelline vessels, such as is found in the young salmon, stickleback and silver gar, but that the venous end of the heart is throughout the whole of embryonic life closely applied to the surface of the yelk sack (see Figs. 14, 15, 16, and 17), so that it appears at times almost like a parasite sucking at the vitellus. Stray colorless blood corpuscles may sometimes be seen in the pericardiac cavity, say about a day after hatching, which would indicate that the genesis of the blood from the vitellus was essentially the same as that in the silver gar, where the fluid surrounding the heart contains multitudes of colored blood disks, and where one can observe them in every stage of metamorphosis from the substance of the vitellus, at the point where the long tubular venous sinus of these embryos joins the hypoblast of the yelk. At the end of the fourth day the mouth of the young mackerel is wide open and the lower jaw, in consequence of the greater length of Meckel's cartilage, reaches nearly as far forward as the upper jaw or snout. The mouth is also frequently opened and closed at this time by the mandibular and hyoid muscles. The chondrocranium has also advanced in development in all of its parts, since the auditory capsule is now clearly inclosed in an investment of cartilage cells, which are joined to the notochord anteriorly by the development of the so-called parachordal cartilages. Under the brain, the primitive cartilaginous bars, the trabeculæ cranii, are developed, and an oval space exists between them, into which the hypophysis cerebri or pituitary body dips downwards. On either side, and below the hinder half of the eyes, the pterygoid cartilages have been developed at the same time hyoid, quadrate, hyomandibular, ceratohyal, branchial, and coraco-scapular elements have advanced in development.

The method which I have found the best to demonstrate the cartilages of the head in fishes as small as the mackerel, which measures only a little over one-seventh of an inch at this stage, is the following: crush a recently killed specimen under a Fol's compressor so as to flatten it sufficiently to let the light through it, then use a one per cent. solution of acetic acid so as to bring out the contours of the cartilages with their cells and nuclei. If this is carefully done there will not be sufficient displacement or disarrangement of the parts of the skull to render their identification at all difficult.

Figure 17 represents the head of a young mackerel nearly a week old dissected so as to show the structure of the skull as nearly as such a difficult subject admits of representation. The parts of the chondo cranium are lettered so that their names may easily be made out from the list of reference letters. I have attempted to show the arrangement of the pavement of choroid cells, *cc*, in the eye as may be shown in preparations mounted in balsam. I would also direct especial attention to the conical teeth represented on the epithelium of the lower jaw. These seem to be developed in epithelial pits and are not in direct connection with the skeleton of the jaw, which is, moreover, not yet bony. They surmount little epithelial papillæ and grow by the addition of material from below; their composition does not appear to be calcareous, but corneous, since I find them to resist the action of acids. Rathke has described teeth of a somewhat similar character in the embryos of the viviparous blenny (*Zoarces*).

In embryos of this age the branchial leaflets are also developed. They at first appear on the posterior border of the gill arches as small papillæ, which, as they elongate, throw out processes from their edges, so that they eventually acquire a bipinnate structure. In these bipinnate fleshy processes capillary loops are formed, which communicate between the branchial arteries and veins. The leaflets with their capillaries are the agents directly concerned in the aeration of the blood of the young fish.

The coraco-scapular rods *csc*, although apparently cartilaginous, have an histological composition different from the cartilages of the head, being much more hyaline. It is also embedded in a vertical fold which extends some way beyond the upper and lower borders of the breast fin. This may be called the pectoral fold. It is not at all improbable that we will yet find embryo Teleosts in which there are continuous lateral folds, for we already know species in which the primitive natatory fold is discontinuous at a very early age. Such is the case with *Hippocampus* and *Syngnathus*, according to my own observations this season. *Hippocampus* never develops a caudal fin, so that we would naturally not expect to find the natatory fold prolonged over the end of the tail; but the posterior position of the early rudiments of the pectorals in *Cybium* and *Parephippus*, it appears to me, is a very strong argument against their origin from a posterior branchial arch; indeed, it is the strongest yet offered against that doctrine by any data derived from a study of the development of the paired fins of Teleosts. In other words, since we now know that the natatory fold, from which the unpaired median fins are developed, is sometimes discontinuous, I see no reason why we should not expect to find the lateral fin-folds discontinuous, as there are more reasons why they should be so in the Teleost than in the Elasmobranch embryo. In fact, it would appear that the greater the longitudinal extent of the unpaired fins in proportion to the length of the body of the adult the more likelihood there is of finding a continuous dorsal and ventral natatory fold developed in the larva, and *vice versa*. The longitudinal extent of the paired fins of Teleost fishes is less, vastly less, in respect to the number of rays, than those of the Elasmobranchs, and in consequence of this difference alone we should not be surprised to find lateral fin-folds of considerable extent in the embryo of the latter and of limited extent in the former. Viewed in this way, we may prove too much for the doctrine of the origin of the paired fins from lateral folds. The truth of the matter appears to be that we ought to quietly wait for more facts before generalizing with the data obtained from only one group.

SUMMARY OF ANATOMICAL RESULTS, BASED ON ALL OF THE SPECIES STUDIED BY THE AUTHOR.

The following are the more important anatomical and embryological facts which have been ascertained; only such as are essentially new to science or which receive new or fuller interpretations are noticed:

1. The segmentation cavity or blastocœl is developed with the growth of the blastoderm so as to almost entirely surround and include the yelk, and persists until late in embryonic life.

2. The somatopleure (muscular layer) and splanchnopleure (peritoneal layer) grow down into the segmentation cavity on either side between the epiblast and hypoblast (*Oncorhynchus*).

3. The heart develops in an extension of the segmentation cavity beneath the head.

4. The blood is of hypoblastic origin.

5. The germinal disk is formed by the aggregation at one pole of the germinal matter covering the vitellus (*Cybium*), or scattered through it as a meshwork joined to the peripheral layer (*Alosa*).

6. Segmentation in the early stages is more or less decidedly rhythmical, with alternating periods of activity and longer periods of rest.

7. The egg-membrane is not always a *zona radiata*, sometimes having no pore canals; a micropyle is always present.

8. The egg-membranes of the ova of certain genera (*Chirostoma*, *Belone*, *Scomberesox*, *Hemirhamphus*) are provided with filaments or thread-like appendages externally, by which they become attached to fixed objects in the water while hatching.

9. The egg-membrane may be absent, and replaced by a highly vascular ovarian follicle, perforated by a follicular foramen in some viviparous forms (*Zygonectes*).

10. The median unpaired fins originate from a dorsal and ventral natatory fold, which may be continuous (*Cybium*, *Gadus*), or be discontinuous at the very first (*Syngnathus*, *Hippocampus*), or be discontinuous very early (*Zygonectes*).

11. The pectoral is the first of the paired fins to be developed from a short lateral horizontal fold, the position of which varies in different genera, appearing very far back at first in some, farther forward and nearer the branchial arches in others.

12. The primitive cartilaginous coraco-scapular arch or shoulder-

girdle is of mesoblastic origin, and appears just beneath what may be called the pectoral fold at the base of the breast fin. Its form and position varies in different genera.

13. The proctodeum or vent of the young fish appears long before the stomodeum or mouth; the intestine develops from behind forward, and it is probable that the intestine and medullary canal are primitively continuous through the intermediation of a neurenteric canal.

14. The number of somites or muscular segments varies in different species at the time the tail is about to be formed, and is greatest in species in which a great number of myocommata are developed in the adult, least in those in which the adult has fewer pairs of myocommata.

PRACTICAL CONCLUSIONS.

In the preceding account of the development of the Spanish mackerel, it has been incidentally mentioned that the eggs of this fish are buoyant, apparently from the presence of a large oil sphere in the vitellus, and not because of the diminished specific gravity of the whole ovum, as appears to be the case with the cod egg. We would now insist upon this character as being of such great physiological import that we cannot afford to ignore it or to conduct our hatching work without taking cognizance of it in the construction of apparatus. The perfectly regular development of the ova was found to take place practically at the surface of the water, while those which sank to the bottom were considered, in the light of experience, as not liable to develop at all. Where the eggs were kept for the whole period of incubation in still water in a marbleized pan, all that sank could be regarded as irrecoverably lost, while those which remained floating at the surface as uniformly hatched out. Changing the water on a lot of ova three or four times in twenty-four hours in a pan gave almost as good results as any other method employed. The active movement of the ova in apparatus devised to hatch other species with heavy ova was amongst the least successful modes, and especially where metals, such as copper, brass, tin, or nickle, were used in the construction of the hatching vessels or screens. Inasmuch as the protoplasm of the living egg is extraordinarily sensitive to the poisonous effects of all metallic salts, such a result is no more than might have been expected. We have therefore been constrained to suggest the use of apparatus which, as far as practicable, was constructed of wood, glass, or of some material indifferent to the action of sea-water. Experiments made, at the suggestion of Professor Baird, with asphalt varnish and rubber paint at Cherrystone taught us that it was possible to coat metallic surfaces with an inert substance which would prevent the corrosion of the metallic vessels used in hatching, and hitherto found to be so fatally injurious to developing fish ova of every kind.

The percentage of losses in every case was large, and I doubt if 25 per cent. of the whole number of eggs was ever hatched out even under the most favorable conditions. The utility of some cheap and effective glass apparatus is very apparent from experiments made by Colonel McDonald, as his system admits of a wide range of application. Other methods, especially those in which the intermittently active siphon principle was applied, seem to afford some promise that a successful apparatus may yet be devised to work on that plan. Some trials of such apparatus made by Colonel McDonald were promising, but I leave the results attained for him to report upon. An equally simple hatching box was extemporized by Mr. Sauerhoff with a Ferguson cylinder set into a tub, the eggs being placed in the cylinder and the constant water supply allowed to escape through its sides and bottom into the tub outside and run off by a proper outlet. With this apparatus a fair degree of success was obtained.

It may be stated as a general principle that buoyant ova must have gentle treatment. That if they are much agitated in the water they tend to be injured and are carried to the bottom, where they die. It appeared that when the normal buoyant tendencies of the ova were interfered with by any of our methods large losses were the result, and that the nearer our methods approached the natural environment of naturaliy spawned ova in the open sea the more successful we were. To forcibly immerse the egg of the mackerel, and keep it immersed, would simply be to thwart what is most palpably a normal condition of its life at the surface of the water.

The fertility of the mackerel, like that of most fishes with floating ova, is very great, and it is to be expected that the mortality of the ova will be in proportion to the fertility of the species. This seems to be borne out by the converse state of affairs in the stickleback and top-minnow, where a small number of embryos are matured, but are developed, with little or no losses, in a nest, and are nursed by the male or viviparously in the body of the female. Viewed in this light, we should expect large losses in hatching the cod, Spanish mackerel, moon-fish, and bonito or crab-eater.

The young fish began to feed on the fourth day after development had begun, and on the third day after hatching. The true nature of the food was not determined, as it was seen in the intestines of only a few specimens. Inasmuch as the young fish by the end of the first week of their life already have teeth, it is easy to believe that their food consists almost entirely of small articulate animals, which, with their quick darting movements, they might readily single out and capture while swimming about in water in which such prey abounded.

EXPLANATION OF THE LETTERS OF REFERENCE USED IN PLATES I–IV.

ab. Air-bladder.
al. Urinary bladder.
at. Atrium or venous sinus.
au. Auditory vesicle or internal ear.
ba. Bulbus aortæ.
bf. Breast or pectoral fin.
br. Branchiæ or branchial arches.
cc. Choroid layer of cells; lamina fusca.
ce. Cerebrum.
cer. Cerebellum.
ch. Chorda dorsalis or notochord.
chy. Ceratohyal cartilage.
cr. Carina.
crs. Cardiac sinus; rudiment of pericardiac space.
csc. Caraco-scapular cartilage or rod.
cv. Caudal vein.
ep. Epiblast or sensory skin layer.
f. Remains of food in intestine.
ff. Fin-fold; rudiment of breast fin
gd. Germinal disk.
gp. Germinal layer or pellicle.
h. Heart.
hy. Hypoblast or inner mucous layer.
hy'. Hyoid cartilage.
i, i'. Intestine.
lv. Liver.
m. Medulla spinalis or spinal cord.
mb. Midbrain or optic lobes.
mc. Medullary canal.
mk. Meckel's cartilage.
mo. Medulla oblongata.
na. Nasal pit.
nf. Dorsal and ventral natatory fold or fin.
op. Operculum.
opv. Optic vesicle.
os. Oil sphere.
pc. Pigment cells.
pn. Pineal gland.
pp. Papular prominences.
q. Quadrate.
r. Rim of blastoderm.

sg. Segmentation cavity.
so. Somites or segments of the muscle plates.
ss. Supracephalic sinus.
tr. Trabeculæ cranii.
v. Vent.
vc. Ventricle.
wd. Wolffian duct.
zr. Egg-membrane.
y. Yolk.

EXPLANATION OF PLATE I.

FIG. 1.—Micropyle and micropylar area, in two positions, of an unimpregnated egg of the Spanish mackerel. × 64.*

FIG. 2.—Unimpregnated egg of the Spanish mackerel, showing the germinal layer or pellicle *gp* which envelopes the yelk or vitellus. × 24.

FIG. 3.—Morula stage of cleavage of the germinal disk of the egg of the Spanish mackerel one hour and forty minutes after impregnation. × 25.

FIG. 4.—Germinal disk of a Spanish mackerel egg shown with its germinal pole slightly inclined towards the observer. The disk *gd* is elevated and biscuit-shaped, and is surrounded at its base by a circle of two rows of flattened cells lying immediately upon the yelk. The cells in the disk are four or more deep at this stage, three hours after impregnation. × 70.

FIG. 5.—Incipient blastoderm of the egg of the Spanish mackerel, viewed from the side somewhat obliquely. The segmentation of the marginal cells has advanced somewhat as compared with Fig. 4, and it is clear that they will become a part of the rim of the blastoderm. × 25.

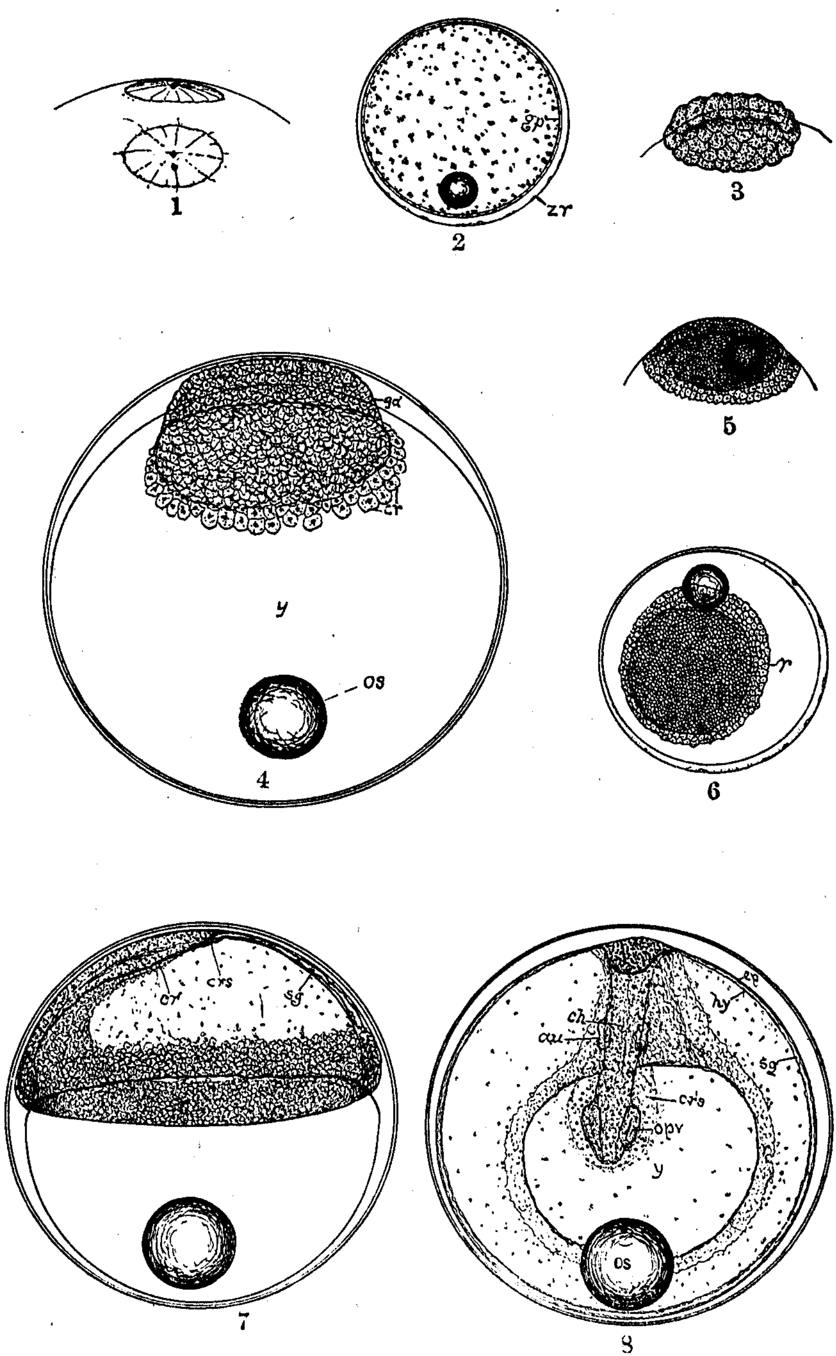
FIG. 6.—Developing blastoderm of an egg three hours and thirty minutes after impregnation, viewed from above, showing the blastodermic rim *r* in its incipiency, while the darker space bounded by it corresponds with the area beneath the blastoderm shortly to become the segmentation cavity. × 25.

FIG. 7.—Blastoderm of the Spanish mackerel egg seven hours after impregnation, inclosing nearly half of the yelk, and showing the lumen of the segmentation cavity on one side at *sg*, which extends beneath the fore part of the head at *crs* to form the rudiment of the cardiac sinus just in front of the carina *cr*. × 48.

FIG. 8.—Embryo of the Spanish mackerel eleven hours after impregnation, showing the rudiments of the optic vesicles *opv*, the chorda *ch*, and the cardiac sinus *crs*. The segmentation cavity *sg* is shown between the epiblast and hypoblast extending nearly all round the yelk. The blastoderm has not yet closed behind the tail. × 50.

---

*In every case the number of diameters to which the figure is enlarged is indicated in Arabic numerals preceded by the sign ×.



SPANISH MACKEREL (*Cybium maculatum*).

EXPLANATION OF PLATE II.

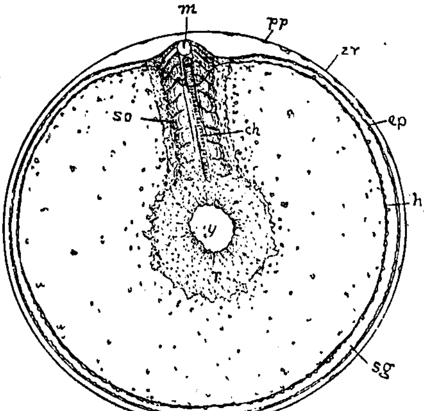
FIG. 9.—Spanish mackerel egg viewed so as to show only the caudal end of the embryo twelve hours after impregnation, showing the lumen of the segmentation cavity *sg*, the rudimentary chorda, and the somites or muscle plates, while the blastoderm has not yet quite closed over the yolk at *y*. The oil sphere is not represented. × 50.

FIG. 10.—Embryo Spanish mackerel fourteen hours after impregnation. × 50.

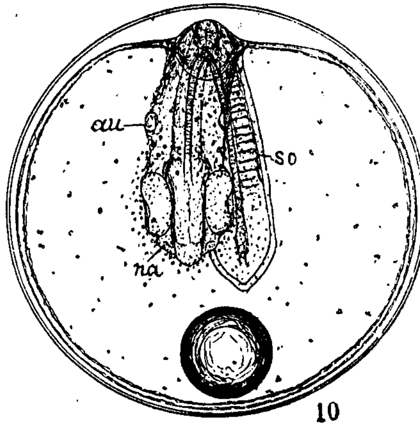
FIG. 11.—Transverse section through the opening *y* of the embryo represented in Fig. 9.

FIG. 12.—Spanish mackerel embryo eighteen hours after impregnation. The rudimentary heart *h* occupies an asymmetrical position in its sinus beneath the head and is a fusiform tubular organ open at the anterior venous end. The oil sphere is attached to the ventral wall of the yolk sack or hypoblast and is partly covered by flattened cells which have budded from it. × 50.

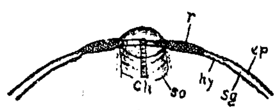
FIG. 13.—Young Spanish mackerel just hatched twenty-four hours after impregnation. The rudiments of the breast fins have made their appearance on each side of the body at *ff*, and the oil sphere is having pigment cells developed on its surface, while those of the body are already aggregated in patches on the tail. × 25.



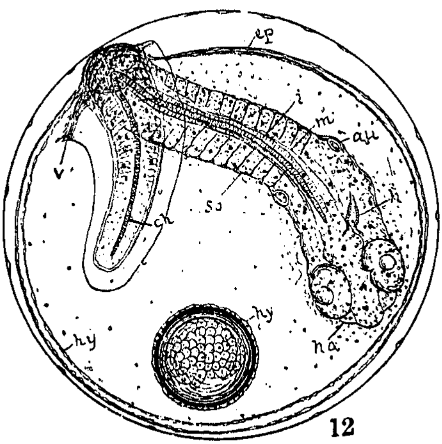
9



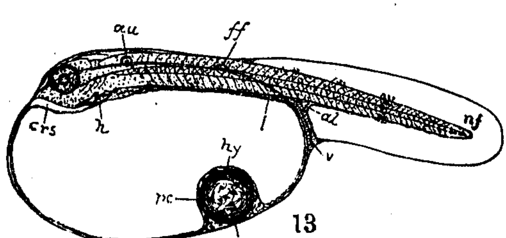
10



11



12



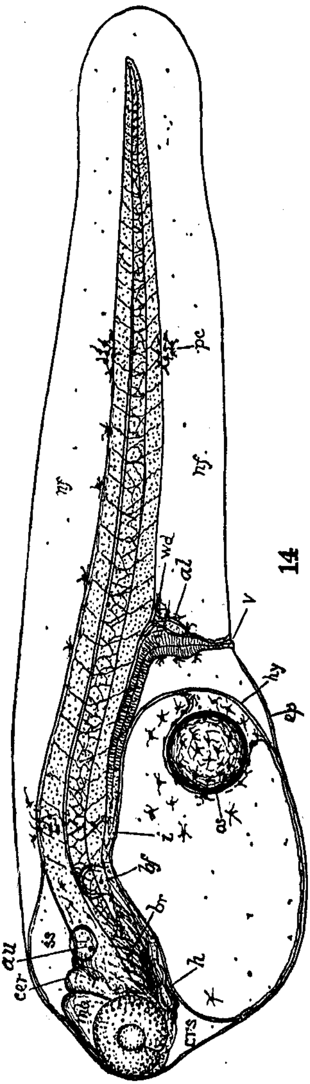
13

SPANISH MACKEREL (*Cybium maculatum*).

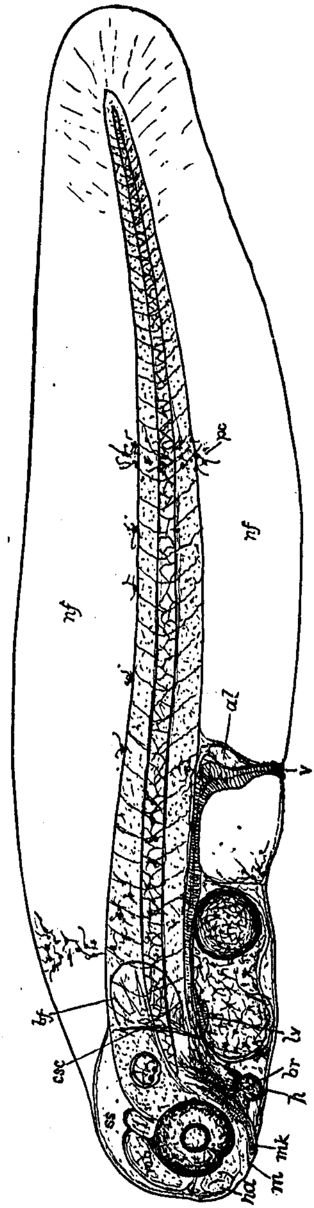
EXPLANATION OF PLATE III.

FIG. 14.—Young Spanish mackerel thirty-six hours after development began, and twelve hours after it had left the egg. The yelk sack has begun to be absorbed somewhat, and in consequence of its gradual collapse its wall is retreating from the outer or epiblast layer so as to leave a larger cardiac space in front and a similar space behind between the sack and the intestine. The latter still retains the form of a much-flattened canal, which is still occluded in the œsophageal and oral regions, where it is not yet broken through as a mouth, though the branchial furrows have made their appearance. The breast fin *bf* now occupies a more anterior position as a vertical semicircular fold a little way behind the ear. The superficial epiblast has been elevated from the brain so as to form a space above the latter, developing the supracephalic sinus *ss*. × 50.

FIG. 15.—Young Spanish mackerel forty-five hours from the beginning of development and twenty-one hours after it has left the egg. The contents of the yelk-sack have been mostly absorbed. The point where the mouth will appear is indicated at *m*, behind which the Meckelian and branchial cartilages are appearing. The heart is more developed, and exhibits an atrium, a ventricle, and a bulbus arteriosus. The liver is appearing as a thickening on the lower side of the fore-gut at *lv*, while the hinder extremity of the Wolffian duct is now plainly visible as a simple canal above the intestine, but is widened behind the vertical portion of the hind gut into a urinary bladder, *al*. The breast fin now occupies a vertical position and the rudiment of the coraco-scapular arch or shoulder girdle has appeared in the pectoral fold at its base. A few colorless blood corpuscles have appeared in the heart, but there is still no systemic circulation. × 50.



14



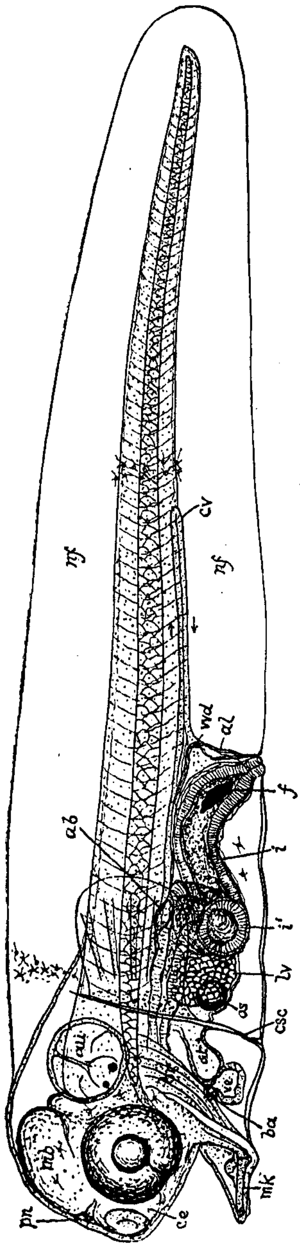
15

SPANISH MACKEREL (*Cybium maculatum*).

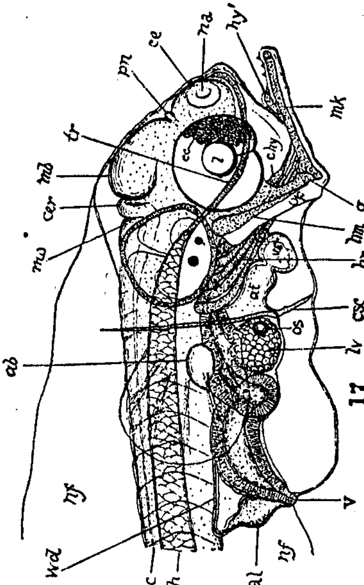
EXPLANATION OF PLATE IV.

FIG. 16.—Spanish mackerel four days after development began and three days after it had left the egg. The chondrocranium is much more developed than in Fig. 15, as is apparent from the development of the mandibular, hyoid, and branchial arches, as well as the trabeculæ cranii, parachordals, and the investment of incipient cartilage covering the ear capsules. In consequence of the advanced development of the mandibular and hyoid arches the mouth is now wide open. The caraco-scapular element *csc* at the base of the breast fin is a slender hyaline rod, different in its histological structure from that of the cartilage of the head bones, and marks the point where a transverse fold of the hypoblast partially closes off the pericardiac cavity from that of the abdomen. The liver *lv* is now more fully developed and is already divided into follicles. There is one turn of the intestine upon itself, which in this instance contains the remains of food at its hinder extremity. The circulation is now fully established, the dorsal aorta extending back for about half the length of the animal; at its posterior extremity, *cv*, it becomes the caudal vein which carries the blood forward to the heart, but not until a considerable part of it passes through a system of vessels which traverse the viscera externally, when it is poured directly into the venous sinus *at*, and so into the general circulation through the heart and gills. × 50.

FIG. 19.—Head of young Spanish mackerel on the sixth day after hatching, partly diagrammatic, showing the air-bladder *ab*, the urinary bladder *al*, the liver *lv*, more developed than in Fig. 16. The oil sphere *os* has been nearly absorbed. The chondrocranium is very much more fully developed, while well defined conical teeth have made their appearance on the papillæ of the dermal epithelium of the lower jaw, which is now longer than the upper. The opercular fold is also more prominent. × 40.



16



17

SPANISH MACKEREL (*Cybium maculatum*).