

XVII.—A CONTRIBUTION TO THE EMBRYOGRAPHY OF OSSEOUS  
FISHES, WITH SPECIAL REFERENCE TO THE DEVELOPMENT  
OF THE COD (GADUS MORRHUA).

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1.—INTRODUCTORY.

The following paper, as far as it relates to the codfish, is mainly the result of studies carried on at Wood's Holl, Mass., during the month of January, 1881, and at Fulton Market, New York, in February, 1882. At Wood's Holl, the writer, as investigator, was associated with Col. M. McDonald and Capt. H. C. Chester, the latter having been previously engaged, together with Mr. R. E. Earll and Mr. F. N. Clark, in an attempt to propagate the cod artificially at Gloucester, Mass., in 1880. It is now claimed by the fishermen in the vicinity of Gloucester and Wood's Holl that the results of the work of the U. S. Fish Commission, in placing artificially reared embryos of this species into the waters of these localities, have already shown themselves as shoals of young fish, the presence of which it is not possible to account for on any other theory than that they are the survivors of those partially reared by the two parties which have been alluded to above as being sent out by the U. S. Fish Commissioner. The fullest measure of success, notwithstanding the gratifying result claimed for us by the fishermen, it has not yet been our lot to attain. The mortality of the artificially fertilized ova is still very great under apparently the best conditions, though this fact need not hinder us in further endeavors to multiply this exceedingly valuable food-fish of our northern coasts. If the annual destruction of ova which accompanies the marketing of the adult fish in New York City alone could in any way be abated, the future of the race of codfish would in a great measure be assured. Inasmuch as a very large proportion of the fish brought to New York are alive and preserved for days in large floating cars in the harbor, the artificial fertilization of many hundreds of millions of ova annually would be a matter of no great difficulty. Such a plan has in fact been already proposed by Professor Baird. The ova after fertilization are to be transported in launches some distance from the filthy and too slightly saline water of New York Harbor, and poured into the waters of the open sound or bay, to undergo further development under natural conditions without further care from the hand of man. This plan seems feasible, and one which will demand but comparatively small outlay in its execution.

The waters of New York Harbor were found to have less than half the normal average specific gravity of those of the open sea, a difficulty which was overcome in some measure by the use of artificial sea water of a specific gravity of 1.024°. Ova fertilized in the artificial sea water not only developed as well as could be expected under the other conditions in which they were placed, but were also shipped from New York to Washington sealed up in one-quart glass jars, packed in cracked ice, and also upon trays covered with dampened cloths. The former method of transportation, however, seemed to give the best results.

Almost all of the observations on the embryo codfish here recorded were made at Wood's Holl in January, 1881, and relate mainly to what could be observed of the development of the living eggs, without further preparation, under the microscope, the small dimensions of the ova and hatched embryos being at the time a great bar to the more thorough investigation which I have since made with other forms with somewhat larger ova. What has been learned by means of sections in other types has been used only in so far as was evidently applicable on general principles. Taken as a whole, these studies must be regarded as dealing with the eggs and embryos of *Gadus morrhua* as living transparent objects, and not as a final monography, such as would be possible had series of sections and other preparations been employed in addition. The outlines of the figures I believe to be approximately correct, all of them having been drawn with care with the aid of the camera lucida. The work of Sars on the same species was unfortunately not illustrated, and is therefore not as valuable as it might have been. If it had been accompanied by figures, I should probably hesitate to publish several of the accompanying sketches, because the deft pencil of the Norwegian naturalist would have portrayed with much greater skill most of the stages here discussed. Mr. Earll's valuable contribution\* to our knowledge of this fish, its habits, and its development, cannot be passed over in silence, as a good many new facts are brought to light not elsewhere recorded. Unfortunately, the observations here placed upon record relate only to a period of about thirty days in all. The later changes beyond the oldest stage here discussed would without doubt be of as much interest and importance to fish culturists as the earlier ones.

The observations recorded throughout the text relating to other forms, not here figured, will, I believe, prove of interest to those engaged in similar studies. These embryological observations have been made at various times and places during the last three years, and embrace as subjects a considerable number of genera belonging to widely separated families. Some of them have been already published, but I have not felt prepared to put the facts accumulated into a connected form, as a

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\* A report on the history and present condition of the shore cod-fisheries of Cape Ann, Massachusetts, together with notes on the natural history and artificial propagation of the species. By R. E. EARLL. Report of the U. S. Commissioner of Fisheries for 1878, pp. 684-740.

general survey of the ground which I have traversed, until quite recently. The observations in addition to those on the development of the cod were made principally at Cherrystone and New Point Comfort, Va., Havre de Grace, Md., and Washington, D. C.

## 2.—THE OVA AND OVARIES OF THE COD AND OTHER FISHES.

The mature eggs of the cod measure 1.3 millimeters in diameter, or one-nineteenth of an inch, and are covered by a vitelline membrane which is not porous or enveloped in adhesive material. It is thin, very transparent, and laminated, as has been stated by Sars, and at one point is perforated by a single minute opening, the micropyle; the membrane is somewhat thicker in the immediate vicinity of this opening. In figures 1, 6, and 7, the micropyle is shown at the lower pole of the egg, and in figure 5, very much magnified, a portion of the surrounding membrane, together with the form of the tube of the micropyle, is seen in optic section. The outer opening is situated in a funnel-shaped depression, the rim of which is defined from the surface of the membrane by a furrow running round it. From the funnel-shaped outer opening a fine canal passes inwards to end in the center of another wider funnel-shaped depression on the inner surface of the membrane at *mi'*, but which is situated upon a considerable internal elevation. As far as the writer has been able to make out with very excellent lenses, this is the only opening into the cod's egg through which communication is established between the water surrounding it and the space inside between the vitellus and the vitelline membrane.

In other species the character of the egg membrane is quite different, since it is often found that the whole surface of the egg membrane is very regularly perforated by very fine canals, so that when it is viewed in optic section under the microscope numerous fine radiating striæ are found traversing the membrane in a direction vertical to its external surface. These striæ are due to the presence of fine canals, which may open at the apices of minute papillæ, as we find in the case of the membrane of the shad's egg; in other cases we may find the surface marked as if by fine lines crossing each other at definite angles. An egg membrane which is minutely perforated, as above described, is known as a *zona radiata*, a name proposed by Waldeyer.

The cod's egg is without the *zona radiata* found inclosing the egg proper of the shad, whitefish, and sculpin, and, inasmuch as it is unquestionably true that a micropyle perforates the *zona* in a number of these cases, it does not appear that sufficient grounds exist for the declaration that a micropyle perforates the *zona radiata* alone, in the face of the fact that the vitelline membrane only is perforated in this one instance.

Waldeyer holds that the vitelline membrane is a secretion from the cells of the follicle in which the ovum is developed. Lereboullet regarded it as a chorion, a conception of it which has now been gen-

erally abandoned. Cellacher argues for its composite nature, as does Kupffer in the case of the herring's egg. Balbiani thinks it best to adopt the name of egg-capsule for the covering of the egg, as by that means he does not commit himself as to its origin.

The micropyle of the fish ovum was first observed in the egg of *Syngnathus ophidion*, one of the pipe fishes, by Doyère, in 1849; the next observations were those of Ransom and Bruch, 1855, and it was afterwards more fully discussed by the former.

The pore canals in the zona radiata were discovered by Johannes Müller in 1854.

Sars has counted the laminae in the vitelline membrane of the cod's egg, but the writer has not been able to assure himself so fully upon this point. Under ordinary conditions, when a portion of the membrane is examined which has been sharply folded upon itself, no such laminar structure is visible until subjected for some time to the action of a 1 per cent. solution of osmic acid, when the laminated structure spoken of is produced, but whether simply by the action of the acid or as a normal feature of the structure of the membrane may be a question.

The immature ovarian eggs of the cod may be studied by taking an ovarian lobule from the ovary of an adult female and placing it in a compressorium under the microscope. Under a power of 150 diameters we learn that, while there are many eggs one-half or fully grown, there are many more which are very immature, and are only revealed to our vision by the aid of considerable magnification. Nor is this all; we find that there may be three well-marked stages of egg development distinguished. These are best seen when the fragment of ovarian tissue has been subjected to sufficient compression to render the ova more apparent by transmitted light, and if the observer will take care to use a 1 per cent. solution of acetic acid, the nuclei of the unripe eggs will be brought into bold relief by the action of this reagent in a few minutes. He will notice, first of all, that the immature ova measure all the way from a little over  $1^{mm}$  down to a very few hundredths. The smallest ova are involved in the fine cellular material of the ovary from which the eggs themselves are slowly differentiated as the growth of the ovary proceeds, when well supplied with blood and nutritive matter, previous to and during the spawning term. When once large enough to be readily distinguished from the indifferent cells which inclose them, the growing egg, or ovicells, are distinguished from the mature ones by inclosing inside them a comparatively large and very granular rounded body, the nucleus or germinative vesicle, which frequently measures half as much in diameter as the whole egg. The protoplasm which surrounds the germinative vesicle is granular, quite transparent, and of a yellowish or pale amber tint, while the vesicle itself is darker in color and more opaque on account of its granular walls. The position of the germinative vesicle is always central in these immature eggs, and it is not until the ova are approaching maturity that any marked change in its form

or position occurs. When the eggs are one-half or nearly full grown, the protoplasm surrounding the germinative vesicle becomes uniformly corpuscular, and, hence, different in character from that found in quite young eggs. Such partly developed ova, when examined with reflected light, appear whitish instead of a clear, transparent, yellowish tint, such as would be noticed in ripe eggs. This difference in color is due to a change in the character of the plasma enveloping the germinative vesicle, for immediately that the eggs are mature and ready to leave the intraovarian cavity they acquire a remarkable transparency. This must be due to a comparatively sudden blending of the protoplasmic corpuscles of the egg into a homogeneous material, very like the white or colorless albumen of a hen's egg, but differs from the latter again in that it becomes whitish,—coagulates when brought into direct contact with water. It would be a matter of great interest to know the chemical composition of the yolks of the ova of a large number of genera. Equally important it is to know what particular proteids enter into their composition besides the oils and coloring matters characteristic of certain species. The yolk material of the cod's egg in its change from the younger granular state is, however, not perfectly homogeneous, any more than that of the shad. Here, as in that species, it is made up of very minute corpuscles, which are themselves very transparent and involved in a clear plasma. I have never isolated the flat, somewhat crystalloid bodies which have been observed in the eggs of Cyprinoids. This proteid has been named *ichthyine* by Valenciennes and Frey. Such ovoidal bodies constitute almost the entire bulk of the ova of the American Cyclogonoid, *Amia calva*, as I have had the opportunity to learn from an examination of a fresh, nearly mature ovary of this fish in New York in February, 1882. It is not a little remarkable that, amidst all the diversity of color and size of the ova of Teleostean fishes, we should also find differences in the microscopic character of the yolk of the different species; a fact which ought, once for all, to be sufficient to silence a mischievous class of compilers who insist upon asserting that the germs of different species of animals are so nearly alike as to be indistinguishable from one another. The office of the yolk is to supply nutrient matter to the embryo which is superimposed upon it, and the membranes of which completely inclose it. We may in ripe ova distinguish, first, yolk corpuscles, these again sometimes aggregated into large granular bodies, which may themselves be involved in a meshwork continuous with the cortical layer from which the germ disk is derived. Finally, oil drops may be present in many other forms, such as *Hippocampus*, *Siphostoma*, *Cybium*, *Parephippus*, and all Salmonoids which I have ever observed, to which we may add Cyprinodonts and some Percoids. Exceptions in regard to structure occur, however, even within the limits of families; for example, the cod's egg is without any oil drop, while in another Gadoid, *Brosmius americanus*, the eggs contain a large pinkish oil drop, placed eccentrically, like that of the Spanish

mackerel, which, as in the latter, no doubt causes the ovum to float during development.

With the further growth and maturation of the ovicells in the lobules of the functionally active ovary it becomes possible, after they have attained the dimensions of about .3<sup>mm</sup>, to discern that each one is inclosed in a more or less well-defined covering of small cells; the ovisac, ovarian capsule or follicle, in its early stages at least, is often, if not usually, composed of flattened or of columnar cells. The history of the development of the ovary in bony fishes is not well known, and little has been written upon the subject of a satisfactory character, except more recently by McLeod, Waldeyer, and Brock, from whose researches it would appear that the ovary makes its appearance as a differentiation of two bands of peritoneal epithelium placed on the dorsal side of the body cavity, on either side of the mesentery. So far as known, the early development of the generative tissues is similar in sharks and true fishes. The bands of primitive germinal cells are known as the germinal epithelium. The reproductive cells are distinguished from the adjacent indifferent epithelium at a very early stage, and are known as *primitive ova*. These primitive ova or germinal cells become either ova or spermatozoa, it being impossible to distinguish what will be their fate when they first make their appearance. In some forms the primitive ova are soon aggregated into masses, which break up into ampullæ, which are afterwards attached to tubes derived from the smaller investing and indifferent cells. In most Teleosts the ovary in a developed state is lobulated, each lobule consisting of great numbers of ova in different stages of development. These lobules may be arranged in a longitudinal or transverse manner; the latter appears to be the most usual mode. It is doubtless true that in some fishes the ovarian lobules, when transversely arranged, correspond more or less closely to the muscular and vertebral segments. In other cases no such arrangement is apparent; the germinal fold may be rolled upon itself longitudinally, or the ovarian rudiment may appear to be derived from the anterior portion of the dorsal peritoneal wall of the abdominal cavity, as in *Gambusia patruelis*, since in embryos of this species which have not yet absorbed their yelk-sacks we may see the primitive generative structures as a pair of cylindrical organs lying in the upper part of the abdominal cavity, attached to the peritoneum only at their anterior ends. Later, when the ovary is developed, no trace of lobulation is apparent, and the small number of ova which are matured remind one of a bunch of grapes attached to the stem, the latter representing ideally the vessels which nourish the growing ova. In other forms the arrangement is very different. In the cod the ovary is enormously developed, and is an internally lobulated and closed, paired organ, opening outward by way of a wide duct behind the vent; the body of the ovary itself extends some distance behind the vent into a prolongation of the abdominal cavity, where its two halves are conjoined. In very immature stages of development of

the ovary, in Teleosts in which this organ is lobulated, I have never seen any evidence of the tubulation met with in some vertebrates. With the male organs it is different; here seminal tubules are developed in a very distinct manner. The lobules in an immature state in reality appear to represent folds of the germinal epithelium, on the exterior surface of which the ova develop in their sacks, which rupture when the ova are mature, allowing the latter to fall into the intraovarian space, where the ovary is a closed saccular organ, or into the abdominal cavity, as in Salmonoids and lampreys. The ovarian leaflets or lobules in an immature state show a very distinct median vascular stem, from which the blood supply for the individual follicles is derived, as may be seen in the immature or undeveloped ovary of *Alosa sapidissima*. The follicles themselves serve at once to contain the growing egg, and by means of a net-work of fine capillary vessels, which traverses its substance, to supply it with proteids, an accumulation of which the egg really represents. The follicle may be greatly modified, as in *Gambusia patruelis*, a viviparous form. Here it is apparently structureless, as far as I have been able to make out; but in reality it is probably covered by a layer of much flattened cells, which, together with an extremely thin vitelline membrane underneath, form the walls of the follicular capillary net-work, the blood cells within which, with their nuclei, are clearly shown in hardened and stained preparations. But these are the only histological elements which can certainly be made out. The capillary net-work is distributed from a thickened annulus at one pole of the follicle, where the afferent nutrient vessel enters and the vein passes off. Through the annulus above alluded to there is an opening, the *follicular pore*, which is variable in size, and answers to the micropyle of the eggs of other fishes. Through this pore the milt of the male apparently finds access during the act of copulation. Impregnation is thus accomplished within the ovary of the female, and development of the embryo proceeds as in oviparous species. The follicle, however, now acquires a new function, in that it not only serves to develop the egg until a mobile embryo is produced, but also functionates partly as the embryonic envelope, and partly as a respiratory structure, by means of which the exchange of gases necessary to the life of the embryo is accomplished. Whether any actual conveyance of nutriment from the maternal organism during this intrafollicular development of the embryo takes place is extremely doubtful, in that we find the yolk-sack with its vessels developed just as in many species which develop oviparously. Respiration is undoubtedly effected, however, by this quasi-placental apparatus of *Gambusia*, for we find the vascular apparatus of the embryos very highly developed long before their escape from the follicle; in fact, the branchial leaflets are already so far developed as to be pinnate in structure, with vascular loops formed in the pinnæ, a condition of affairs not usually attained by the embryos of oviparous forms until

after a considerable time, several days or even two weeks after hatching, according to the species.

The mucus usually found in the functionally active ovary, completely covering the ova which have fallen into the ovarian cavity, is of considerable interest. Its origin is somewhat obscure, but it is in the highest degree probable that it is derived from the follicles at the time of their rupture and the escape of the eggs. It is evidently a lubricant to facilitate the escape of the ova from the ovarian cavity or abdomen into the open water. In other cases, where the ova are enveloped in an adhesive mucous material, as in the cases of *Perca*, *Cottus*, *Idus*, *Esox*, *Clupea*, *Pomolobus*, *Apeltes*, etc., its function is altogether different, and serves, in addition, either to glue the eggs together in masses or bands, or to cause them to adhere firmly to fixed objects in the water. Sometimes the eggs adhere in ribbon-like masses, such as is said to be the case with the eggs of the perch (*Perca flavescens*). Mr. G. P. Dunbar reports in the American Naturalist that the eggs of *Atractosteus* are held suspended in a thick, jelly-like substance, forming long ropes several inches in diameter, which are hung on old snags, roots, or branches of trees that have fallen into the water. The spawn has much the appearance of that of the frog, with the exception of the form it assumes and the size of the eggs. Other contrivances for the suspension of fish ova have been described by Haeckel and Kölliker, and more recently more fully investigated by myself in *Tylosurus*, *Hemirhamphus*, and *Chirostoma*, consisting of a garniture of fine filaments which partially clothe the surface of the membrane. These filaments are developed within the ovarian follicle as processes of the envelope of the egg, around which they are closely coiled until brought into contact with water. The peculiar mucus which hardens under water is probably also developed as a secretion of the ovarian follicle in most cases; in others there doubtless exist glands which secrete this material, in the same manner as we find a special secretory sack in the male of *Apeltes*, from which the material for the fibers is derived with which the animal binds together the parts of its nest.

As far as my own investigations enable me to judge, the history of the process of the maturation of the ova of bony fishes in general is very similar, but there are modifications of the process, the nature of which can only be ascertained through a study of the growing ovaries of representatives of the various families known to the ichthyologist. It is probable that even this will not suffice, for the most unexpected peculiarities are found to be very characteristic of the eggs of a species closely related to some other. As an example of this, the egg of the shad may be mentioned as differing very greatly from the egg of the herring, in that the former has a very much more spacious breathing chamber surrounding the vitellus or yolk than the latter. It is not adhesive, a characteristic by which it may be again distinguished from the egg of the herring. Such peculiarities are no doubt related to some



special physiological characteristics, about which it is very important that practical fish-culturists should be informed, in order that they may be enabled to handle the eggs intelligently.

The existence of a second nuclear body in immature fish ova has been asserted by Balbiani and Van Bambeke. Balbiani speaks of it as the *vésicule embryogène*, a name given to it by Milne-Edwards, and he figures it in the immature ovarian eggs of *Pleuronectes limanda*, lodged in a depression at one side of the vitellus. He has also detected it in the unripe ova of the carp, pike, perch, and *Cottus lævigatus*, by the use of acetic acid. The writer has not seen this accessory vitelline nucleus in the ovarian eggs of any species studied by him, although working with the same reagent; there is a possibility, however, that it may have been overlooked. This body corresponds to the accessory nucleus originally discovered by Von Wittich in 1845, in the eggs of spiders, and called a vitelline nucleus (*Dotter-kern*), by Carus in 1850, which name is in general use by German investigators; Van Bambeke speaks of it as the nucleus of Balbiani. Reiterated attempts at a demonstration of this accessory nucleus have failed with me; I have seen what might be taken for it, but would not venture to assert that what I saw was normally characteristic of young ova, such as some of the investigators alluded to above have evidently used. The immature ova of *Anguilla vulgaris* have given me good opportunities to study the structure of the nucleus or germinal vesicle, in which, however, I find nothing very different from what has already been described by Beale, Rauber, and others. The nucleoli adherent to the wall of the germinative vesicle in the ovi-cell of the eel are, I find, very numerous. Amœboid movements and changes of form of the nucleolus or germinative spot have been described by Eimer\* in the immature eggs of *Silurus glanis* and the carp. In these the germinative spot, which is included by the germinative vesicle, underwent great changes of shape in comparatively rapid succession, throwing out prolongations in different directions, and then withdrawing them again, like an *Amœba*. The germinative spots of the immature ova of *Gambusia* show these movements. The germinative vesicle itself, in fact, may undergo slight spontaneous changes of form, and in stained preparations, especially where safranin has been used, a considerable variation of form may be noted, and evidence of a thick nuclear wall also becomes pretty clear in many immature ova. Nuclear changes are, however, now known to be very generally manifested by all kinds of cells.

Recently a more careful study of the germinative vesicle of ova in general has revealed the fact that its substance is traversed by granular threads, which anastomose with each other and tend to connect the walls of the vesicle and the germinative spot or spots together, just as has been found to be the case with ordinary cells of all kinds where the wall of the nucleus and the globular nucleoli are found to be joined

\**Arch. f. Mik. Anat.*, XI, 1875, pp. 325-328.

in this manner. This structure has been called the nuclear net work or reticulum. Such reticuli have been observed in the germinative vesicles of the eggs of *Hydra*, star-fishes, sea-urchins, and fresh-water mussels; by the writer in the eggs of the clam, oyster, common slipper limpet, and gar-pike; by Van Beneden in the ovarian eggs of bats; by Balfour in the ovarian eggs of sharks; by Rauber in the ova of osseous fishes; so that their occurrence is probably almost universal throughout the animal kingdom. Not only are egg-cells found to have such reticuli developed in their nuclei, but they are also found developed in white blood corpuscles and many other kinds of histological elements. The extraordinary similarity in the character of many of these reticuli is very striking, and argues for the existence of a similar cause in their production in the diverse forms in which they occur. In Flemming's elaborate researches nuclear forms of such complexity have been described as to put one's credulity to the test, but more recent investigations of my own with his methods have convinced me that such structures do exist, and that we have hitherto missed them only for want of the means of demonstration.

The fate of the germinative vesicle when the egg has reached maturity is still involved in some obscurity as far as concerns the eggs of fishes. That it disappears before the egg leaves the follicle in which it was developed there can be but little doubt, for as soon as the ripe eggs have fallen into the intraovarian cavity they are found to have lost all trace of the conspicuous germinative vesicle or nucleus which was so characteristic of them in their immature condition, and to have acquired a transparency and homogeneity in which no trace of a nuclear body can be made out with the most cautious use of reagents. In this, Hoffmann has been more fortunate than myself. This is conspicuously the character of the ripe egg of the cod. When first removed from the ovary, the vitelline membrane is somewhat lax, but as soon as it is placed in water it slowly absorbs it through the micropyle, and soon becomes tense from an imbibition of that fluid, which occupies a space all around the vitellus between it and the vitelline membrane. Impregnation seems to be necessary in some species before any water can be absorbed; this is especially the case with the ova of the shad, but is in a lesser degree necessary in the case of the cod. Practically, these facts are of great use, as in the instance of the shad, where the egg acquires several times its original dimensions, and when impregnated and "water-swollen" it is said that the eggs have "risen," which may be taken as a very sure indication of the fact that impregnation has taken place. The same eggs unimpregnated will not become "water-swollen" until a much longer time has elapsed, a very large proportion of them not at all, which shows the remarkable influence exerted by the entrance of the spermatozoa through the micropyle upon the power of the egg membrane to absorb water. It would appear as if the spermatozoön, in making its entry, had opened a passage-way for the water.

Such an effect upon the micropyle is conceivable, though we are at a loss to understand the function of the pore-canals, should it be true that the entrance of the spermatozoön would cause the micropyle to remain permanently open. Johannes Müller believed that he had demonstrated that they were tubes, and with careful manipulation it has appeared to the writer that he could see through the individual canals when a piece of canalculated egg membrane was placed flatwise under the microscope and viewed with a high power of good definition. This is also the opinion of Allen Thomson, expressed in the article "Ovum" in Todd and Bowman's *Cyclopediæ of Anatomy and Physiology*.

The imbibition of water by an unimpregnated fish egg appears to be largely due to osmotic action. If an impregnated egg is placed in a fluid having a great affinity for water, such as alcohol or glycerine, the membrane tends to collapse at first, owing to the rapid extraction of the water from the cavity of the enveloping membrane; as soon, however, as the balance of osmotic action is restored and the contents of the envelope become as dense as the surrounding fluid, the membrane becomes full and tense as at first. This tendency of the membrane to reassume the tense or full condition is doubtless due to the capillary action of the pore canals of the membrane, where such exist.

In the freshly laid unimpregnated ova of both the cod and the shad one may look in vain for a germinal disk such as had been described by various authors in the freshly laid eggs of other species of fishes. Even after the egg of the cod has lain in water for some hours there is very little change in the relation of the germinal matter and the yelk. The former covers the yelk as a thin layer of absolutely uniform thickness, as shown in Fig. 1, and differs from the yelk only in color, being yellowish, tending towards amber in tint. The distinctness of this external germinal or cortical layer of authors in the cod's egg is more decided than in any Teleostean ovum which it has been my privilege to study. The germinal protoplasm shows a double contour under the microscope outside of the yelk, and is as sharply limited as if it were an independent envelope. This germinal layer is further distinguished from the yelk which it incloses in that it has refringent globules or vesicles imbedded in its substance, as shown in Fig. 1. In Figs. 2 and 3 these vesicles are shown more magnified, and are seen gathered together in clusters. In Fig. 4 a portion of the germinal layer is shown in optic section, and represents the vesicles lying next its outer surface. The disappearance of these vesicles is comparatively rapid, and appears to be effected in the following manner: Those in close proximity to each other coalesce and form larger vesicles, and are thus reduced in number, and finally disappear altogether by rupturing their outer walls next the outer surface of the germinal layer, possibly expelling their contents into the respiratory or breathing chamber surrounding the vitellus. This view is only theoretical, however, as the writer failed to discover what became of them by actual observation. As long as the egg was

not brought in contact with the male spermatic fluid the outer pellicle of germinal matter retained the disposition shown in Fig. 1, with its included vesicles, but as soon as the egg was fertilized, or shortly thereafter, the vesicles were seen to grow larger and less numerous, because the clusters were coalescing and apparently being expelled from the germinal matter. So great was the influence of the presence of spermatozoa that those eggs in contact with them began to change almost immediately, while unfertilized ova retained their vesiculated germinal layer unchanged for four hours after their removal from the ovary of the parent fish.

*The disappearance of the vesicles above described was therefore an indication of the fact that impregnation had taken place.*

Since the foregoing was written the writer has observed similar phenomena in the ova of *Tylosurus*, *Elacate*, and *Cybium*, in all of which the development of the germinal disk is effected by the aggregation of the germinal pellicle at one pole to form the germinal disk. The same is true of the eggs of the branch herring, *Pomolobus vernalis*, and the shad. The germinal pellicle is, moreover, part and parcel of the intermediary layer of Van Bambeke, *couche hæmatogène* of Vogt, and parablast of Klein; the germ disk and the yolk hypoblast are both derived from it, as we shall learn farther on. It includes both of the latter, and the names bestowed upon the cortical layer by different embryologists simply serve to denominate what was at first a part of the germinal matter of the egg and afterwards becomes the envelope of the yolk.

Balbani states that Agassiz and Burnett recognized evident traces of segmentation in the unimpregnated eggs of certain American fishes of the cod family. As the writer has been unable to find the original of this statement, it will be of little use to discuss the matter in the absence of all evidence to confirm the observation, for, as not even a germinal disk was developed in unimpregnated eggs of the cod after four hours had elapsed, in impregnated ova it was appreciably developed one and a half hours afterwards; it follows that it is not probable that any true cleavage of the germinal disk of this species ever takes place without the influence of the spermatic particle.

That the germinal disk is formed independently of the influence of the spermatozöon in many other species there cannot, however, be the slightest doubt. I have witnessed this phenomenon in the eggs of *Chirostoma*, *Morone*, *Parcephippus*, and *Ceratocanthus*, while it is known to take place in many other species investigated by European authors, but the disk appears in some cases at least to have been differentiated before it leaves the intraovarian cavity, as in some *Salmonida*, for example.

In order to ascertain more definitely the nature of the minute vesicles inclosed in the germinal layer, a number of unimpregnated ova were placed in dilute acetic acid, which had the effect of freeing the outer germinal pellicle from the yolk. The pellicle was then carefully removed, and stained with hæmatoxylin and mounted; the protoplasm interven-

ing between the vesicles was the only portion which would stain, the vesicles remaining perfectly transparent. From this it was concluded that the contents of the vesicles were not protoplasmic or nuclear, but some indifferent fluid. When immersed in alcohol or ether no change was noticed, so that it was highly improbable that the vesicular contents were oily.

His has observed that the oil globules of the salmon egg are surrounded by an albuminous envelope; this is probably only a continuation of the protoplasm of the germinal layer, as the oil always has a superficial position in these forms and is not deeply imbedded in the yolk, as in *Brosmius*, *Cybiium*, *Parephippus*, and *Elacate*. Meischer has also shown that His is wrong in his belief that the rose-colored oil of the salmon egg is composed of lecithin, but is an oil not coagulable at 100° C. nor under the influence of concentrated acids, insoluble in alkalis, very soluble in ether and alcohol, and is invariably removed from ova preserved in a strong solution of the latter. If the egg has been previously hardened in chromic acid, and sections are prepared, I find that the places formerly occupied by the oil spheres are shown as circular openings around the edge of the section next the yolk envelope. The oil is almost instantly blackened by osmic acid; in the unchanged state it swims upon the surface of the water when a fresh egg is crushed and the oil allowed to escape.

### 3.—FATE OF THE GERMINATIVE VESICLE.

The observer sought in vain in these stained preparations of the ripe unimpregnated eggs for the germinative vesicle, and equally fruitless were his endeavors to discover this structure immersed in the yolk beneath the germinal disk after the latter had been formed. It is believed, therefore, that it has been broken up before the egg has escaped from its follicle in the ovary, and that its remains have rearranged themselves in some way in connection with the germinal matter. No advance of the germinative vesicle towards the periphery of the egg was ever observed in the immature egg, or in those nearly mature, so that it is surmised that the process of breaking up takes place with comparative rapidity. Kupffer's search for the germinative vesicle of the ovum of the herring was, according to his own account, as fruitless as my own with the cod's egg. Like the ova of birds, those of fishes seem to lose their germinative vesicle before they leave the follicle in which they were developed. It is a most remarkable fact that in some types, mollusks, *e. g.*, the germinative vesicle—egg nucleus—should persist in a central position after maturation, and in others, as, for example, in the ova of Elasmobranchs, Teleosts, and *Aves*, it disappears or is metamorphosed by the time of maturity and before the egg has left its parent follicle. Again, it is equally remarkable that in some forms the *polar cells* are developed independently of impregnation, while in others the nuclear metamorphosis attendant upon the development of the polar

cells never precedes the conjugation of the egg and the spermatozoon, as appears to be the case with the eggs of *Ostrea virginica* and *O. angulata*. The account given by Cellacher of the disappearance of the germinative vesicle in the egg of the trout will evidently not hold for that of the cod, where there is no germinal disk developed at the time of the maturation of the egg. Here, as in the shad, the vesicle has already disappeared as such while the egg was still within the follicle, but my efforts to study the metamorphosis of the germinative vesicle in ova of varying grades of maturity in the lobules of the ovary led me to no definite or valuable conclusions. The ripe eggs lose the whitish color of the less mature ova which still have the germinative vesicle imbedded in their centers.

Cellacher has given the following account of the disappearance of the germinative vesicle in the egg of the trout: "The germinal vesicle approaches the periphery of the egg and is enveloped by the germinal matter while still within the follicle. The germinal vesicle of the trout egg recently escaped from the follicle is, according to my observations, wholly eliminated. I have described the whole series of phenomena which occur during this elimination in Max Schultze's *Archiv für mikroskopische Anatomie*, Vol. VIII, and figured in different phases of the same. The process is briefly as follows: In the egg recently freed from its follicle the germinal disk is aggregated at a certain time in a depression on the surface of the yolk sphere. In it the germinal vesicle is embedded, opening on its surface by a fine pore. The thick wall of the vesicle, which is traversed by fine pore canals, and in close contact with the germinal matter, begins to manifest contractions, is ruptured, and is finally spread out upon the surface of the germ in a circular form. By this means the contents of the germinative vesicle, in the form of a finely granular spherule, are eliminated from the germ. I once observed that the contents of the germinative vesicle were divided into two unequal spheres."\* Cellacher then figures the case of a trout egg in which the germinal vesicle has divided, and has been expelled from the germinal disk as two dissimilar globular bodies. These two bodies may well be the two polar cells of other embryological writers. It seems to me highly improbable that an actual elimination and dissolution of a portion of the substance of the egg takes place, such as is here described. We will describe further along what may possibly be regarded as polar cells in the cod's egg. The early contractions of the germ disk of the trout are well described by Cellacher, and seem to be usually met with by investigators who have studied the development of fishes. The polar cells of the shad egg, or what I at one time regarded as such, I now think are probably a result of abnormal development, and not to be considered in this connection.

Salensky,† in his preliminary account of the development of the ster-

\* *Zeitsch. f. wiss. Zoologie*, XXII, 1872, pp. 406-407.

† *Zoologischer Anzeiger*, I, 1878, pp. 243-244.

let, has given the following account of the changes undergone by the germinative vesicle:

"The germinal vesicle is embedded in the germ disk, and is so large that it may be seen with the naked eye; it is without a wall, and consists of a glairy substance, which hardens in spirits, and is only separated from the surrounding plasma of the germ by a denser layer of protolencite [germinal protoplasm].

"During the first hours after oviposition one can no longer distinguish the germinal vesicle; and in its place a number of small islands may be observed, consisting of more transparent matter, which are scattered through the germinal mass, and which in their structure are quite similar to the germinal vesicle. The identity of the substance of these islands with that of the germinal vesicle indicates that the latter, even before impregnation, breaks up into a number of parts; a phenomenon which is analogous to what occurs in other animals, as in echinoderms, for example, as described by several investigators (O. Hertwig and Fol).

"Impregnation is indicated by the appearance of a clearer discoidal mass at the upper pole of the egg, and consists of a transparent, almost homogeneous substance, which corresponds to the veil-like body of the amphibian ovum described by Hertwig. We may retain the name proposed by Hertwig for this structure. On the surface of the veil-like body a vast number of spermatozoa may be noted, with their heads directed to the outside and their tails in the opposite direction. The veil-like body lies so closely against the surface of the egg that it is difficult to make out a separation between them; it attains its greatest thickness at the upper pole of the egg, and grows downwards in the form of a strand into the germinal mass; towards its margin the veil-like body gradually thins out. The surface of the germ at the time of impregnation appears to be very strongly pigmented. At the upper pole of the egg this pigmentation is most marked. The pigmented mass, which appears in that position as an elevation, depends inwards into the germ and forms a band, which, from its analogy to that described in the ova of amphibia by O. Hertwig and Bambeke, may also be called the pigmented tract (*Pigmentstrasse*). It is very possible that this indicates the pathway by which the spermatozoa penetrate the egg.

"The entrance of the spermatozoa I could not observe. For the observation of this phenomenon the eggs of the sterlet are not well adapted. In the earliest stages observed by me I found a clear spot at the lower end of the pigmented tract which was evidently nothing more than a portion of the future segmentation nucleus, and therefore the male pronucleus. This body was without a wall, and consisted of a finely granular, transparent substance, and was covered above by pigment granules. The formation of the female pronucleus (*Eikernes*, O. Hertwig) occurs at the expense of the islands already alluded to, one

of which approaches the male pronucleus, becomes more nearly round, and finally assumes the ovoidal form of a nucleus. In the course of further development the two pronuclei approach each other more closely, and finally blend together into one nucleus, which represents that of the first cleavage, and in its histological characters is perfectly like the pronuclei; it is without a wall, and consists of finely granular, almost homogeneous matter."

Schenk's account of the metamorphosis of the germinal vesicle of the ray is somewhat like that of Cellacher's regarding the trout, except that he does not state that it is eliminated in a similar manner. Alex. Schultz asserts that the appearances seen by Schenk are the result of the action of reagents.\*

#### 4.—DEVELOPMENT OF THE GERMINAL DISK.

As already remarked, the vitellus of the cod's egg is composed of a thin external layer or pellicle of germinal matter, which incloses the yolk substance, which forms by far the largest proportion of the whole vitelline mass. The outer pellicle (*Dotterhaut* of Cellacher; *couche intermédiaire*, Van Bambeke) may be regarded as the *protoplasm* from which the germ is formed, while the contained yolk, which is broken down into leucocytes during development and later embryonic growth, is the *deutoplasm*. These structures are the homologues of similar parts in other Teleostean eggs as well as in those of the *Chondrostei*, or sturgeons, as we perceive by the description of the ova of the latter by Salensky. The layer *pr* and the contained mass *d* of Fig. 1, pl. I, correspond to these two elementary portions of the vitellus of the cod's egg.

The formation of the germinal disk of the cod by a kind of amœboid migration of the peripheral germinal matter towards the lower pole of the egg is one of the most remarkable phenomena in the history of the development of Teleostean eggs which has hitherto been recorded. The amœboid movements which accompany the development of the disk are most striking, and cannot fail to arrest the attention of the observer, in that as soon as a perceptible thickening or accumulation of germinal matter has gathered at the lower pole, the germinal protoplasm manifests active changes of form, due to its contractility. These pass over the incipient disk as waves of contraction, and accompany the process of development of the disk long before any sign of segmentation has

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\* In this connection I may remark that chromic acid, followed by alcohol, or the first alone, will sometimes produce changes in the yolks of fish ova of a very remarkable character. In illustration, I recall the alterations so induced in the yolks of the eggs of the shad. Sometimes the effect produced by the shrinking and coagulation of the deutoplasm is to develop a complex system of anastomosing canals and spaces, which at first look like as if they were truly normal features in sections. Further investigation has convinced me that these tubular cavities are purely the result of the action of chromic acid upon the proteids of the yolk. Doubtless, structures of this kind have misled other investigators, judging from observations which are upon record.



been manifested. With this accumulation of the germinal matter the corresponding pole of the egg also becomes heavier.

In its singular progressive movement the germinal layer is observed to become thicker at the lower than at the upper pole of the egg, and to bulge upwards into convex elevations on its inner surface next the yolk. This migration proceeds until the yolk at the upper pole is almost exposed, as in Fig. 6, which shows the relation of the germinal matter to the yolk one and a half hours after impregnation. While there are no oil spheres present to buoy up the eggs, as in the mackerel and moon-fish, the specific gravity of the germinal matter is greater than that of the yolk, so that it always assumes a position on the lower side of the yolk. Should the egg be turned round so as to bring the disk uppermost, the yolk will be gradually turned by the gravity of the disk until the latter regains its nethermost position. During its migration the germinal protoplasm eventually arranges itself in radiating bands, which sometimes anastomose, and all trend towards and join the edge of the incipient disk below. Later these bands develop nodes or enlargements, *prn*, along their courses, as shown in Fig. 7, and pour their substance into the disk, which is now defined three hours and forty minutes after impregnation. If a granule in one of these bands is watched for a time it will be noticed that it exhibits a more or less decided progressive movement.

At the time the disk is defined its inner surface at first presents irregular rounded elevations, which gradually subside, when the under side of the disk becomes flat. Then the outer surface of the disk is elevated into one or more large rounded, prominences, which in like manner eventually disappear. These are some of the amœbal phenomena already alluded to.

It is very important for us to make a distinction here between the mode of formation of the germinal disk of the cod and that of the Clupeoids, as worked out by Kupffer and myself, and of *Tinca*, as described by Van Bambeke. It would appear that we concur in the opinion that in the latter more or less protoplasm destined for the development of the germinal disk is derived from the center of the vitellus, into which, as may be seen in the ova of *Alosa*, *Clupea*, and *Pomolobus*, the external germinal layer sends processes which in the shad and branch herring look like hyaline roots passing down amongst the yolk spheres. At a later stage of development this arrangement seems to disappear, and the yolk spheres, although still evidently involved in a meshwork of germinal matter, do not have the same amount of the latter insinuated between them as at first in the vicinity of the developing germinal disk. On the other hand, in the eggs of *Gadus*, *Tylosurus*, *Cybium*, *Elacatè*, and *Parephippus*, the germinal matter forms a distinct outer coating over the vitellus, and these forms do not have any distinctly marked yolk spheres, as in *Alosa*, involved in germinal protoplasm, except in the case of *Elacate*, where the yolk spheres are very

large and few in number. What is meant here by the term yelk spheres must also be explained. We denominate as yelk spheres those large, finely granular masses of protoplasm which are involved in a delicate matrix of germinal protoplasm. The ultimate granules or spherules which compose these spheres or yelk masses are altogether different, vastly more minute, and are the same as what we have called yelk spherules and granules. The large yelk masses are very clearly seen in the shad's egg, that of the herring, and in *Elacate*, but not so clearly in any other forms known to me. In many types the yelk is almost homogeneous under a low power. In Cyprinoids it is finely granular, and again wholly different from what is seen in the eggs of Clupeoids and Gadoids. These distinctions are important, and not less so is the distinction drawn between the mode of formation of the germinal disk of the Gadoids and Clupeoids. The germ disk of *Gadus* is wholly developed from the external germinal layer of the vitellus, that of the Clupeoids is apparently not entirely so formed, but derives part of its substance from the protoplasmic matrix involving the large yelk spheres or masses below it.

The germinal pellicle, cortical layer, has certain common characters in all the forms, however; these are its superficial position and the vesicular or refringent bodies it incloses prior to the development of the disk. In the eggs of the shad and of the herring the cortical or germinal layer contains large refringent bodies, different from the vesicles which occur in the same layer in the ova of *Gadus*, *Tylosurus*, and *Cybium*, in the last of which I have watched their slow disappearance and apparent absorption into the surrounding germinal plasma. In this process they become gradually smaller and finally disappear under the influence of impregnation. It would seem as if these refringent structures were somewhat similar to those seen in the germ of *Acipenser ruthenus*, and described as *islands* by Salensky. In *Clupea* and *Alosa*, as the peripheral germinal matter is gradually gathered into a depressed, somewhat conical germinal disk, the refringent globules or spherules which were originally distributed over the whole vitellus become less numerous and mostly disappear, except over a portion of the vitelline surface and in spots usually near the margin of the disk. After the disk has segmented into a large number of cells and advanced somewhat beyond the morula stage, these refringent spherules have disappeared entirely. The space figured underneath the germinal disk in the yelk of the egg of *Clupea harengus* by Kupffer is probably a product of the hardening reagents used, and is not to be regarded as a *latebra* in the sense of the structure of that name found in the ovum of birds. Actual sections of several species of fishes of very early stages of development have served to convince me of the correctness of this interpretation. Germinal matter which does not take part in segmentation lies below the disk, as shown by Cellacher, and extends over the vitellus as an almost structureless membrane, *Dotterhaut* of Cellacher. It is this membrane which protects the vitellus and forms

a coating over the yelk while the egg is developing within its membrane or capsule, and even includes the yelk after the blastoderm has closed over it. This yelk membrane or pellicle derived from the germinal matter forms the floor of the segmentation cavity. In the substance of the membrane free nuclei also develop; these doubtless contribute by a process of intussusception to the formation of hypoblastic structures at an early stage of development, but later these nuclei appear to give origin only to blood cells, mainly in the form of leucocytes, except in the case of *Tylosurus*, where the blood cells of the early stages are colorless, but hæmoglobin is soon developed, and finally, it would appear, concurrently with their free germination from the membrane they are already colored. The free nuclei are most numerous in the portion of the yelk membrane or hypoblast in the neighborhood of the heart, near the head, and at the anterior portion of the yelk-sack.

#### 5.—IMPREGNATION OF THE EGG.

Of the history of this process in the Teleostean egg we as yet possess very few trustworthy observations, except those of C. K. Hoffmann. This is in part due to the difficulty attending its study in an egg of such a disproportionately large size as that of the fish with its large yelk, and no observations have yet been made upon this point with such success as upon the eggs of mollusks and echinoderms, as, *e. g.*, *Limax campestris*, by E. L. Mark; *Asterias glacialis*, by Fol; and *Toxopneustes variegatus*, by Selenka. Hertwig, Flemming, and Bambeke have also contributed essentially to our knowledge of the process of impregnation, the former and latter especially in relation to what occurs in the amphibian ovum. Almost all observers seem to be agreed that a single spermatozoon only is requisite. This enters the egg either through a penetrable membrane or through a micropyle, and blends at once with the plasma of the egg, producing in this process of coalescence a clear space, surrounded by granular rays in the vicinity, which has been designated the male pronucleus. This male pronucleus then blends with the female pronucleus to form the first segmentation nucleus, but the origin of the female pronucleus, as described by Fol, is somewhat complicated. It is derived from the germinative vesicle by a complex metamorphosis of the latter. The following is the series of events attending impregnation and the order of their occurrence, essentially, as observed by Fol and summarized by Balfour:

- “1. Transportation of the germinal vesicle to the surface of the egg.
- “2. Absorption of the membrane of the germinal vesicle and metamorphosis of the germinal spot and nuclear reticulum.
- “3. Assumption of a spindle character by the remains of the germinal vesicle, these remains being probably in part formed from the germinal spot.
- “4. Entrance of one end of the spindle into a protoplasmic prominence at the surface of the egg.

"5. Division of the spindle into two halves, one remaining in the egg, the other in the prominence; the prominence becoming at the same time nearly constricted off from the egg as a polar cell.

"6. The formation of a second polar cell in the same manner as the first, part of the spindle still remaining in the egg.

"7. Conversion of the part of the spindle remaining in the egg into a nucleus—the female pronucleus.

"8. Transportation of the female pronucleus towards the center of the egg.

"9. Entrance of a single spermatozoön into the egg.

"10. Conversion of the head of the spermatozoön into a nucleus—the male pronucleus.

"11. Appearance of radial striæ round the male pronucleus, which gradually travels towards the female pronucleus.

"12. Fusion of male and female pronuclei to form the first segmentation nucleus."

The foregoing account is essentially the sequence of events as observed by Fol in *Asterias glacialis*. This series of events evidently does not hold for all forms. Thus, in *Hirudinea*, *Mollusca*, and *Nematoidea* impregnation takes place normally before the extrusion of the polar bodies is completed (Balfour), so that the event which stands as ninth in the preceding scheme would actually stand first, as in the case of the egg of *Ostrea*, where no disposition is manifested to extrude polar globules until the ova are brought into contact with the spermatozoa. In the case of the lamprey, Kupffer and Benecke have shown that only one spermatozoön enters the egg, but that others pass through the vitelline membrane, and are taken into a peculiar protoplasmic protuberance of the ovum which appears after impregnation. In ova of *Ostrea virginica* which have been killed and hardened in osmic acid the pellucid tract which penetrates the egg for some distance in the vicinity of the polar globules probably represents the axis of the amphiaster formed at the time of the development of those bodies. I am well assured of the fact that no tendency toward a reorganization of the centrally placed nucleus of the mature egg of the oyster is ever manifested until it is brought into contact with spermatozoa. The egg of the osseous fish is scarcely referrible to either of the foregoing categories; it has lost the germinal vesicle as a central structure before it leaves the parent follicle, and coincident with the development of the germinal pellicle or germinal protoplasm which covers the yolk or deutoplasm, its substance has probably been mostly transferred to that layer. As we now know that the germ disk is formed at the time of impregnation or independently of it, it is to be supposed that in this process the germinative vesicle or its remains may not improbably undergo a complex metamorphosis. Although I have not yet met with anything that I could regard as undoubted polar cells, a minute prominence occurs on the disk of the cod which may be regarded as such, or as an apparatus for the

reception of the spermatozoon, such as has been described by Kupffer and Benecke in the egg of the lamprey. This structure is represented in Fig. 7, Pl. I, at *pp.* in an egg of the cod, in which the formation of the germinal disk has not yet been completed. Repeated observation convinced me that I was not looking at an accidental feature, but that it was constant during this and somewhat later stages of development antecedent to segmentation. The actual ingress of the spermatozoon into the ovum I have never witnessed, although the cod's egg is one of the best adapted of all fish ova for this purpose on account of its transparency and small size. If Fol's compressor is used, experiments may be very readily carried out under the microscope, and if the upper and lower plates of the compressor are kept far enough apart so as just to allow the eggs to remain free and mobile within a few drops of water, encircled by a ring of block tin or hard rubber, clamped by the cover, the eggs will always arrange themselves in one position, with the germinal disk downwards and the yolk uppermost. This peculiarity enables one to see only the lower face of the disk through the large transparent yolk above it when the tube of the microscope is placed vertically, or its edge, or in optic section, when the tube of the microscope is placed horizontally, with the stage and compressor upright. To see the upper surface of the germinal disk of the live egg it is most convenient, in fact necessary, to have an inverting prism attached to the microscope, into the mounting of which the objectives may be screwed, so as to view the eggs from below. Nacet's inverted microscope, used in chemical investigations, would answer well for this purpose. The sketches which I have made were obtained from living eggs treated in this way, without compression while confined within the area of a hard rubber or metal ring, which served to hold the water and eggs in place when the cover of the compressor was screwed down. Attempts made to witness the entrance of the spermatozoa by the help of the above-described apparatus, using very dilute mixtures of milt with water, were not successful. The proper mode of procedure, in order to demonstrate the changes by histological methods, would be to take a batch of ova fresh from the ovary and divide them into two lots. Impregnate the one lot and allow the other to remain unimpregnated. Then take of both a series of specimens at intervals of two or three minutes and place them in a dilute chromic acid solution to fix the nuclear and other protoplasmic changes, so as to afterwards facilitate staining and the preparation of sections, and the satisfactory study of all the changes which the nuclear matter of the germ has undergone until the time of the first segmentation. A similar series of the unimpregnated ova would throw some light upon the history of the process of the migration of the nucleus from the center of the egg, if taken in connection with the investigation of the mature and functionally active ovary with its products in different conditions of maturity.

The complete disappearance of the germinative vesicle from ova in

general previous to impregnation, had, for many years, received the assent of many distinguished investigators, though not a few still hold to the belief that it did not wholly disappear. The latter view is the one now generally accepted by embryologists and rests upon several series of investigations carried out by several observers with the greatest care. Notwithstanding this some very eminent investigators still hold to their belief in the total disappearance of the nucleus of the ovum, and they base upon this supposed fact a very weighty argument for their favorite hypothesis, which demands that all eggs during their development shall pass through the moneron or non-nucleated stage of development, in accordance with the doctrine that the development of the individual must briefly recapitulate the development or evolution in time of the race to which it belongs. This grand generalization properly conceived is truly important, but its more recent defenders have overstepped the bounds of legitimate deduction and induction in their efforts to establish a consistent theory of animal evolution, in that later researches have shown that the *monerula* stage of animal development is not yet demonstrated.

Latterly it has been affirmed by Strasburger\* that not only is *omnis cellula e cellula* true, but that the truth of *omnis nucleus e nucleo* is nearly equally well established. The chaotic *monera* and *urschleim* of the Haeckelians are justifiable only if they carry no grievous errors and mischief into the sacred realm of science. The extensive discussion of such points to the exclusion of the true methods of investigation has called for several digests of the existing state of the facts in the case, one of the best of which is that given by Mr. C. O. Whitman, in his *Embryology of Clepsine*.† Modern histology, that is to say, what we have learned to know of cell development within the past decade, discountenances most emphatically the doctrine of the existence of *structureless* cells devoid of nuclei or nuclear matter. In fact, in addition to the dictum of Strasburger, we owe it largely to Professor Flemming that we have proof of the exceedingly complex metamorphosis of nuclei in the most ordinary processes of growth. The dividing line between the phenomena of growth, cell development, and the early phases of embryonic and embryogenetic development is certainly not as easily made out in many cases as might be supposed, and if there were no other argument against the monerula hypothesis, the facts of embryology and histology alone would be sufficient to impel a candid person to at least suspend judgment for the present.

Recent investigations upon the impregnation of the eggs of the lamprey (*Petromyzon*) by Kupffer and Benecke show that there are two polar cells extruded from the germ; one is formed previous to, the other

\* In an address delivered before the congress of German naturalists, at Danzig. Published in French in the *Revue Internationale des Sciences Biologiques*, IV, No. 3, 1881.

† Quarterly Journal of Microscopical Science, July, 1878.

after the entrance of the single spermatozoön which effects the fertilization of the egg. Beside these there is a polar process, *zapfen*, which originates from the middle of the germinal disk as a hyaline protoplasmic band (*axenstrang*, Calberla), drawn out by adhesion to the inner surface of the *zona radiata* from the center of the germinal disk when the *zona* is lifted up from the latter by the imbibition of the water drawn in through the micropyle or pore canals. Through this process a single effective spermatozoön makes it way into the vitellus, when the hyaline process is withdrawn; a number of spermatozoa may enter the egg through the micropyle and be enveloped by the hyaline process, but they take no share in the impregnation. Calberla, on the other hand, asserts that but one spermatozoön enters the micropyle and that the tail is left behind, closing up the opening of the latter. In this Calberla agrees with Fol's description of the entrance of the spermatozoön into the egg of the star-fish. Scott says he has seen the second polar cell described by Kupffer and Benecke at the germinal pole of the egg. Calberla confirms almost fully the previous observations of A. Müller on the mode of impregnation of the eggs of the lamprey, but his views in regard to the fate of the germinative vesicle are not accepted by Scott.

The prominence which we noted on the germinal disk of the cod's egg is probably the representative of the extruded polar cells which have been derived from the germinative vesicle. It is not hyaline, as the polar process of the egg of the lamprey has been described to be, but is composed of granular protoplasm. The separate first polar cell adherent to the *zona radiata* of *Petromyzon*, upon its inner surface, is something very different from the polar prominence observed in the cod's egg, which reminds one in its main features of the polar cells of molluscan eggs. It is not so regular in form, however, as these, since it is scarcely ever, if approximately, the same shape in different eggs, often having a jagged appearance or with minute points projecting from the two principal portions. But since the writer did not succeed in witnessing the actual entrance of the spermatozoön into the egg on the breaking up of the germinal vesicle, the final interpretation of the nature of the prominence remains to be elaborated.

Prof. C. K. Hoffman, of Leyden, in the *Zoologischer Anzeiger* for 1880, pp. 607-610 and 629-634, gives the following as the result of his investigations, at the zoological station at Naples, of the early stages of development of *Scorpana*, *Julis*, *Crenilabrus*, *Heliopsis*, *Fierasfer*, *Syngnathus*, *Hippocampus*, *Gobius*, etc., but more especial attention was paid to the first two on account of the great transparency of the eggs of those genera.

"As to ovogenesis my observations fully agree with those of Waldeyer, Brock, and Kolessnikow; the primordial ova originate from tubular invaginations of the germinal epithelium of the ovary. The ovarian egg during its whole development is covered by a *granulosa*,

egg follicle, consisting of but a single layer of cells. At the time of maturation, a fatty metamorphosis of the cells of the granulosa takes place, which promotes the escape of the egg from the follicle. The micropyle is always an open pore; it has a very wide external opening and a very narrow inner one. The latter ends on an internal prominence of the egg-membrane, which is a *zona radiata*. The lumen of the internal micropylar opening is so constricted that not more than a single spermatozoön can pass through at one time, as was first observed by His in the salmon.

“It is well known that the eggs of many bony fishes adhere to fixed objects as soon as they fall into the sea-water. This is not always accomplished in the same way. In *Hcliasis*, *Gobius*, *Blennius*, *Belone*, etc., this is accomplished by long filaments, excrescences of the *zona radiata*, which are not distributed over the whole of that structure, but which are found only on certain portions; viz, in the vicinity of the micropyle. In the herring and *Crenilabrus* the whole of the surface of the egg is covered with an adhesive material. In the case of all ova which attach themselves by these contrivances, the *zona* consists of two layers, an inner and an outer one, the latter being adhesive, and split off from the former to embrace the whole egg, or is developed in the form of fibers, processes, and similar appendages. In contrast with such forms, those ova which float or sink to the bottom, of their own weight, do not seem to have such a differentiation of the *zona* into two layers. It is highly probable that the *zona* is a secretion from the surface of the ovarian egg, and that it is to be regarded as a vitelline membrane.

“The primordial ova consist of a homogeneous mass, coagulable in acetic acid, when it becomes granular, and they inclose a large nucleus and a single large nucleolus. In very young ova, of which the contents are similar to the foregoing, several nucleoli may already be discerned in the nucleus. In the ova designed for the next brood, the yolk corpuscles are gradually developed, until they finally occupy the whole mass of the egg except the space taken up by the nucleus, and only the small spaces between the granules and corpuscles are filled up by the protoplasm still present. The yolk granules and corpuscles consequently appear to be developed at the cost of the contained protoplasm of the egg. The cells of the granulosa were never observed to give off protoplasmic processes passing inwards into the egg through the pore-canals.

“In the foregoing stage all of the eggs are dull and opaque; even the eggs of *Scorpxna*, *Julis*, *Serranus*, and *Pteraser*, of crystalline transparency at maturity, pass through the same opaque stage of ovarian development. In the very large nucleus the nucleoli continue to multiply.

“Towards the time of maturity, the nucleus begins to move from its original central position to the periphery. During its change of position, the smooth, tightly distended nuclear membrane commences to



wrinkle, becomes gradually thinner, and finally disappears altogether; the already numerous nucleoli become still more so, but also smaller and smaller, until they are finally indistinguishable, so that one is obliged to conclude that they have been dissolved in the intranuclear fluid. Finally, the nucleus is brought to lie close against the zona radiata, as a wall-less, irregular, viscous, almost homogeneous mass; the intranuclear fluid in which the nucleoli have been dissolved now begins to mix with the egg contents. Accompanying this blending important changes occur, and, as a result of the process, the direction spindle, the nucleus, and the yolk are differentiated. In the pellucid ova of *Scorpana* the yolk spherules are again broken down and the food-yolk then forms a clear, semi-fluid mass; in *Julis*, *Serranus*, and *Fierasfer*, the clear yolk also contains a large, shining oil-sphere; in *Crenilabrus* the yolk is not entirely pellucid, but contains some, not very numerous, yolk granules; in the herring and *Heliasis* the yolk contains a great number of yolk spherules, but which, in consequence of their larger size and less bright appearance, look very differently from the yolk spherules of the immature egg.

"The direction spindle [polar spindle] has its peripheral pole placed immediately against the inner opening of the micropyle. It is best seen in *Scorpana*, in which it has a length of .025<sup>mm</sup>, and a diameter of .0145<sup>mm</sup>; its longitudinal axis forms an angle of 45° with that of the axis of the egg. It is less easily made out in *Julis*, while the eggs of *Crenilabrus*, *Heliasis*, *Gobius*, *Blennius*, *Belone*, *Clupea*, are too unsatisfactory and opaque as objects in which to seek for the direction spindle.

"The form of the germ in the mature ova taken from the females of different species, without being brought into contact with water, is very different. In *Julis* it surrounds, as a relatively thick layer, the entire yolk, and is thickest at the micropylar pole of the egg; in *Scorpana* it covers in a cap-like manner the micropylar pole of the yolk where it is thickest, and becomes gradually thinner towards the equator, where it disappears almost wholly, but is continued over the opposite pole as a distinct but very thin layer. In the eggs of the herring and *Heliasis* it does not form a very thick stratum beneath the micropyle, and extends from this region as irregular thick and thin processes down amongst the yolk spheres of the whole egg. The direction spindle also is always imbedded in the germ disk. Kupffer's statement that the germ of the herring is developed under the influence of the sea-water and the sperm rests upon inaccurate observations; in the unimpregnated egg of the herring, as in *Heliasis*, the germinal matter still remains in great part strewn amongst the yolk spherules, as is apparently the case with all eggs in which the yolk does not consist of fluid material, but for the most part of larger and smaller yolk spherules.

"The fact that the yolk spherules of pelagic eggs should again be broken down and in mature eggs become clear and pellucid throughout must probably be regarded as a phenomenon of adaptation, the whole

developmental history agreeing fully with this interpretation, in that it is unusually rapid. For example, the eggs of *Julis* hatch in fifty-two hours, *Scorpana* in fifty-eight hours, *Fierasfer* in fifty eight to sixty hours, without the slightest trace of pigment having yet appeared on the eyes, so that when the eggs are ready to hatch, they are still as transparent as at the time they were laid.

"I observed the following in watching the first phenomena of impregnation. In bony fishes, the first segmentation nucleus is formed as in numerous other animals, by the conjugation of two nuclei. One of these two nuclei is the egg nucleus (female pronucleus, *pronucleus femelle*), the other the spermatic nucleus (male pronucleus, *pronucleus mâle*). The conjugation of these two nuclei is best seen in the beautiful, pellucid ova of *Scorpana* and *Julis*, as well as in the less transparent eggs of *Crenilabrus*; in the cases of other species of osseous fishes investigated (*Heliasis*, *Gobius*, *Clupea*, etc.), the ova are not clear enough to enable one to reach a conclusion in regard to this important point.

"As soon as a spermatozoön has penetrated so far into the micropylar canal that it reaches the germ disk, or perhaps the direction spindle itself, the first phenomena involving the spindle and germ appear in their order. Around the lower pole of the spindle there is formed a small, clear protoplasmic mass; whether a similar feature is developed at the upper pole of the spindle it is difficult to say, since this lies so close against the inner opening of the micropyle that it is not possible to be certain as to just what occurs here. The protoplasmic granules which are scattered irregularly through the egg gradually aggregate more and more around both poles of the spindle in distinct radial lines, especially around the small, clear protoplasmic area around the lower pole; shortly afterwards the development of the well-known caryolytic figures is accomplished forming the *amphiaster de rebut* of Fol. Scarcely have the radial figures [asters] become distinct, or at about the same time, it may be observed that the germ begins to contract [aggregate] at the micropylar pole. The first changes in the spindle now also begin, and it becomes at first somewhat shorter and thicker. The same remark applies also to the nuclear plate, when it again assumes its earlier form, and thereupon again elongates, becoming gradually thinner and thinner, before finally disappearing altogether. As soon as the spindle begins to elongate, the division of the nuclear plate occurs. The nucleus formed from the central half of the spindle is the egg-nucleus [female pronucleus]; that formed from the peripheral half of the spindle is the polar body, which in *Scorpana*, *Julis*, and *Crenilabrus* escapes from the egg through the micropylar canal. Inasmuch as the division of the spindle begins as soon as the spermatozoön has penetrated deep enough into the micropylar canal to come into contact with the germ, and the lumen of the canal being so narrow that never more than a single spermatozoön can pass through it at one time, and the polar body being budded off at the same instant, as in *Scorpana*, *Julis*, and *Crenilabrus*,

prevents the entrance of other spermatozoa. In the *three above-mentioned genera of osseous fishes more than one spermatozoön cannot therefore enter the egg.*

“Immediately below the inner opening of the micropyle, and at the point where the spermatozoön entered the germ and before the spindle has completely disappeared, there appears, although extremely small, yet plainly visible, a new aster or radial figure, and in its clear center a second small nucleus is developed—the male pronucleus. Around both nuclei the protoplasmic granules are arranged in sharply defined rays. Both nuclei then become gradually larger and larger, approach each other, to finally blend together to form the first segmentation nucleus. Before this conjugation takes place the germ has been completely aggregated by contractile movements of its substance at the micropylar pole of the egg. The eggs of *Scorpæna* and *Julis* only are adapted to the study of the phenomena here described. In the eggs of *Scorpæna* a very inconsiderable space is formed around the vitellus, between the latter and the yolk, which becomes noticeable only when the egg is getting ready to segment. The same remark applies to the eggs of *Julis*. In *Crenilabrus* the water space is, on the other hand, more spacious, but in that it is developed very late in the neighborhood of the inner micropylar opening, the germ remains in close contact with the inner opening of this canal, so that in these three genera of osseous fishes the polar body can be pushed out only through the canal, in that there is no space between the germ and the zona radiata. In other cases, as soon as the spermatozoön has come in contact with the germ a large paravitelline cavity or water space is formed, as in *Heliastis*, for example. In consequence of this, the polar body which is extruded cannot be thrust out into the micropylar canal, but remains within the paravitelline space. Since, in the cases of *Scorpæna*, *Julis*, and *Crenilabrus*, but one spermatozoön can enter the egg, it is highly probable, that the same is true of the ova of all osseous fishes, although it may not be possible at this moment to say with certainty at what time the entrance of other spermatozoa is interrupted. In those cases also in which a large water space is formed between the germ and micropyle, perhaps the tension of the zona radiata in such instances is effectual in closing the inner opening of the micropyle which ends on a papilliform internal prominence of the zona. In the water space within the zona I never saw any spermatozoa. The spermatozoa can enter the egg only through the micropyle. The phenomena which manifest themselves in mature eggs when simply placed in water without spermatozoa are very variable. Of one and the same lot of eggs of which a part were fertilized and developed regularly, the other part, after lying in water for twenty-four hours, did not show the slightest alteration; in others again, after four hours, the spindle had disappeared, the polar body was extruded, and the germ was as well developed as if the egg had been fertilized, with only this difference, that the germ was formed much more slowly than in the fertilized egg; in

other eggs of the same lot after four to six hours the spindle was still present, and the germ had aggregated and acquired an unusual thickness. Whether eggs in the two last mentioned conditions are still capable of impregnation I do not know, since in each instance the opportunity was wanting to make the test. In the cases in which the germ was developed after four hours and the polar body extruded, I never was certainly assured that I saw a nucleus—egg-nucleus. The aggregation of the germ, the extrusion of the polar body, and the disappearance of the spindle are phenomena which may occur independently of each other and of impregnation. Why a portion of one lot of eggs should remain unchanged in water without spermatozoa, while others pass through the changes above described, it is not easy to say; perhaps it is, that the most mature ova pass through the stages already described, while those not so mature remain unchanged, though capable of being impregnated.”

#### 6.—THE ORIGIN OF THE YELK HYPOBLAST.

This structure, in its relation to the genesis of the blood, is of the greatest interest physiologically, and the evidence of its true character, which has been gradually accumulating in my hands, is of the most conclusive nature. It has been named the *parablast* by Klein, *couche hæmatogène* by Vogt, *couche intermédiaire* by Van Bambeke, *couche corticale*, *germinal layer*, *germinal pellicle*, *Dotter-haut*, etc., but these terms are thoroughly synonymous, and their significance need not trouble us any further. I have followed its history through the later stages of development to its complete disappearance, and am delighted to be able to add the remarkable observations of Prof. C. K. Hoffmann\* upon the origin of the free nuclei in it, as observed by him in *Scorpena*. Ziegler has also studied its development in *Salmo salar*, so that the evidence as to the rôle it plays in development is almost complete. Further discussion of it in this essay will be postponed until I come to consider the history of the blood-vascular system of the yelk-sack of several forms. Under the head of the structure of the egg we have already described its main features as found in several species. Hoffmann's observations as to its early history are as follows, and were made upon the ova of *Scorpena*, *Julis*, etc.:

“In respect to the segmentation I can communicate the following: While the male and female pronuclei are blending to form the first segmentation nucleus, the latter has already begun to again become spindle-shaped. The newly-formed spindle lies in the germ with its axis in conformity with the axis or diameter of the egg, and stands vertically against the end of the micropylar canal. The granules of the germ then group themselves in distinct rays around both poles of this spindle. The well-known caryokinetic phenomena then occur, which manifest themselves during each and every cleavage, and after

\* *Zoologischer Anzeiger*, 1890, pp. 632-634.

some minutes the spindle-shaped stage has again vanished and two new nuclei have been formed, both of which lie in the plane of the axis or diameter of the egg. One of them lies at about half the depth of the axis of the germ, the other deeper and nearer the yolk. With the division of the first segmentation nucleus into two new nuclei, the consequent cleavage has led to the division of the egg into two very unequal portions, the upper and smaller lying near the micropyle and consisting of protoplasm, which, at the level of half the height of the axis of its substance, the germ, contains a nucleus; this portion I shall call the archiblast [the germinal disk of this essay], the other very much larger portion, the parablast [the yolk hypoblast; germinal layer]. The parablast consists indeed for the greater part of food-yolk, but it approaches in character the germ, the protoplasm in which lies a nucleus close to the yolk, and is continued over the whole yolk as a thin envelope. The archiblast [germinal disk] only segments; its nucleus is the parent of all the cleavage nuclei; the parablast [germinal layer] does not segment; nuclear division only takes place in it; it is developed into a multinucleolar cell.

"Before the separation of the archiblast from the parablast, each nucleus of both these parts has been transformed into a new caryokinetic figure or spindle, the position of the axes of which are at right angles to the axis or diameter of the egg. The spindle or caryokinetic figure formed by the nucleus of the archiblast (germinal disk) is a magnificent spectacle; that of the parablast is less distinct on account of its more central position. Before two new nuclei have been formed from the spindle in the archiblast the first segmentation furrow begins to divide the archiblast into two equal sized parts. Both are divided from each other by this furrow, but at their bases or lower surface they are still continuous with the underlying parablast. Each nucleus of the two portions of the archiblast soon begins to prepare for another division, and in the parablast two free nuclei may be observed, which are also getting ready to divide. At the same time the separation of the archiblast from the parablast, at the base of the former, begins to manifest itself, and when the archiblast has been divided into four segments they have become quite free in that they have now separated themselves from the parablast below. They then lie on the protoplasmic layer of the latter, and in this layer four free nuclei may now be noted. The cleavage or segmentation now proceeds regularly. When the nuclei of the archiblast have been transformed into new spindles, the same takes place with the nuclei of the parablast. All of the free nuclei are ever undergoing similar phases of division, or are synchronously in a state of rest, and the nuclei of the segments of the archiblast pass through the same phases, at least during the first few hours of segmentation, whilst at a later period the free nuclei of the parablast pass into a resting stage.

"With the completion of segmentation, the Teleostean egg consists of

a great number of segmentation spheres, comprising the segmented germ-disk or archiblast, and the multinucleated parablest. From the archiblast all of the germinal layers are developed; never does the parablest take part in their formation. That this is the fact is best shown by means of sections, prepared from embryos in much more advanced stages of development, in which the intestine has been developed, and in which we still find these same free nuclei as at first. We may then rightly inquire what is the significance of the protoplasmic covering of the food yelk in which a great multitude of free nuclei are imbedded. The only answer which I can give to this question is the following: The parablest, so rich in free nuclei, assimilates the constituents of the food-yelk, in order to convert them into a form suitable for the growth of the cells of the archiblast, or to convert the yelk into the embryonic layers developed from the archiblast; in other words, the multinucleated parablest assumes the rôle of provisional blood. This view I would justify by the three following sets of facts: (1.) The germ-disk already begins to grow during segmentation. This growth can only take place by the incorporation of nutritive material which can only be supplied by the food-yelk. (2.) During the later stages of development, underneath the embryonic layers, that is, under the embryo itself, one finds the free nuclei heaped upon each other in several layers, and the protoplasmic layer in which they are imbedded very strongly developed, while around the other parts of the yelk they are sparingly developed. (3.) If one places the eggs under conditions injurious to their development, allows them to remain, for example, in stagnant instead of running water, they become abnormally affected. If such eggs are more closely investigated, it is learned that it is the free nuclei which are first affected, in that a fatty degeneration occurs in them, and as soon as abnormal changes occur in the free nuclei, one may be certain that in a short time the germ, or embryo, will be found dead. The free nuclei are also of great importance in nourishing the germ; that is, the embryo.

“What is the fate of the free nuclei, whether they have only a transient existence, or whether the protoplasm in which they are imbedded divides into definite tracts, or, in other words, whether the free nuclei become differentiated into cells at a later period, I do not know, as my investigations have not proceeded so far. If the view should be confirmed which has been taken of them by His—who at any rate erroneously regards these nuclei as originating from leucocytes, which have entered the immature egg—that they become blood corpuscles later, an opinion with which Balfour concurs in regard to their fate in the ova of cartilaginous fishes, would be the strongest evidence in favor of my own view, that they functionate as provisional blood during development. It would then throw light, in a remarkable way, upon the genesis of the blood, as the first blood corpuscle would then be formed at the moment when the egg was divided into the archiblast and parablest or into germ and food-yelk. Kupffer’s statement that in just hatched embryos of the her-

ring there was no trace of the presence of blood corpuscles, I can confirm. The same is true of *Crenilabrus*, *Julis*, *Scorpxna*, *Fierasfer*, etc."

In that the yelk hypoblast contains free nuclei in what appears to be a continuous and homogeneous sheet of superficial protoplasm, without any evidence of division into cells or segments, it is truly of the nature of a syncytium as defined by Haeckel, or it may be defined as a multinucleated protoplasmic layer. A. Rauber also speaks of it as a plasmodium, a term borrowed from cryptogamic botany, and first applied to that most remarkable of substances to be met with under rotten wood and damp leaves in moist glens and representing a stage of development of a very singular order of *Fungi*. Plasmodium is as near the ideally structureless non-nucleated condition as one of the *Monera*, but in the case of the germinal layer of the egg of osseous fishes, while it is apparently devoid of nuclear bodies up to the time of the first cleavage, after that it acquires them and really becomes a syncytium, as before stated. The point insisted upon by Hoffman is of great weight in relation to the part it plays in the development of the blood, and in the later stages of embryonic development we shall find that the blood actually develops from it and that the larval blood-vascular system of the yelk is in part actually channeled out of it superficially. This idea was first tacitly formulated by Professor Carl Vogt, now of Geneva, in 1842, when he made use of the term *couche hematogène* in describing the development of *Coregonus palaa*, where it could scarcely escape observation if the lowermost germinal layer in that form shares in the development of the blood as conspicuously as in the embryos of our own whitefish, *Coregonus albus*.

#### 7.—SEGMENTATION OF THE GERMINAL DISK.

When the protoplasmic streams over the surface of the yelk have carried the principal portion of the germinal protoplasm to the disk, these disappear and the cod's egg no longer presents the appearance shown in Fig. 7. When the aggregation of the disk is completed it presents a discoidal, biscuit-like form, with the edges blunted or rounded off and thickest in the center. While all evidence of the strands of germinal matter radiating from the edge of the disk has disappeared, a thin veil of germinal protoplasm still remains behind to cover and include the yelk sphere. This stratum in the cod's egg is exceedingly thin and is continuous with the germinal protoplasm of the disk all round the margin of the latter previous to the advent of segmentation. The same condition appears to hold in the case of Salmonoids, Clupeoids, and Cyprinodonts, but in all of these the layer of germinal matter left behind as an envelope for the yelk seems proportionally thicker.

After the germinal disk has been fully developed and has assumed the biscuit form already alluded to, at about the sixth hour, in eggs which hatch in twenty days, it begins to elongate, becoming wider in one direction than in the other, and at the same time more depressed,

with the margins more attenuated than when the formation of the disk was first completed. At the middle of its shortest diameter a transverse furrow now appears, which, by the eighth hour after impregnation, has caused the disk to assume an hour-glass shape when observed from above or below, the two halves being almost exact counterparts of each other. If one will now arrange the mirror of the microscope so as to cause the light to fall upon the eggs obliquely without passing up the tube to the eyepiece, the transparent germinal disk will be found to inclose a very large number of fine granules, which show a disposition to arrange themselves in a definite manner. These granules are found to have aggregated somewhat towards the center of each half of the disk, with a clearer space in the central portion, as shown in Fig. 9, Plate II. The clearer spaces  $n$  in the opposite halves of the disk may be regarded as the nuclei of the two segments which must have resulted from the first segmentation nucleus included in the germinal disk before it had exhibited any sign of division. The first cleavage may now be regarded as complete, and if the reader will observe the granular bands running across the middle of the disk in Figs. 9 and 10, he will notice clear spaces between them. This is due to an equatorial arrangement of the granules of the germinal matter and indicates the point of separation between the two cells resulting from the first cleavage. The first cleavage of the disk may now be considered complete.

In an hour and a half more, as shown in Fig. 10, the second cleavage has been completed and is indicated by an emargination at either end of the disk and the differentiation of granular bands and a clear space along a furrow traversing the disk at right angles to the segmentation furrow of the first cleavage. The granular bands in both cases being due to the same causes, namely, a polarity which is manifested in the process of cell division in general, from which it results that the granular matter of the protoplasm is arranged in the form of a kind of double partition or plane coinciding with the direction of the cleavage furrow. The cell-plate so defined is represented in Fig. 10, but is not so easy to make out after the segmentation has advanced still farther so as to divide the disk into much smaller segments. We also for the last time have the nuclei distinguished when the disk has been cleft into four segments, afterwards these are not discernible without the use of reagents.

In the course of two to four hours more still further advances have been made in the segmentation or cleavage of the germinal disk, but usually in a very regular way, the segmentation furrows cutting each other at right angles and dividing each of the four cells of the first and second cleavages into at first two and then four masses of nearly equal size. By the twenty-third hour after impregnation, the germinal disk of the cod's egg will have been divided into fourteen to eighteen segments, as shown in Fig. 12 from below and 11 from the side. In Fig. 12 the large cell at the right is just beginning to divide, the incipient



cleavage being merely indicated. When the large cells of the disk commence to divide somewhat later and earlier than each other an irregularity in the form of the cells ensues which tends to restore the circular form of the germinal disk, which after the second and up to and including the third and fourth cleavages had a subquadrate form when seen from above or below. When this stage of development is completed, that is, when the germinal matter of the disk has been split up throughout its entire thickness into a single layer of cells, the morula or mulberry stage of development has been completed.

After the lapse of forty-five hours and a half, or twenty-two and a half hours later, the morula stage has been replaced by another condition of things represented in Fig. 13. Cleavage of the segmentation spheres or cells of the disk also takes place now in a plane parallel to that of the disk itself, so that by this time three layers of cells may be very distinctly made out, which are superimposed upon each other. These layers are the first indication of the development of the germinal layers, and foreshadow the conversion of the germinal disk into a blastoderm in which the epiblast first appears, then the mesoblast and hypoblast. At this stage there is at first, however, no regularity such as might be expected in the disposition of the layers, because the arrangement of the cells is somewhat modified by mutual pressure.

Later still, or on the fourth day, the advance in the cleavage is very marked, the individual cells being only a fraction of the size which they presented on the second day, and they display, moreover, an arrangement into a number of very irregular layers as shown in Fig. 14. Another change has been suffered by the form of the whole disk; it no longer presents the concave inner face shown in Fig. 13, but has become very convex on its inner side and has contracted considerably in transverse diameter and become thicker in the center.

A little way back we hinted that certain irregularities in cleavage manifested themselves at about the time the germinal disk was divided into twelve to sixteen cells or segments. This phenomenon has been noted in the segmentation of an undetermined fish egg by Prof. W. K. Brooks, of Johns Hopkins University. It is also very strikingly developed during the early stages of cleavage in the eggs of the Clupeoids. It would appear that segmentation is a rhythmical process, and that between the phases of actual segmentation there usually, if not always, intervene periods of rest or quiescence. Within the past year I have had very good opportunities upon several occasions to study the transformations of nuclei during the process of segmentation in the early development of the germinal disk and blastoderm of fish ova. These phenomena I have noticed more particularly in hardened and stained preparations, treated with acid carmine, by the use of which the details of the process at various stages may be made palpable. Some of my observations have formed the subject of a short paper published in the *Bulletin of the Fish Commission* for 1881. That paper, "On the nuclear

cleavage figures developed during the segmentation of the germinal disk of the egg of the salmon," was the result of a study of a comparatively early stage. I have since assured myself by an investigation of the more advanced blastoderms of the ova of the trout and white-fish that complex nuclear metamorphoses continue to manifest themselves much later and are a constant accompaniment of cell division or multiplication in the blastoderm. I have also elsewhere pointed out the effect of fluctuations of temperature in accelerating or retarding the division of nuclei, and consequently its influence upon the rate at which development proceeds. Although these studies are anatomico-physiological in character, their bearing upon the labors of the Fish Commission are important in that they afford us a rational interpretation of a very obscure process, viz, that of growth and development and its dependence upon temperature in cold-blooded vertebrates. If it is agreed that the force which determines development is affected by changes of temperature, it must follow that growth force is in some way dependent upon heat, one of the forms of molecular motion. The facts show that this is true, and that growth,—cell division, appears as if it might be regarded as a form of physiological work exhibited by protoplasm under the direction of determinate laws of nuclear change. It appears that irregularity or asymmetry of development of the cells of the germinal disk is common in meroblastic ova with a large yolk, as is noteworthy in the eggs of birds, reptiles, Elasmobranch and Teleostean fishes, especially during the early stages. This appears to be dependent upon the behavior of the nuclei after the disk has been segmented into several cells. Their metamorphoses from some cause do not exactly keep pace with each other; some divide sooner than others, so that it results that some pairs of incipient cells have already divided before division has begun in others, giving rise to a disk composed of irregular and unequal sized cells at the time when its cleavage into a single layer is completed.

As already stated, the metamorphoses of the nuclei, which have in reality descended from the first segmentation nucleus, are rhythmical. At first round and containing, besides a nucleolus, numerous granules and even granular reticuli, at the time segmentation is about to begin its contents rearrange themselves; the nucleus, in large cells of early stages often showing as a clearer rounded body imbedded in the center of the cellular protoplasm, slowly acquires a more elongate form; at the same time its granular contents tend to arrange themselves in bands nearly at right angles to the plane of cleavage. These lines then seem to undergo a further metamorphosis, in that their substance becomes slowly more homogeneous and is finally aggregated into very refringent rod-like bodies arranged in the form of a wreath or crown at either pole of the nucleus. These wreaths or crowns of refringent rods are then repelled more and more from the plane of cleavage, and at the same time tend to become more densely packed together in a parallel manner.

Their appearance at this time reminds one of an exceedingly minute bundle of cigars, transparent, and now forming the poles of the very elongate nucleus. By this time the equatorial division of the cell into two is practically completed and at right angles to the axis of the controlling center or nuclear body just described. The retrogressive or resting stage now supervenes. The two poles with their bundles of refringent rods are now the nuclear centers of two new cells which have been the result of the cleavage. These two bundles of refringent rods undergo a retrogressive metamorphosis, by which they become globular and take up a larger space in the center of the new cells of which they form a part. The refringent rods disintegrate and the new nuclei, which have passed into the resting or quiescent stage, undergo a repetition of the changes above described during the next stage of segmentation.

The rhythmical phenomena which accompany these internal changes are manifested in the outward changes of form of the protoplasm involved. The cleavage furrows are usually developed with comparative rapidity, accompanied by a tendency to heap up the protoplasm of the two new cells in a conical form. This conical form then gives place to a more depressed one, which coincides with the resting stage. The development of wrinkles in the cleavage furrows, as shown in Figs. 35, 36, 39, and 44, are also to be referred to active movements of the germinal matter, involving more especially the paraplast or superficial cell substance. In fact these appearances show that contractility of the protoplasm is manifested during development.

Pathological or abnormal phenomena are also manifested during very early stages of the segmentation of the germinal disk. Of such early irregular forms of segmentation, I have represented three in outline in Figs. 36, 37, and 38. The proof that these are abnormal is the fact that the protoplasm of the component cells has become brownish, more distinctly granular and dead. The symmetry seen in Figs. 9, 10, 35, 39, and 44 is wanting. Such appearances are the preludes to the disorganization of the egg, and are as fatal in their results as the appearance known as "rotten spawn" in ova freshly taken from the ovary.

The "rotten spawn," by the way, is interesting as showing that ova may become injured while still within the ovary. Microscopic examination reveals the fact that such ova contain masses of clotted or dead plasma, which is brownish by transmitted light, or whitish like boiled rice by reflected light. This dead or injured protoplasm may involve portions of the yolk only or parts of the outer germinal pellicle as well, and the existence of such a condition in a lot of ova is sufficient to warrant their rejection, for, as a rule, when any eggs from a female fish show this condition, the whole of her spawn is worthless.

To return to the subject proper to this part of our discussion, it is also important to note that the first cleavage furrows do not cut entirely through the germinal disk. The proof of this fact we have in the

existence of a stratum of unsegmented germinal matter lying beneath the disk as a thin layer continuous with that which covers the yolk. This has been noticed by Oellacher, Van Beneden, Rauber, Hoffman, and Van Bambeke, and I have demonstrated the fact to my own satisfaction on the ova of Clupeoids, Salmonoids, and Cyprinodonts. Van Beneden is the only observer who has apparently noted it in what appears to have been the egg of a Gadoid fish. While I have not demonstrated the structure in question in the ovum of the cod, the existence of a primitively structureless yolk membrane is evidence enough of the fact, taken together with what I have demonstrated in the eggs of other species.

The later appearance of free nuclei in the yolk membrane or yolk hypoblast would appear to warrant the inference, which has been shown to be the fact by Hoffmann, that in some way nuclear matter had been left behind in its plasma which had been derived from the first segmentation nucleus, which would account for the germination of blood-cells from its outer surface, as witnessed by Gensch and myself. I am not, at any rate, inclined to believe in the theory of the spontaneous development of nuclei in this layer.

The later phenomena of segmentation of the germinal disk cannot be so well observed in the live egg as the earlier ones, in that the cells become successively smaller and less distinct, until finally the whole disk assumes a lenticular form and is composed of a great multitude of very small cells. Each of these cells, however, when the disk is hardened and stained, reveals the nuclei distinctly, and sometimes one may meet with a cell in the act of division with the nucleus in a condition of metamorphosis. The cells are arranged in very irregular strata, as shown in Fig. 13. This stratification becomes less distinct in Fig. 14, in which the epidermal or epithelial layer is developed as a somewhat thinner stratum than any of the cells below. There is at this period no distinct differentiation of any of the germinal or blastodermic layers, if we except the epithelial differentiation of the outermost layer of the germinal disk.

Beyond this point the differentiation of the germinal disk into the blastoderm, in a portion of which the embryo fish makes its appearance, is very gradual. In fact every step of development is but a prelude to that which is to follow, but of the hidden force or impulse which determines the invariable mode in which it takes place we know very little beyond the fact that it has been named *heredity*. With Whitman we may quote Bergmann and Leuckart: "Jeder einzelne Entwicklungsmoment ist die nothwendige Folge des vorausgegangenen und die Bedingung des folgenden."

#### 8.—TRANSFORMATION OF THE GERMINAL DISK INTO THE BLASTODERM.

The next event in the history of the disk is its metamorphosis into the blastoderm, at one side of which the first indications of the embryo make their appearance. This is not fairly accomplished until three

days later than in the stage represented in Fig. 14, or on the seventh day of development. The disk spreads somewhat and becomes decidedly concave on its inner face, at the same time a cavity appears which occupies an eccentric position at one side of and within the blastoderm, as shown in section in Fig. 15. This cavity appears to be the result of further cleavage and is filled with a serous fluid; the cells which inclose it frequently jut into the cavity somewhat irregularly. In Fig. 16, the space occupied by the segmentation cavity is shown by the lighter area *sg* somewhat crescentic in shape and bounded by a thicker rim of cells around its outer margin and the thinner portion of the embryonic disk above. The portion of the blastoderm from which the head of the future embryo will be developed is shown just below *A*, as a rounded promontory of cells projecting into and forming the concave margin of the segmentation cavity. This promontory from *A* to *B* is composed of a number of layers of cells and represents the embryonic disk or shield of authors, in which the first trace of the axis of the body of the embryo becomes apparent. An outline, Fig. 17, more magnified and somewhat older, shows comparatively little change in the form of the blastoderm and segmentation cavity.

The origin of the segmentation cavity as well as the character of its walls has engaged my attention considerably.

Klein \* represents it as originating by the elevation of the blastoderm at one side, so that it is freed from contact with the parablest layer lying just below it. In this way a space, filled with fluid, is developed. As far as my own observations enable me to reach a conclusion it appears that the above view of its origin is probably the correct one. The imperfect floor of the cavity is afterwards apparently developed by an ingrowth and budding of scattered cells at its edges and bottom, probably from the yelk membrane (parablest); this floor disappears during a later stage. It is singular that no investigators have recognized the homology of this cavity with the segmentation cavity in the eggs of Elasmobranchs and Amphibia, as pointed out by Balfour, with whose conclusions in this regard I was wholly in accord long before the appearance of the second volume of his monumental work on embryology in 1881. With regard to the details of its development, however, I differ with this authority; and of his statement that it disappears "shortly after the appearance of the medullary plate" I can only say that I have accumulated a very large amount of evidence in proof of the contrary. Inasmuch as the whole of the evidence on this point is now in my possession in the form of sketches from living ova as well as sections, it may be well to give a summary of my views regarding this point with references to previous investigators. H. Rathke is the first to have described the growth of the blastoderm over the yelk and its complete inclosure of the latter as observed by him in 1832 in the development of *Zoarces*.

\* Quar. Jour. Mic. Sci., No. LXII, 1876, pp. 113-131, plate VI.

In 1865 Stricker discovered this cavity in the egg of the trout, in consequence of which Cellacher has proposed to name it after him. This cavity is altogether different from what has been described by several authors as appearing in the center of the cellular mass of the disk, and which, as suggested by Cellacher, is, in all probability, a product of reagents. Cellacher also appears to have been aware of the persistence of this cavity up to the time when the yolk blastopore closes. So that so far as I may have any claims to priority in the matter it simply relates to a proof that it persists in a considerable number of genera and is characteristic of the blastoderm of Teleosts in general. Cellacher, however, does not regard it as a segmentation cavity, so that it has remained for Balfour and myself to establish its homology. I at one time believed with Balfour that this cavity was at first closed below by a more or less complete stratum of cells, but if its development is followed to the time when the yolk blastopore is closed it will be found that such is not the case, and that the yolk hypoblast, which is not truly hypoblastic, corresponds simply to the *granular layer* of Balfour. When I say that the yolk envelope, *Dotterhaut, couche intermédiaire*, yolk hypoblast, etc., as it has been variously named, is not truly hypoblastic, I mean to imply that no portion of it is in the relation of a hypoblast to the embryo and that it seems to serve simply to inclose the yolk and effect its metamorphosis into blood. The few scattered nuclei in its substance, except just below the embryo's head, cannot be regarded as forming a cellular floor, since in preparations stained with borax carmine these nuclei are seen to be free, much scattered, and simply involved in the plasma of this layer.

It will be seen from the above that my views have undergone some change since the publication of my paper on *Tylosurus*; but these changes of opinion relate entirely to the history and fate of the yolk envelope or intermediary layer of Van Bambeke. I still hold to my interpretation of the homology of the whole amphibian ovum with the disk only of the Teleostean egg; but a discussion of this and other theoretical matters may be more fittingly reserved for the close of this paper.

In other types the segmentation cavity is unquestionably originated as a direct result of cleavage. This is apparently the case in the Teleostean egg; after the blastoderm is fairly formed the germinal mass of cells immediately involved in the development of the embryo, having been detached during segmentation from the germinal matter comprising the yolk envelope, they are freed in great measure from complete contact with the latter except underneath the embryonic disk. Fluid finds access into the cavity beneath the thinner non-embryonic portion of the blastoderm, but as the blastoderm grows the cavity increases in dimensions transversely and diminishes in depth, so that finally a film of fluid of extreme tenuity is interposed between the non-embryonic portion of the blastoderm and the homogeneous yolk membrane. Viewed

in optic section, normal ova sometimes show the segmentation cavity as a space of considerable depth, as is indicated in the outline sketches of sagittal sections of two early stages of the blastoderm of the cod, Figs. 47 and 48. With the further development the depth of the cavity diminishes, often to such an extent as to apparently vanish in ova in which the yolk has been included by the blastoderm. It is to this fact that we may ascribe the belief, current amongst investigators, that it wholly disappears.

It is quite impossible to reconcile the account given by Haeckel with the facts as presented by others, when he implies that the whole under surface of the blastoderm is lifted up from the yolk and remains in contact with the latter only round its margin. The margin at the same time, he says, is reflected inwards, a single layer of cells growing inwards from all sides, to finally close in the center, forming the hypoblast in that manner. This is not in accord with what the writer has seen in the egg, of the cod, nor can it be substantiated by the classical researches of Cellacher made two years prior to Haeckel's,\* nor by my own more recent investigations during the past two years. There is no evidence to show that the epiblast of the blastodermic disk is reflected inwards to develop the hypoblast. Haeckel says further, that the clear fluid in the segmentation cavity is resorbed and that the cavity itself disappears entirely. This statement the writer disputes *in toto*, with an abundant support of facts in his behalf. He will only mention here that not only does this cavity persist, but that it also actually increases in size during the later stages of development, as may be observed after yolk absorption has begun, as may be seen in *Coregonus albus*, *Elacate canadus*, *Cybbium maculatum*, *Parephippus faber*, etc.

In Fig. 18 the left half of a blastoderm of a cod's egg of the latter part of the seventh day is seen in median section along the plane of the axis of the embryo from A to B; the thickened portion or embryonic area has divided into two thick lamina or strata, each several cells deep. The whole of the upper surface of the blastoderm is covered by a very thin single layer of epiblast cells which pertain to the epithelial layer. The bilaminate condition of the blastoderm extends also into the rim or annulus *r*, which extends around about one-half of the disk and widens just below A, where it is blended and confounded with the embryonic shield or area. The thinner portion of the blastoderm, extending from A to the upper border of *r*, is composed of two layers of cells which roof over the very shallow segmentation cavity. The outer of these is the epithelial layer already referred to, and the inner one composed of rounded cells answers to the sensory layer of embryological writers. Such, in brief, is an outline of the history of the blastoderm immediately after it has clearly become such and before there is as yet any distinct differentiation of the axis of the embryo which is now marked only by the thickest portion of the blastoderm in the median region

\* *Jenaische Zeitschrift* IX, 1875.

from A to B. This figure also shows the very narrow slit-like lumen of the segmentation cavity to the right of A, and its extent over the left half of the blastoderm is shown by the line below and to the left of *sg*.

#### 9.—THE DEVELOPMENT OF THE GERMINAL LAYERS.

This portion of the subject is one upon which I cannot, unfortunately, throw much light on the basis of observations made upon the development of the cod's egg, and I shall therefore place under contribution the labors of Cellacher and others on the trout, and my own observations upon those of several other species. It is evident that in respect to the developmental changes which the blastoderm undergoes in different species, there is considerable variation. In the trout, for example; the embryonic shield or area, corresponding in the cod's egg to the space from A to B in Figs. 16, 17, and 18; there is a considerably less prominent development of the embryonic shield at a correspondingly early stage. In *Tylosurus*, at an early stage, the conditions of the two in respect to the size of the embryonic shield is about the same.

The embryonic shield, as development advances, grows farther and farther inwards towards the center of the blastodermic disk, or rather, as it grows in length before and behind, the disk at the same time spreads in consequence of the continued segmentation of its component cells, so that these are spreading themselves over a greater and greater area while they are at the same time undergoing a definite rearrangement into strata or layers, each of which has a definite share in building up the different parts of the embryo's body. This mode of spreading, however, never affects the relation of the embryo to the edge of the disk. Its tail-end lies at the edge, its head at the center for a considerable time (in small or moderate sized ova constantly), with the axis of the body of the future embryo lying in one of the radii of the disk. In unusually large ova, like those of the salmon, *Tylosurus* and *Arius*, the blastoderm spreads so fast after a while that the embryo does not grow in length rapidly enough to maintain the position of the head near the center of the blastoderm. These last facts explain Cellacher's position in regard to this phenomenon in the trout's ovum.

With the development of the embryonic shield the differentiation of the lower layers commences. The first to be split off is the sensory or epiblastic layer; in the cod's egg this is formed on the seventh day in ova which hatched in sixteen days. The process is truly one of delamination, and cannot be regarded as produced by a true gastrulation at all, as Haeckel has tried to show in a paper already noticed. The process of the differentiation of the layers we saw began with the development of an epithelial layer of epiblast over the surface of the germinal disk before the appearance of the segmentation cavity. Immediately after the appearance of the latter the embryonic disk begins to be developed and the layers differentiated. The sensory layer is split off first from the underlying stratum of cells at the head end of the embry-



onic shield and is continued backwards towards the tail-end of the embryo. This splitting also involves the rim of the blastoderm, which is found to be composed of three strata of cells, viz, the epithelial, the sensory or truly epiblastic, and the lowermost or mesoblastic and hypoblastic. This relation holds throughout the whole disk except at the tail and rim, where the sensory layer and lower one appear to pass over into each other, they appear in fact to be folded upon each other, as shown diagrammatically in Fig. 18 at B and *r*. The two principal layers are at first quite thick; as the disk spreads, however, the portions in the vicinity of the embryonic disk alone maintain their original thickness to a marked extent, and then only along the axis of the embryo as development advances. The two principal strata in the rim *r* also remain thicker, and they are really continuous with those involved in the formation of the embryo. As development of the blastoderm advances, however, its rim becomes narrower and a less marked feature as well as somewhat thinner. The segmentation cavity is roofed over by the epiblast alone, consisting of epithelial and sensory layers only. At first the sensory layer which covers the segmentation cavity is two or more cells deep (*Alosa*), but later this thins out, so that finally when the blastoderm has entirely inclosed the yelk, it is sometimes quite difficult to demonstrate positively that there is more than a single layer of cells present. The layers covering the segmentation cavity in the Salmonoids are thicker than in other species with small ova without a vitelline circulation, and in such forms a mesoblastic stratum may be added to the epiblastic covering of the yelk at a late stage of development. The stratum covering the segmentation cavity in the young codfish just hatched is like that last described. The hypoblast is differentiated later and is confounded or blended with the mesoblast up to the time when the muscular and the peritoneal layers are differentiated, which does not take place till about the time the muscle segments commence to be developed; even after that time it is somewhat difficult to make out the limits of the hypoblastic layer in sections.

The foundations of the embryonic structures of the young fish have been laid down with the development of the epiblastic and mesoblastic tracts of tissue, and the events which follow, especially the development of the brain and spinal cord, which together we will call the neurula hereafter, play a most important part in still further modifying the history of the primary layers. It is somewhat difficult to give a clear account of the development of the neurula without the aid of figures, but this we will now attempt to do as briefly as possible.

#### 10.—DEVELOPMENT OF THE CEREBRO-SPINAL AXIS OR NEURULA.

The development of the brain and nervous system or neurula of the teleostean embryo presents some very remarkable peculiarities, the principal of which is that it is at first quite solid and only develops a neural canal at a relatively late stage, or after the neural cord (*axen-*

*strang*, His) has been split off distinctly from the epidermal layer overlying it. The formation of the neurula occurs, as will be inferred from what has already been said, in one of the radii of the blastodermic disk. The sensory layer thickens perceptibly along this axis at a very early stage. In the cod's egg this is perceptible on the eighth day of development, as shown in Fig. 19, pl. IV, where the band of cells *P st*, which pass inwards towards the center of the disk of the blastoderm, are the rudiment of neurula or medullary plate from which the nervous system will be differentiated. This rudimentary nervous cord continues to become thicker in a vertical direction as development proceeds, and at last begins to be apparent as a ridge on the under side of the blastoderm. The ventral ridge or keel of the neurula becomes more apparent on the ninth day at the fore part of the embryonic axis, as may be seen in a side view of an egg of that age with its blastoderm in optic section represented in Fig. 20. Another view of an egg of the same age is given in Fig. 21, but here the head end of the embryo is directed toward the observer, and in the darkly shaded portion, which is an optic section of the fore part of the body of the young embryo, shows the neural keel prominently developed on the side next to the yolk. In the embryo of other animals, with two or three exceptions, the development of the neurula takes place in an entirely different manner. In most forms in fact, when the neurula is forming, the epiblast becomes grooved on its external face, while, as the furrow so developed deepens, its sides fold over toward each other to join in the middle line, leaving at the same time a canal throughout the whole length of the neurula and even continued at the hinder end into the primitive intestine or archenteron. Not so with the neurula of the embryos of osseous fishes. Here the neurula is at first an absolutely solid cord or strand of cells which by a slow thickening of the sensory layer at last forms a deep laterally compressed mass of cells which juts down into the yolk, pushing the hypoblast before it. It is even difficult to prove that there is ever any such a thing as a medullary groove or furrow developed at all in the sense in which we know it in the embryos of Amphibians, for example. However, our Fig. 21, pl. IV, shows a depression in the median dorsal line of the embryo at *mg* which we may regard as representing the medullary groove in the blastoderm of the cod of the ninth day. Such a feature is also shown by Oellacher in his figures of sections of the blastoderm of the trout; it is also developed in that of the shad. Calberla in his studies of *Syngnathus* states that the epidermal layer of epithelium is carried down by invagination into the medullary plate along its mesial axis as the neurula is developed. In such sections of similar stages of Clupeoids as I have had the opportunity to study I have seen no evidence of anything of the sort. In this respect they are like the embryos of *Lepidosteus* investigated by Professor Balfour. In this regard Oellacher's investigations upon the trout seem to coincide with the results of Balfour and myself. As

development advances the medullary groove becomes less and less marked in depth, and by the time the embryo's body has been fairly outlined there is nothing more of it visible in sections. For my part I now have serious doubts as to whether any actual infolding of the sensory layer of the blastoderm of Teleosts ever takes place to form the neurula. It would almost seem as if the formation of the medullary plate took place rather by the slow heaping up of the cells of the sensory layer along the neural axis by an amœboid or migratory process. It is at any rate difficult, if not impossible, to find the evidence of any process of infolding of the nervous layer to form the neurula such as has been observed in other types. The mode of development of the neurula, however, as it may be observed in Teleosteans, Amphibians, Elasmobranchs, Marsipobranchs, *Amphioxus*, birds, and mammals, differs so widely in detail and essentials in these different groups as to hinder us from framing any general theory of development for the nervous system of the vertebrates. Such a procedure is all the more to be regarded as premature, in view of the fact that we do not yet know the full history of the development of such forms as *Myxine*, *Lepidosteus*, and *Amia*. What there may still remain to be revealed of a startling or unexpected character, in a study of these forms, we do not know, for the development of none of them is thoroughly known. In fact, the development of comparatively few animals is as thoroughly known for all of their stages as will be demanded by the comparative embryology of the future, so ably heralded by the late Dr. Balfour. The science in its present state may, on account of the imperfection of most of the developmental histories of the principal types, be compared to an ancient manuscript of which just enough has been preserved to give us an idea of the way it treats its subject. Great gaps in our embryological knowledge are apparent in even some of the best studied forms. In one form we know the early history; in another the later. In others we know the development of the germs in the reproductive organs before impregnation; in others we do not. In some cases we know the late phases of development when the embryo or young passes into the adult condition; in others our knowledge in this respect is a blank. Not only is this a serious difficulty, but there is also the still more serious one of reconciling the contradictory statements and observations of honest investigators each of whom has usually added some important information to that which we previously possessed, but who have rarely missed falling into errors of interpretation due to the nature of the subject, defective opportunities and methods; or on account of the finite nature of the mind itself they have been more or less mistaken in making inferences and deductions from the observed facts. This is no discredit to the science, but only a necessary condition through which it must pass in the course of its development.

The blastoderm of the cod's egg, like that of other Teleosts, continues

to spread out and grow over the surface of the yelk sphere underlying it. The blastoderm in fact is molded upon the yelk sphere as a hollow spherical membrane, which finally includes the latter. It grows over the yelk by what is called *epiboly*, the yelk itself remaining passive. The rim *r* of the blastoderm shown in Figs. 20, 21, 22, and 23, Pl. IV, moves progressively toward the naked pole of the yelk, and in its progress more and more of the yelk sphere is covered up. As long as the rim is spreading over the upper pole of the yelk its circumference continues to increase; as soon as it has passed the greatest diameter of the yelk, as shown in Fig. 22, it begins to diminish in circumference. This diminution of the diameter of the rim of the blastoderm continues until it finally closes at the exposed pole of the yelk. At the moment of closure, the free margin of the rim presents a wrinkled appearance, the wrinkles radiating from the center of the small pore which remains up to this time. After the closure of the blastoderm the radial wrinkling disappears. The pore which remains up to within a short time of the closure, is the *yelk blastopore* of authors. The rim, which has closed at this point forms a discoidal plate of cells continuous with and forming a part of the tail of the embryo. The cellular disk developed by the closure of the rim may be called the *caudal plate*. It takes a share in the development of the tail of the embryo and also in the muscle plates, neurula, and hind-gut, for the whole of the substance of the rim is appropriated in building up the caudal extremity of the body of the young fish. In Figs. 24 and 25, pl. V, I have represented the appearance of the tail of the embryo cod on the eve of the closure of the blastoderm at the pore *bl*. Other representations of its appearance are given in Figs. 28 *a* and 29 *a*, 29 *b*, and 30 *a* and 30 *b*, the last four being side views. These figures also show, in three instances, the relation of the problematical vesicle *kv* first described by Kupffer, to the blastopore. Their identity is difficult to follow, and I now doubt whether it is anything more than an evanescent structure which has nothing to do with the development of the urinary vesicle or the anal end of the intestine. It is at any rate apparently inconstant in position, and in some fishes evanescent and of temporary importance. Sometimes it appears to be involved in cells, at other times it is clearly surrounded below by the homogeneous yelk membrane or hypoblast alone. In Fig. 26 the blastopore of the yelk is also shown with Kupffer's vesicle lying below the blastoderm as a lenticular vacuole.

As the blastoderm of the cod's egg has spread over the yelk and included it, the neurula has also further developed; the most marked feature of its advancing evolution being the increased ventral prominence of its keel or carina, as shown at *cv* in Fig. 22. At the same time it also becomes more prominent dorsally, as is shown by the same figure, the fore or cephalic end of the body of the embryo is now pronounced in outline, and the principal paired sensory appendages of the neurula become more prominent. The only sensory appendages of the

neurula, which are in direct connection with it from the first, are the optic vesicles or rudiments of the eyes. They are the first of the sensory structures of the young fish to be developed. They, in fact, are already apparent at a very early stage of development, and are formed as lateral outgrowths of the extreme anterior end of the neurula, after the eighth day, and by the ninth day, as shown in Figs. 20 and 21, *op*, they are conspicuous as thickened lateral lobes of the anterior portion of the cephalic end of the rudimentary neural system. Their development in Teleostean embryos is to a certain extent characteristic, in consequence of their great relative size in proportion to other parts of the nervous system at this time. At first their connection with the neurula is quite lateral and anterior; as development proceeds, however, the down growth of the carina or keel of the neurula carries their stalks or points of attachment downward. The place where they arise from the keel marks the position of the origin of the optic nerves, in the vicinity where the cerebrum is continued into the more posterior portions of the brain or thalam-encephalon. During the early stages of their development, they, like the neurula itself, are composed of a solid depressed ovoidal mass of cells. As development proceeds, this mass acquires a lumen or cavity, which is at first a mere cleft like the primitive cerebral lumen or cavity. The cavity of the optic vesicles in section is at first somewhat oblique to the plane of the blastoderm, but this feature is lost with advancing development, and the lower wall of the optic vesicle is finally pushed inward, upward, and more towards the axis of the embryo, while the hind wall itself is also raised so that both together assume a more nearly vertical position. This condition is shown Fig. 27, taken from the head of an embryo eleven days old. The rudiment of the eye-ball is now more nearly vertical and oval as seen from the side. It is now a very depressed double-walled cup as viewed from above, and is connected by a hollow stalk with the lower forward part of the brain. In Fig. 27 its stalk lies just behind and below the rudiment of the nasal or olfactory pit *na*, and its outer lamina *op* is afterwards transformed into the retina, while the inner thinner lamina becomes covered by the choroid or pigmented layer, and the thickened epithelial tract of epiblast *l* is carried inwards with the further development of the eye-ball, and transformed by invagination and further metamorphosis into the lens. The central part of the thickened rudiment *l*, of the lens, becomes the transparent, posterior highly refractive fibrous part, while the surrounding thinner margin of the layer *l* is reflected over the thicker hinder part, as a thin layer of epithelial cells. After this the lens is constricted off from the epithelium and the construction of the eye is essentially completed. The further development, however, of the eye involves the consideration of still other events in the history of its layers. The development of the lens is shown in Figs. 26, 27, 29, and 30. In Fig. 29 the outer epithelial layer is shown, inclosing, as it were, the columnar internal refringent layer within. The optic cup is still open below on

the fourteenth day as shown in Fig. 31. The rim of the cup seems, in fact, to grow down from above and, gradually becoming more globular in form as a whole, its free lower borders approximate and coalesce, forming the choroidal fissure *fc*, through the proximal part of which the optic nerve enters the eye. The inner walls of the choroidal fissure at its hinder part may be regarded as continued into the optic stalk, and it so happens that the optic nerve finally loses all connection with the outer wall of the optic cup and perforates it to connect itself with the inner and thicker layer which has become the retina. The optic stalk itself is, for the most part, if not entirely, converted into the optic nerve and crus. With the further development of the optic cup, its rim is reflected inwards and more fully covers the lens and becomes thinner. After this stage it is said that in other forms the iris, with its muscles, pigment, etc., are derived from the mesoblast around the choroid at the rim of the optic cup. The cornea grows from a ring of cells of uncertain origin just below the epithelial layer of the epiblast at the time the lens is invaginated, according to Balfour. The vitreous humor is developed in much greater proportion at a very early stage in some forms than in others. In *Tylosurus* and *Apeltes* it develops relatively early; in other forms, as *Gadus* for example, it is not perceptible until about the time of hatching. The vitreous humor appears to be gradually developed and seems to me to be a fluid transudation; perhaps it really arises as an ingrowth through the choroidal fissure, as held by Balfour. The aqueous humor develops, according to the last authority, within the ring-like rudiment of the cornea, the cavity of which enlarges as development advances. The brilliant silvery pigment of the iris of the young cod is very probably of mesoblastic origin. Of the development of the muscles of the eye little can be said here, as little is known of the subject. The study of the development of the muscles, by the way, is a department of embryology not yet sufficiently well cultivated. The blood-vessels of the eye enter the organ through the choroid fissure. The eye is already functionally active at a very early stage or upon the eve of hatching, as I have detected movements of the eye-ball even just before the young fish had left the egg. Its complete pigmentation is accomplished by the time the embryo frees itself from the egg-membrane, but most species of fishes do not begin to swim actively for some time afterwards. In others of very rapid development, the movements which are made are not sustained but fitful in character, and often executed with astonishing velocity. That the eye is already functionally active is proved by the ability to instantly recognize an approaching object in the water manifested by young fishes a day or so old. They already see well enough in most cases to try to avoid being caught with a pipette or small skim net.

The account which precedes has dealt in large part with the evolution of the eye of the young fish and has carried us far beyond the time when the neurula has been fully formed. Coincident with the increas-

ing prominence of the ventral keel of the blastoderm, as shown in Fig. 22, another process has been going on by which the tissues of the neurula have been more markedly differentiated from those adjacent. A very distinct line of demarkation is established on either side of the neurula separating it from the muscular mesoblast on either hand; this is shown in Fig. 23. This demarkation was, however, already established when the sensory layer was split off from that lying below it, but as the carina of the neurula is developed, the mesoblast is gradually separated off into two lateral masses, the stratum of hypoblast and mesoblast below the neurula being now only a relatively very thin layer which it has apparently pushed down before it. The line of separation between the sensory and muscular layers at first coincided with the plane of their upper and lower surfaces, but as the neurula grew in depth, this line of separation became more and more nearly vertical at the sides of the latter. In the cod's blastoderm this is accomplished about the tenth day in embryos which hatch in from sixteen to eighteen days, and before the closure of the blastoderm over the yelk. The muscular layer has by this time also been quite separated from the peritoneal layer below it, and the *muscle plates*, as we may designate the three-sided, longitudinal, lateral masses of cells on either side of the neurula, extend from behind the optic vesicles up to the tail end of the embryo and are continued into the lower layer of the rim of the blastoderm at *r*. The substance of this rim seems, in fact, to be entirely incorporated into the building up of the embryo's body, up to the time the blastoderm has inclosed the yelk. The neurula has meanwhile also been undergoing further differentiation. At its front end in the vicinity of *cv*, Fig. 23, a distinct median split or cleft has appeared in it which extends forward and backward some way, but is not developed in the tail end of this structure. This cleft or cavity in the fore part of the neurula represents the neural canal of other types, and from it in the head region the cerebral vesicles will arise at a later period. At the tail end, on the other hand, the neurula is solid, and on the caudal swelling no trace of the medullary groove is visible. The caudal swelling is a mass of cells in which it is impossible to discover any traces of the differentiation of laminae or layers, except that of the outermost epiblastic or dermal layer. The development of the neurula proceeds therefore from the head towards the tail end of the embryonic axis, where it also grows in length as the rim *r* of the blastoderm advances to finally close over the yelk. Once the closure is accomplished the neurula becomes more defined at the caudal region; it was greatly depressed here on the eve of closure, but the caudal end of the embryo rapidly thickens as it incorporates the caudal plate derived from the rim of the blastoderm. The now more pronounced development of the caudal extremity of the neurula is due to a process very similar to that concerned in the formation of the keel or carina at the head end of the embryo; in fact the keel of the neurula develops from before backwards just as does its lumen or cavity.

The next event in the history of the development of the neurula is its separation from the epithelial layer of the epiblast. This occurs cotemporaneously with the development of its internal lumen, and proceeds from before backwards. The epithelial layer, in fact probably the subjacent layer of the corium itself, has the same history, being developed somewhat sooner in the cephalic than in the caudal region. With this the hitherto flat upper portion of the neurula becomes rounded off except at the tail end. It is now separated from the skin. During this time the skin has commenced to develop pigment in its deeper layer, as shown in Fig. 31. These pigment cells are stellate and exhibit a slow amoeboid or migratory movement as development proceeds, becoming aggregated at a later period by this means into patches upon definite regions of the body.

With the further progress of development the tail commences to bud out from the caudal end of the embryonic axis, which at this point continues to become gradually thicker and more prominent and finally swells out into a hemispherical prominence just above the point of closure of the blastoderm. This is the rudiment of the tail of the embryo. In an embryo sixteen days old represented in Fig. 32 the tail has grown out for a considerable distance, and it has been bent over to one side on account of the confined space in the egg-membrane, so that its dorso-ventral axis is turned at nearly right angles to that of the body. A slight fold extends over its end and dorsally and ventrally which is entirely composed of the skin layer. This fold, *nf*, may be regarded as the beginning of the embryonic natatory fold and develops in height from behind forwards as embryonic evolution advances.

With the outgrowth of the tail the development of the neurula is continued backwards and its extreme hinder extremity remains solid as in Fig. 31, and continuous with the same mass of cells from which the chorda dorsalis or notochord *ch* takes its rise. This continuity of the extreme posterior extremity of the notochord with the neurula is maintained until the tail of the embryo is fully developed. Indeed, after hatching even, in the young cod and most other forms studied by the writer, the chorda is lost in a caudal cellular mass and its end is not included within the urostyle until a considerable time after the young fish is free. I have never met with young cod old enough to see the development of the bones of the tail. The muscular layer is also continued backwards into the tail and clasps, on either side, the neurula and chorda. Lastly the skin or dermal layer is also developed in extent in order to keep pace with the tail as the latter lengthens. The whole process of the growth of the tail is a very remarkable one, and in the cod's embryo, for example, it is hard to understand how the material for so much new structure is transported to its new location without the help of a vascular system, no trace of which has yet appeared. The new matter seems to be added by apposition and intussusception. How much of this process may be due to the amoeboid properties of the



germinal matter concerned in building up new structures in the way in which we see that the tail is evolved we do not know. And yet it is very hard to see how it is possible for new material to pass through and around the cells and cellular structures already built up, to reach the extremity of the tail in order to add to its length and bulk.

#### 11.—THE DEVELOPMENT OF THE BRAIN.

The brain or encephalon of the embryo cod, on the fourteenth day, has somewhat the form of a vertical flat rhomboidal sack, its interior bluntly pointed extremity being the rudiment of the cerebrum, and its hinder part is continued into the anterior end of the neurula or neural tube, as the embryonic spinal cord may now be called. On the tenth day it already begins to become thicker behind the posterior borders of the optic vesicles. By the eleventh day a distinct constriction of the cephalic end of the neurula behind the eyes divides the latter into an anterior and posterior portion, as shown in Fig. 27, but it can hardly as yet be said that a cerebral vesicle has developed, for there is now present only a vertical cleft in the center of the cerebral end *cv* of the neurula. The walls of the brain of Teleostean embryos of this stage, unlike those of other types, are now very thick. They consist in fact of two thick flat plates of cells placed vertically between the eyes. The first constriction *tf* of Fig. 27 marks the boundary between the mid-brain and the cerebellum. At a later period a constriction appears a little way behind this one which marks off the cerebellum and medulla oblongata from each other. This occurs about the twelfth day, when the fore part of the brain rudiment also acquires another constriction which separates the mid-brain from the cerebrum or fore-brain as shown in Figs. 29 and 30. By the fourteenth day the cerebral regions have been developed and the first, second, third, and fourth vesicles or cerebral cavities are present, but they still retain the laterally compressed form characteristic of the early stages of Teleostean brain-development.

The so-called ventral bend or flexure of the encephalon in the embryos of other types is almost null in the Teleostean embryo. In fact, I much doubt if it can be shown that any flexure occurs, as the development of the brain of the osseous fish can be accounted for on another principle. With the great development of the ventral keel of the neurula at the head end of the embryo the vertical depth of the encephalon is almost as great as when the cerebral vesicles are developed. If an invagination upwards and forwards of the floor of the brain now takes place, so as to develop the infundibulum in front of it, the construction of the brain of the late stages is attained. My reason for holding that the infundibulum is developed in this way, is the fact that no perceptible downward flexure of the encephalon ever occurs prior to the development of that portion of the brain. Moreover, the infundibulum is developed long before the head becomes free from the yolk-sack, and therefore before a downward flexure of the brain is possible to any

marked extent. Doubtless, the infundibulum is also partially developed as a downgrowth of the floor of the brain, but this need not involve the whole encephalon in a downward flexure. During the earliest stages of the brain development here described, the whole brain region is but little wider than that of the body; with its more advanced development, however, the cephalic end of the embryo widens, in consequence mainly of the rapid growth of the mid-brain in a lateral direction. If a section of an embryo's head is prepared of the age of Fig. 30, cutting through the region of the mid-brain, the mid-brain cavity will be shown as a cruciform opening, the lateral portions passing into the hollow lateral lobes, *mb* Fig. 30, which are pushed out towards the eyes. When, however, sections of much later stages are prepared this arrangement disappears; the optic thalami have acquired greater development; in fact, the whole ventral portion of the mid-brain has augmented in volume, and the origins of the lateral lobes seem to have been elevated while the lobes themselves curve down over the underlying brain substance, abutting laterally against the eyes and behind against the cerebellum. The lateral lobe of the right side of the mid-brain is shown at *ll* in Fig. 28.

Behind the infundibulum, shown in Figs. 28, 29, 30, and 32, the medulla oblongata has a very thick floor, in just hatched embryos, while the roof of the fourth ventricle, contained in it, is quite thin. The thick roof of the medulla in embryos of *Alosa*, three days old, passes straight forwards over the infundibulum to just above the optic thalamus. The bundles of commissural fibers connecting the various parts of the brain, especially those arising from the floor and traversing its substance in various directions, I have not traced. This portion of the medulla is shown in the optic section represented in Fig. 28.

The pineal gland appears as a mesial outgrowth from the anterior portion of the mid-brain, being fairly developed on the eve of hatching or shortly before it, as indicated in Fig. 32, at *pn*, in a side view of an embryo cod on the sixteenth day of incubation. This organ has recently received a good deal of attention on account of the relation it has been supposed to bear to the primitive mouth of the vertebrates. Goette has described it as being a product of the point where the roof of the brain remains longest attached to the external skin in amphibian embryos, and he compares the pineal gland to the long-persisting pore which leads into the neurula of the embryo of *Amphioxus*.

Some days after hatching I have taken the embryos of *Alosa* and prepared longitudinal vertical sections of the head in order to discover what might be the true relation of the hypophysis to the infundibulum. Dohrn \* has recently investigated this feature of the development of Teleostean embryos and has arrived at the conclusion that the hypophysis is really formed from the hypoblast and not from an epiblastic

\* *Studien zur Urgeschichte des Wirbelthierkörpers. Mitth. aus der zödl. Station zu Neapel, III, 1881.*

oral invagination, as is the case with birds, mammals, Elasmobranchs, and Amphibians. I have a number of such longitudinal sections of *Alosa* which show the hypophysis connected with the oral epithelium by a narrow stalk. In sections of much earlier embryos I find it exceedingly difficult to detect this structure with certainty. I believe, however, that Professor Dohrn is quite right in holding to the belief that it does not originate from an epiblastic involution of the stomodæum. In fact it would almost seem to be demonstrated that the mouth of the young fish is developed, as Dohrn shows, from behind forwards and that it really has no stomodæum as we know that structure in other forms. According to the above mentioned authority the mouth is developed from the anterior part of the mesenteron and that it at first grows out as two narrow, pointed, horizontal clefts which break through at two points on either side of the middle line even before the head has grown out over and beyond the epiblast which is continued over the snout and down over the yolk. This is a very singular state of affairs, but I am not assured from my own investigations that what was observed in vertical longitudinal sections of *Hippocampus* and *Belone* will apply to *Alosa*. The origin of the mouth of the Teleostean embryo is a very difficult subject to work out. In embryos in which the mouth is just on the eve of opening I cannot convince myself positively that I can see what Dohrn claims to have done. It is true there seems to be a less pronounced development of the oral tract of hypoblast near the point where the mouth ought to open in the middle line, but I cannot convince myself of its total absence. In sections off of the middle line the walls of the oral opening are more pronounced, but I can not yet agree that it is decided that the mouth of the Teleostean embryo first opens at two points a little way off of the middle line. In living embryos as well as in hardened ones, treated with chromic acid, the mouth opens as a small tranverse opening, and as development proceeds the rim of the upper jaw is carried forward beyond the line of the lower. But this is digressing again from the subject of the hypophysis, which it appears we cannot regard in the present state of our knowledge at least, as certainly originating from an oral invagination. Although the hypophysis is not a part of the brain, as has been positively demonstrated by Rathke, Goette, Balfour, Dohrn, and others, its development naturally falls within the limits of a description of the formation of that organ. It is a very diminutive structure in Teleostean embryos, even after hatching, and is pushed up between the cranial trabeculæ, still retaining its connection with the oral epithelium even on the third day after incubation in *Alosa*. It rests in a bowl-shaped depression on the lower face of the infundibulum and only two or three of a series of thin sections through the mesial region will usually strike it.

The pineal gland, on the other hand, is clearly a part of the brain; sections through the middle line often strike it and show it as a depressed, biscuit-shaped body with a stalk at its anterior portion connecting it with the forepart of the roof of the mid-brain. It is very

difficult to see this part of the brain distinctly in living embryos, on account of their transparency and the consequent impossibility of making out its limits with distinctness, surrounded as it is by structures very similar in optical character.

In a few instances I have succeeded in getting horizontal sections through the plane of the optic crus of embryo fishes. It presents nothing different, at a relatively late stage, from what is seen in the structure of the same part in the adult. The development of the cerebral hemispheres seems to take place relatively late in embryonic life, and they also retain a remarkable solidity. The cerebral hemispheres growsome-what in size after the larval period of development is past, but in most *Teleostei* they never attain the dimensions of the mid-brain.

## 12.—THE OLFATORY AND AUDITORY ORGANS.

At a comparatively early stage of development the nasal organs are differentiated from a pair of small circular tracts of the sensory layer of the epiblast lying between the anterior extremity of the neurula and the optic vesicles as shown at *na* in Figs. 26 and 27. At a late stage, with the advance of development and the consequent growth and shifting of the parts in relation to each other at the forepart of the head, the nasal involutions or sacks are displaced downwards so that as the head of the embryo grows in thickness and juts forward more, they are carried down nearer to the point where the mouth will open. They thus finally come to lie nearer the margin of the upper jaw than would be supposed possible, judging from their original position. They are at first simple thickenings of the sensory layer; by the fourteenth day in the young cod they have been completely involuted as thick saccular depressions continuous at their borders with the skin. The thick columnar epithelium which clothes them is the olfactory or Schneiderian membrane, which during the later larval or even, in most cases, probably, the post-larval stages become involuted into folds which have a radial arrangement on the floor of the nasal sack, with secondary ridges connecting them. During this time also the nasal membranous bridge is developed across the pit, in consequence of which it acquires ventral and dorsal openings which communicate with each other. The bridge itself may in some cases be developed as a considerable flap-like external process, or there may be a considerable space intervening between the dorsal and ventral openings of the nasal organ. The other changes which the olfactory organs undergo during the post-larval development of the young fish are principally those of position and changes in the length of the olfactory nerve. This last is at first extremely short. I believe it to originate primarily from the upper hinder portion of the neurula destined to form the cerebrum, from what Marshall and Balfour have called the neural crest; at any rate, there is much to favor this view from what we may learn from an examination of stages such as those represented in Figs. 26 and 27. As development proceeds, its

origin would seem to be carried downwards like that of the optic nerves or stalks as they may at first be called; but of course the olfactory nerve develops later than the optic. In relatively late stages, or after hatching, transverse sections show the roots of the olfactory nerves arising from the sides of the cerebral lobes and passing to the nasal pits. As the snout develops, however, the nasal organs of the young fish assume a more elevated and posterior position, while in some cases, in consequence of the great forward development of the vomer, parasphenoid, ethmoid, premaxillary, prefrontal, and maxillary bones, the nasal organs are carried very far forwards so that the olfactory nerve may require to be prolonged several inches in the adult before it reaches the nasal organ.

The auditory organs, like the nasal, are involutions of the sensory layer of the epiblast. In the cod their rudiments begin to develop on about the tenth day; by the twelfth, at first apparently solid, their involution has been completed, and they are present on the fourteenth as a pair of very thick-walled ovoidal vesicles, with a very small cavity, as shown in Fig. 26, *au*. This internal cavity gradually increases in size, while the walls themselves become gradually thinner. The contents of the vesicle is apparently a fluid lymph; on the fifteenth day two very refringent bodies make their appearance in the auditory vesicle on its internal wall; these are the otoliths, the *asterisk* and *sagitta*, as they are named, respectively. With the development of the auditory vesicle they increase somewhat in size; they are calcareous in composition and have a depressed spheroidal form, with a radiate fibrous structure. The exact mode of development of the otoliths is not well known. With the progress of development the auditory vesicles elongate somewhat antero-posteriorly, the inner portion becomes vestibular in character, and the otoliths lie against its inner wall, near the ventral border of the sack. At the time of its involution it would appear that the auditory nerve or its rudiment was developed from the side of the hind brain. Sections of the later stages through the auditory vesicles show the roots of the auditory nerves arising from the side of the medulla, pretty high up, and curving down on the inner face to the lower anterior part of the inner side of the auditory vesicle. They enter the vesicle on its lower inner side, and terminate in a cushion of columnar epithelial cells, which are surmounted by fine, hair-like protoplasmic filaments, which project freely into the endolymph of the utriculus, as we may call that portion of the auditory vesicle at this stage of its development. The sensory terminal cushion, in which the auditory nerve ends, is evidently an acoustic macula; in its vicinity pigment cells are usually developed in considerable numbers some time after incubation. The horizontal anterior and posterior semicircular canals are apparently developed by the infolding of the walls of the auditory vesicle. These folds first appear as ridges, which apparently grow inwards in such a way as to shut off the semicircular canals from the vesicle, except at

their ends. Beyond this stage I have not followed them, and it only remains to suggest that the canals are further developed by growth in length, in the course of which the curved tubular portions are elevated and separated from the utriculus. As regards the sacculus, I have no observations of value to record; this structure, as far as I can make out, seems to be developed during the post-larval period. The connection of the auditory vesicle with the air-bladder seems also to take place at a late period, for in all the forms observed by me the diverticulum of the fore-gut, which gives rise to it, is quite rudimentary, even up to the time when the semicircular canals of the ear have been formed. It only remains for us to call attention here to the fact that both the auditory and olfactory organs are less intimately connected with the evolution of the neurula or larval nervous system than the eyes, which are connected with it from the first moment of their development, seeming, in fact, to be mere outgrowths of that system. The internal ears and the nasal organs, on the other hand, are formed as paired involutions of the epiblast, their connection with the nervous system being established in a manner entirely different from that of the eyes.

### 13.—THE LATERAL SENSORY ORGANS OF THE LARVAL COD.

These organs have a rather singular distribution in the young cod-fish just hatched. There are five of them to be seen on either side of the body, as may be noticed in Figs. 40 and 42, but on the head and on the side of the body they are not placed on the middle of the side, as they are on the tail. About three of them are placed on either side of the tail, as may be seen in Fig. 42, at *sh*. A nerve filament, *nf*, Fig. 43, passes out to each of them, and is presumably connected with the nervous system, but the exact relations of these nervous connections I have failed to make out. The nerve fiber which passes out to the one on the head, Fig. 40, *sh*, appears to arise from the medulla oblongata; in the one behind the pectoral fin, on the side of the body, at the base of the dorsal natatory fold, the nerve going to it seems to arise from the spinal cord. In both cases faint filamentous prolongations from these two nerve eminences are seen to be prolonged anteriorly and posteriorly in the skin. These filaments, I take it, represent the nerve of the lateral line, evidence of the presence of which is seen in the serial arrangement of the sensory eminences, *sh*, themselves. They are not nearly as numerous as the muscular segments, a feature in which the larval cod differs greatly from the larva of *Gambusia patruelis* where these sensory elevations correspond exactly to the number of muscular segments. This segmental arrangement of the sensory eminences or nerve hills has also been noticed in other larval fishes by Schulze, and is an exceedingly interesting fact. In a good many other forms of larvæ of osseous fishes these lateral sensory eminences are not developed at all at the time of hatching. This is the case with *Alosa* and *Pomolobus*. We have therefore all grades of their development in known types, from none to a few

in *Gadus*, on to that in which every muscular segment has its corresponding pair of nerve hills or eminences. Their function is evidently a sensory one, and their serial relation to the auditory, optic, and olfactory organs is at least suggestive, if nothing more. The structure of these hills, however, bears a most remarkable resemblance to the ending of the auditory nerve in the auditory vesicle, even as regards details.

They appear to be mere local lenticular thickenings of the skin, connected by a nerve filament with the spinal nervous axis, and with careful illumination one may see that these bodies are surmounted externally by very fine, hyaline protoplasmic filaments, which extend freely into the surrounding water, but which are perfectly rigid and immobile. In this they exactly resemble the similar filaments which are met with surmounting the macula or cushion-shaped termination of the auditory nerve. There is much room here, for one disposed to speculate, to suggest a probable explanation of such remarkable resemblances. Dercum has suggested that they may possibly serve to appreciate vibrations not perceptible to the ear, serving perhaps to enable the animal to detect the approach of another body, which starts the surrounding fluid medium into slow vibration. Their columnar epithelial structure has been determined by observers, but the nature of the process by which they become converted into the covered system of the lateral line, as found in the adult, still remains to be worked out. In the adult, one or more rows of scales are often involved in the structure of the canals of the lateral line system, these scales having a tube developed along their longitudinal axis, or it may even be branched. Within these tubes, which also open in various ways to communicate with the outside, a complex system of nerve buttons or eminences are found, which are evidently akin to the nerve hills found in the larvæ as naked dermal elevations. Some of the mounted preparations of these structures of the adult, treated with osmic acid and hæmatoxylin, prepared by Dr. Dercum, have a remarkable likeness in some respects to the ending of the eighth nerve in the auditory vesicle of larval fishes.

Balfour found the lateral line system of Elasmobranchs to be innervated from the ninth pair or vagus nerve. Such a relation of the first nerve hill on the head of the young cod is conceivable, but I am assured that nervous fibers pass inwards separately to the nervous axis from each of the others behind it, so that such a relation to the vagus is here scarcely possible for the latter. The inclosure of the lateral line system is probably accomplished by the development of folds of the skin above and below the series of nerve hills, these folds coalescing finally to form a canal open to the exterior at intervals.

The single median barbel on the lower jaw of the adult cod is also a sensory organ of a special kind, but is not developed until the young are older than the oldest figured in the plates accompanying this memoir. It is therefore developed during the post-larval stages. Leydig has in-

investigated these structures in other forms microscopically, and shown them to contain sensory end-organs of a highly specialized character.

Besides the foregoing nervous dermal structures, there are present upon the skin of the larvæ of some forms a very singular type of structures which have apparently not been much studied. These are the goblet cells of the skin. Their function is probably not sensory, but secretory, pouring out a mucous substance over the skin. In the early stages of young fishes generally which I have studied they are usually absent; in fact, I have never met with them except in the larvæ of those Salmonoids with large ova and embryos. They are apparently unicellular, (Gegenbaur); at least this is their appearance in the young of *Salmo* and *Oncorhynchus*. In form they are globular, with a wide, trumpet-shaped mouth or external extremity, which apparently represents the efferent opening of these unicellular glands. They are very numerous, imbedded in the epithelium of salmon embryos, and are found all over the head and body, extending even over the whole of the yolk-sack, which is thickly studded with them.

#### 14.—DEVELOPMENT OF THE NOTOCHORD.

The disputed question of the particular layer from which this organ in the Teleostean embryo is derived I am unable to settle definitely. Some authorities hold that it is derived from the ventral edge of the neural keel by delamination, being split off from before backwards as a chord of cells. It is not certain, however, that it may not originate from the lower layer or hypoblast, and not from the neural keel; at any rate, a more exhaustive study of the subject is still required before a definite conclusion can be reached in regard to the origin of this organ. It is developed before the intestine acquires a lumen, and while that structure is still a solid median band of hypoblast cells, lying just below the primitive chorda. Transverse sections show it as a distinct, slightly depressed rod or cylinder, which extends from just behind the infundibulum, below the medulla oblongata, to the caudal plate or mass of cells, in which its posterior extremity is completely lost. The cells of which it is at first composed are not distinguishable from those of the mesoblastic muscular and splanchnic layers on either side of it, but by the time of the closure of the blastoderm over the yolk its presence may be very easily detected in the living embryos as well as in transverse and longitudinal sections of the same, having been distinctly segmented off from the adjacent structures in the vicinity. With the downgrowth of the neurula to form the carina, the mesoblastic tract or layer has undergone changes of development, or rather localization, on either side of the middle line, by which the muscular layer becomes completely separated into two lateral longitudinal masses, with the chorda lying between them, and with only a very thin stratum of splanchnic mesoblast underlying, from which it would appear that the aortic and venous trunks of the body and the peritoneum arise at a later period. This splanchnic layer is in



fact, at this period, almost if not quite continuous with the hypoblast below; at any rate, it is not fairly differentiated as a separate layer until after the chorda has been clearly defined. Up to this time the muscular mesoblast is quite solid and composed of closely packed equal-sized cells. After this stage has been reached the chorda cells themselves commence to enlarge, and the whole chorda acquires a gradual augmentation of volume, affecting most conspicuously its diameter. It also loses its depressed oval form as seen in section and becomes cylindrical. With the increase in diameter, the chorda cells also undergo other changes of shape, in the course of which they become columnar, with their longest axes arranged transversely to the axis of the chorda itself. They are finally so disposed that in longitudinal sections the chorda cells appear as if they were arranged into a series of disks placed transversely within the chorda sheath, which has by this time appeared, or at the stage of development shown in Fig. 31. The changes which now follow are very singular indeed; between or within these discoidal masses of chorda cells cavities appear filled with fluid. These cavities, like the disks or cellular septa, are also placed transversely to the axis of the notochord, and are at first lenticular in form, but by degrees they enlarge and displace the chorda cells, as if they were being pushed to the notochordal wall or sheath. The protoplasmic basis of the notochord gradually disappears from the axis of the organ, until it is wholly replaced by the fluid cavities, which have increased enormously in volume. The walls of the cavities which make up the axial part of the chorda are exceedingly thin, and in just-hatched embryos of several genera I have as yet failed to discover any trace of nuclei in those portions of their walls which extend into the body of the chorda. The walls of the cavities must have been derived from the protoplasm of the cells of the primitive chorda, and their nuclei have probably been transported to the walls of the chorda sheath, where they seem to be very much flattened and spread out upon the inner surface of the outer walls of the great vesicular cells composing the chorda. Lieberkühn\* compared the great vesicular cells of the chorda filled with fluid to the vegetable cell with its parietal layer of protoplasm contained in a cellulose wall, the whole inclosing a large sap cavity. This comparison would seem to be fully borne out by the foregoing description of what may be witnessed in the development of the notochord of osseous fishes. The probability—in truth, the fact—must be this: The lenticular vacuoles which we find to originate within the chorda at an early stage are not developed interstitially between the disk-like tracts of primitive chorda cells, but in the cell substance itself. As the vacuoles enlarge they become covered by a layer of plasma, which becomes gradually thinner as the vacuole enlarges. The fluid contents which are found in chorda cells have been accumulated by a process of transudation from the surrounding tissues. As the vacuolated

\* *Ueber Bewegungserscheinungen der Zellen. Schr. d. Gesellsch. z. Beford. d. gesamt. Naturwissensch. z. Marburg. Vol. IX, p. 337, 1870.*

chorda cells enlarge they lose their lenticular form and gradually become polyhedral, and but two to three of them are found side by side in any one diameter of the chorda. As the growth of the tail of the embryo proceeds, the chorda not only increases in diameter, but it also lengthens, together with the other parts of the caudal extremity, and the metamorphosis from the solid condition to the vacuolated one proceeds from before backwards to the slightly swollen caudal end of the chorda, where it is still in connection with a caudal mass of undifferentiated cells, even after great advances in its development have been made at its anterior end, as shown in Fig. 34. The sheath of the chorda also becomes thinner as development advances, and in *Alosa*, for example, the sheath seems to be formed mainly of the walls of the vacuolated cells which come to the surface. This view of the fate of the walls of the chorda cells lying next the surface of the notochord, with their parietal nuclei, seems also to be in accord with those of Gegenbaur and Balfour. The contents of the vacuolated cells of the notochord in the embryos of osseous fishes are not gelatinous, but quite fluid, and may for the most part be abstracted by alcohol or glycerine, causing the chorda to collapse more or less notably.

The volume of the chorda as observed in the embryos of different genera of the same relative age is subject to a very marked variation. In proportion to the bulk of the remainder of the embryo it is most voluminous in the just hatched young of *Alosa* and *Pomolobus*. In other families I have never met with it in anything like the same proportionate size as compared with the other parts of the body. In cross-section in the Clupeoids it will measure quite three times as much proportionally in area as in *Gambusia*, *Cybium*, *Tylosurus*, *Gadus*, *Parephippus*, *Salmo*, *Idus*, *Esox*, *Morone*, and *Hippocampus*. In the last-mentioned genus no caudal fin is developed, and hence its terminal end undergoes no upward flexure, as is the case with many other forms of Ganoids, Teleosts, and Elasmobranchs. In *Siphostoma* also there is apparently no alteration in the direction of its caudal end, for here the five or six caudal-fin radii are formed homocercally in the tail fold without affecting the direction of the notochord. The tail of *Hippocampus* is prehensile, however, before the differentiation of the vertebral bodies, and while its skeletal axis is still entirely notochordal.

It would appear that in some forms, at least as development advances, the vacuolated cells of the notochord divide and become smaller. This is apparently the case with *Gambusia patruelis*, in which we may also note a general acceleration in the development of the notochord, by which it presents a tendency to form the external skeletogenous layer and become constricted at points corresponding to the muscle segments, to form the vertebral bodies, even before the yelk-sack is absorbed. In the embryos of this species we also find the cartilaginous sheath very thick at period, and the constrictions, which are visible as the first indications

of the commencing development of the vertebral bodies, have a shape somewhat like an hour-glass with a wide neck, the narrow portion representing the middle of the future vertebral centrum. In *Alosa* of the same relative age as *Gambusia* the sheath of the notochord is exceedingly thin, and cannot be made out even in sections as anything more than the merest film. In *Salmo*, on the other hand, the notochordal sheath, at the time of hatching, is a thick homogeneous membrane several times the thickness of that found in embryos of *Alosa* of the same age, and thicker even than that of *Gambusia*. It follows from what we have learned, from the foregoing comparisons, that we are not warranted in proposing any general theory of the development of the notochordal sheath, even within the limits of a group as restricted as that of *Teleostei*. Of the development of the *membrana elastica externa*, which covers the notochordal sheath, I have nothing to say, not yet having been able to convince myself of its presence in *Alosa*, for instance.

It has been insisted that *Teleostei* "may fairly be described as passing through an Elasmobranch stage, or a stage like that of most pre-Jurassic Ganoids, or the sturgeon, as far as concerns their caudal fin" (Balfour, Comp. Embryol., II, 64). We have already noted two exceptions to this rule in the singularly modified pipe-fishes and *Hippocampus*. It now remains to call attention to another type in *Gambusia*, where the extreme tip of the notochord is bent upwards to only the slightest degree; so slightly, indeed, that its extremity is not raised above the level of the dorsal line of the notochord, although the hypural, the urostylar cartilages, and the rudiments of the neural and haemal arches are developed. This is in a comparatively late stage, but when we come to study still earlier stages we do not even find any evidence of the dorsal prominence the same as at the margin of the tail fin of embryos of *Salmo*, which is clearly the margin of the embryonic caudal fin, where the tip of the notochord grows backwards and obliquely upwards into this rudimental structure. Balfour is also in error when he says that in *Salmo* the rays of the caudal fin appear simultaneously above and below the end of the notochord. This is actually the case, however, in *Gambusia*, where at least three fin rays arise even above the end of the still cartilaginous urostyle, while six develop above the level of the notochord itself. This subject has been most fully discussed by A. Agassiz, to whose invaluable memoirs on the development of the caudal fin embryologists will in future be obliged to refer for data.

The anterior flexure of the notochord also varies considerably in different forms. In *Alosa* it is slight after hatching, but in *Hippocampus* it is excessive, and is accounted for by the extensive downward flexure of the head in the region of the neck of this singularly modified Teleost.

The subnotochordal rod is developed as a strand of cells in *Alosa* and *Salmo*, just below the notochord. Cellacher calls it the *aorten-strang*, by which he seems to imply that it shares in the development of the aorta.

## 15.—DEVELOPMENT OF THE RIBS.

The frame-work of the trunk in *Teleostei* varies greatly in character; the heads of the ribs also vary in different forms in relation to their positions with respect to the vertebral axis and the notochord itself, and it is on this account that it will not be possible to frame a general theory of their development from the study of any one form. In some the heads of the ribs articulate with the hæmal processes; in others some distance below the vertebral axis, or directly with the sides of the latter. All that I propose to do at present is to record what I have observed in relation to their development in a single type not hitherto the subject of embryological investigation. In this case the relation of these skeletal appendages to the notochordal axis was so intimate that their discussion may appropriately follow that of the notochord itself.

The form in question in which I have observed some of their stages of development is *Gambusia patruelis* of Baird and Girard, which, as already remarked, tends to develop its skeletal frame-work very precociously as cartilage, even long before the complete absorption of the yolk-sack, which is an unusual feature, and one to be accounted for probably by the fact that this species, like Cyprinodonts generally, develops its young within the ovary viviparously to a remarkable degree of advancement. The embryos used were in an advanced state of gestation in the ovarian follicles, from which they were removed and cut into transverse and longitudinal vertical sections.

In this genus the cartilaginous rudiments of the ribs were found to abut directly against the notochord at its side and above the middle of the side, where the head was somewhat larger in circumference than the distal portions. At the hinder portions of the body cavity their origins were somewhat more ventral than in the middle and anterior regions. They arise in pairs and extend obliquely outward, backwards, and downwards between the muscular and splanchnopleural layers, following the intermuscular septa as perfectly cylindrical rods, and appear to be surrounded by a stratum of connective tissue, which is continuous with that surrounding the notochord, and in which presumably the ossification of the vertebral bodies and the first superficial sheath of bone of the ribs themselves will take place at a later period. From their origin at the sides of the notochord, in the middle region of the body, they bend downwards and follow the courses of the septa between the muscular plates, just where these terminate on the splanchnopleure. The foregoing describes fairly their relations to the surrounding tissues, but their finer structure is somewhat remarkable and calls for special notice. They do not present the appearance of cartilage as seen in the cartilaginous rods of the branchial arches of the same embryo, nor that of the parachordal plates or trabecular cartilage of the base of the skull. They, in fact, recall nothing of the structure of any

other part of the embryonic skeletal frame-work of any form with which I am acquainted. Perfectly cylindrical rods from their origins at the sides of the notochord, they consist of a single row of hollow, discoidal, apparently vacuolated cells, apposed by their flat surfaces. In their vacuolated condition their component cells resemble the notochord, of which they are evidently appendages, as already stated. The question now arises, do they originate from a single line of solid cells along the intermuscular septa? Their condition as observed by us would appear to favor such a view. The question is also raised as to their appendicular relation to the notochord; and what is the significance of their direct connection with the sides of the chorda? They appear like miniature lateral repetitions of the chorda, but, unlike it, to be formed of but a single linear row of vacuolated cells. Their points of insertion I have not certainly determined to be intervertebral, but such they probably are, since their courses follow the muscular septa. Hoffman has urged a similar relation of the ribs and chorda in the embryos of other forms, but I have not seen his paper on the subject. At these stages of development of *Gambusia* the muscular plates were far advanced in development and already presented the condition of a congeries of fibrillated, cylindrical, or oval bundles of muscle fibers, and the distinction into dorso-lateral and ventro-lateral masses, with the horizontal lateral septa of connective tissue developed between them.

No observations on the development of the ribs of the young cod were made, for the reason that no embryos of a sufficiently advanced state of growth could be obtained.

#### 16.—DEVELOPMENT OF THE SKULL.

Upon this subject little can be said here, since, on account of the very minute size of the embryo cod, I have not yet subjected the head of the larva to a thorough examination by means of sections, the only practicable method of studying this part of the skeleton of such a form. Dissection is out of the question. Stated in general terms, my investigation has been conducted as follows: By carefully compressing the embryos under a compressor of the proper form, the cartilaginous basis of the chondrocranium may be revealed if a dilute solution of acetic acid is used to develop the cell contours. I have also found that the chondrocranium of larger forms of osseous fish larvæ could be isolated with tolerable success with the use of a weak solution of caustic potash, which destroys the other soft parts, but does not so readily attack the structure of the embryonic cartilage.

As a result of such modes of investigation, it may, I believe, be stated as generally true that the basicranial plate, perforated by the pituitary space and ensheathing the anterior end of the notochord, is the first portion of the true skeleton to be developed in osseous fishes, but the skeletal axes of the branchial and hyomandibular arches develop their rudiments about the same time. The branchial arches are formed from

the mesoblast of the inner medullary portion of the fleshy branchial arches; the branchial blood vessels are formed in the outer and hinder part of the same medullary tract, which is continuous above with the same layer of tissue from which the cartilaginous basis of the cranium is developed. The remarkable researches of Prof. W. K. Parker upon the development of the skull of the salmon leave nothing to be desired upon that group, but I am assured that many details of the process still remain to be worked out for other forms. For instance, the palatopterygoid bar does not seem to develop relatively so early in other forms (*Alosa*) as we find in the salmon. Nor is the rostral portion of the basis of the skull nearly so precociously developed; the supraorbital bar is also weaker and arises at a comparatively later stage in *Alosa*. In fact this tendency to manifest a later development of the skull in these types appears to be related to the general backwardness of the condition of development of the median and paired fins. In *Alosa*, for example, there is no sign of cartilaginous fin rays at the time of hatching, while in *Gadus* they are even later in making their appearance. In the salmon, on the other hand, evidences of rudiments of fin rays have already made their appearance at this stage in the tail, in the dorsal, anal, and pectoral fins, while the rudiments of the ventral fins are prominently developed while there is still no sign of them in the embryos of *Gadus* and *Alosa* of the same stage. In *Gambusia* again, in conformity with the generally accelerated condition of the development of the skeleton of the embryo, the skull shows a like tendency to be more fully formed at an early period. In it the cranial tegmen, labial cartilages, the intermaxillary rudiment, supraorbital bars, branchial and laryngeal apparatus have reached a stage much more fully differentiated than in other forms of the same relative age. In the embryos of *Alosa*, for example, at the time of hatching, the basihyal and glossohyal cartilages are still in the form of an unsegmented plate, while in *Gambusia* they have been developed long before incubation is complete. The Meckelian cartilages of the lower jaw, however, develop concurrently with the oral opening and grow in length as its gape increases. The quadrate cartilage retains its solid junction with the metapterygoid in the slow-developing forms above alluded to, just as in the salmon. Conscious of having added but little that is new to this part of the developmental history of osseous fishes, we leave this portion of the subject for fuller treatment at some future time.

#### 17.—THE DEVELOPMENT OF THE UNPAIRED OR MEDIAN FINS.

The development of the unpaired fins from a median dorsal and ventral natatory fold seems to be general amongst osseous fishes, with only a few unimportant exceptions, mainly amongst Lophobranchs. In the cod embryo the natatory fold here alluded to appears soon after the tail buds out from the caudal plate. It is at first a low fold of the skin, as at *nf*, Fig. 32, which extends over the end of the tail and forward on

the dorsal and ventral median line. With the progress of development it becomes more conspicuous, growing in height, so as to soon be very much wider, as may be seen in Fig. 34. Its first appearance is heralded by a faint doubling of the skin upon itself, so as to project outwards as a median ridge, extending from the point of origin of the tail at its ventral margin from the yolk-sack back over the caudal extremity and progressively forward over the median dorsal line towards the head. By the time the embryo leaves the egg this fold extends forward on the back as far as the pineal gland, or to a point just behind the forebrain, as may be seen in Fig. 40. Its development, however, is continued even somewhat farther forward fourteen days after hatching, as shown in Fig. 45, until it ends almost immediately between the nasal pits. At this time its extreme anterior extent gives to the young cod a very singular appearance as viewed from the side, such as is not met within any other form which I have studied. In outline, as viewed from the side, the young fish now bears a resemblance to the conventional representations of the dolphin in old sculptures.

The natatory fold is now actually wider than the caudal portion of the trunk, but it is quite thin and comprises only the skin folded upon itself, its whole thickness being mainly, if not entirely, derived from the epiblast. At first, in all forms known to me, the caudal portion of the natatory fold is rounded in outline, as seen from the side, but may assume a fan-shape, even before a single caudal fin-ray has been developed in it, as is the case in *Alosa* and *Pomolobus*. In others, again, the rays begin to develop before the caudal portion of the primitive median natatory fold has become fan-shaped, as may be seen in *Salmo* and *Oncorhynchus*. In still others there is no continuous median fin-fold developed at all, as in *Gambusia*, *Siphostoma*, and *Hippocampus*, and the median fins grow out at first as short, local, dermal folds, in which fin-rays soon afterwards develop. In those forms in which the unpaired fins are developed from a continuous median fold, the dorsals, anal, and caudal are evolved only in certain regions of the fold itself, the portions of the latter, which do not become fin rudiments, atrophy. Balfour says (Comp. Embryol. II, 63) that "the dorsal and anal fins are developed from this fold by local hypertrophy." The process, however, when narrowly studied, presents features the significance of which cannot be fully apprehended under the term hypertrophy. As stated at the outset, the median larval fin-fold is at first a mere outward duplication of the skin containing no mesoblastic tissue between its laminae, but as soon as the positions of the fin-rudiments are defined we may note that there has been an outgrowth of mesoblastic tissue into these regions, causing them to become thicker and less transparent. With the progress of this process, the mesoblastic tissue gradually advances toward the margin of the fold, insinuating itself between the epiblastic walls of the continuous fin of the larva. Soon afterwards it becomes evident that the fin-rays are becoming differentiated by the mesoblastic tissue

arranging itself in parallel bands from which the cartilaginous matrix of the rays is developed. The rudiments of the bony sheaths of the rays of the caudal fin in sections of that portion of larval salmon are found to lie almost in contact with the skin and to be crescentic in section. These are evidently the lateral pieces which develop into the bony segmented sheaths of the rays of the adult, which are of much the same form except that they more completely ensheath the cartilaginous matrix of the rays than in the larvæ. At the base of the caudal fin of the salmon embryo the sheaths of the fin-rays lie deeper than in its distal portion, and a stratum of tissue is interposed between the skin and the sheaths of the rays, which is afterwards apparently developed into the flexor muscles of this fin. The cartilages, which afterwards ossify and become the hypural bones, are mesial in position and a considerable thickness of tissue is interposed between them and the rudiments of the rays lying on either side. Such, in general terms, appears to be the process of caudal and median fin development in osseous fishes. In some forms there is a tendency manifested to develop more or less mesoblast in the median fin-fold, and vascular loops also appear in its mesial substance at an early stage of development, as may be seen in the embryos of *Apeltes* and *Tylosurus*. In other forms, again, the median fin-fold retains its thin, transparent, dermal, non-vascular character for a long time after hatching; this is noteworthy in *Alosa*, *Pomolobus*, *Cybiium*, *Parephippus*, and *Idus*, while in *Apeltes* vascular loops are present in it by the time of hatching. In *Siphostoma* and *Gambusia* the fin-folds of the unpaired fins grow out as local dermal folds into which mesoblastic tissue is almost immediately insinuated to develop the rays.

It will be evident, from what has preceded, that the theory of the origin of the unpaired fins from continuous folds does not hold in Teleosts; that there are exceptions to it where we should least expect to find them; in fine, that the form of the caudal fin is sometimes outlined before the rays appear or the reverse. It is worth while, however, to point out that in those forms with two or three dorsals (*Gadus*), or where there is a series of dorsal and ventral finlets in the adult (*Cybiium*), the continuous larval fin is most apt to be developed, while in those forms where there is a reduction (degeneration) of the fin system, as in the Lophobranchs and *Gambusia* (with but one dorsal), the continuous median fin-fold is not always developed. The last qualification does not hold, however, in all cases, for in the larva of some Cyprinoids, *Idus* and *Carassius* (one dorsal in the adult), I find the continuous fin-fold developed to the same extent as in *Apeltes*; and amongst the Clupeoids, which have but one short dorsal in the adult, it is surprising to find the natatory fold extensively developed. The query arises, why should *Gambusia* form such an anomalous exception? We can understand the cause of the peculiar development of the median fins of Lophobranchs as resulting from their extreme specialization, but in the first case the explanation is not so clear.



The mesoblast, from which the median system of fin rays of the larvæ of osseous fishes is developed, appears to be an outgrowth from between the mesial, dorsal, and ventral points of meeting of the muscle plates, and that it is pushed out into the natatory fold during the development of the skeletal and muscular elements of these fins, and that it is continuous dorsad of the spinal chord, and ventrad of the notochord, with the tract of tissue, from which the interspinous elements of the skeleton are differentiated. It is, therefore, a part of that mesoblastic tract from which the haemal and neural arches, interposed between the dorso-lateral and ventro-lateral plates of muscle-segments, are differentiated, and was primitively continuous with it.

#### 18.—THE DEVELOPMENT OF THE PAIRED FINS.

The paired fins of *Teleostei*, like the limbs of the higher vertebrata, arise locally, not as blunt processes, however, but as short longitudinal folds, with perhaps a few exceptions. The pectorals of *Lepidosteus* originate in the same way. Of the paired fins, the pectoral or anterior pair seems to be the first to be developed; the ventral or pelvic pair often not making their appearance until after the absorption of the yelk-sack has been completed, in other cases before that event, as in *Salmo* and *Gambusia*. The ventral undergoes less alteration of position during its evolution than the pectoral pair.

In that the development of the pectoral or breast fins of *Gadus* is typical of the group we can do no better than describe their evolution in that form, as observed prior to and after hatching. The date of appearance of the first sign of the pectoral fin-fold varies somewhat in different genera, but in *Gadus* it appears as a slight longitudinal elevation of the skin on either side of the body of the embryo a little way behind the auditory vesicles, as shown in Figs. 30, 32, 33, and 34, at *ff*, and shortly after the tail of the embryo begins to bud out. At the very first it appears to be merely a dermal fold, and, in some forms, a layer of cells extends out underneath it from the sides of the body, but does not ascend into it. It begins to develop as a very low fold, hardly noticeable, and as growth proceeds its base does not expand antero-posteriorly, but tends rather to become narrowed so that it has a pedunculated form, as in Fig. 40. With the progress of this process, the margin of the fin-fold also becomes thinner at its distal border, and at the basal part mesodermal cells make their appearance more notably within the second or inner contour line of *bf*, Fig. 40. In some species I am quite well assured that there is at an early period a mesodermal tract or plate of cells developed just behind the auditory vesicles, just outside the muscle plates of this region, on either side, which may be regarded as the source of the mesodermal cells which are carried up into the pectoral fin-fold. This is developed at about the time of the closure of the blastoderm, and these lateral mesodermal tracts of tissue may be called the pectoral plates. The free border of the fin-fold grows out laterally and

longitudinally expanding the portion outside of the inner contour line of the fin, as shown in Fig. 40, into a fan-shape, so that the whole fin becomes much more distinctly pedunculated as viewed from the side. This distal thinner portion is at first without any evidence of rays, further than that there is a manifest tendency to a radial disposition of the histological elements of the fin. This radial disposition of the histological elements of the fin-substance has an undoubted relation to the growth in length and expansion of the organ and is conspicuously manifested in the development of the dermal lobes of the caudal fin of *Alosa* before the development of rays. The distal lamina, as we may call the thinner extremity of the fin, is the portion in which the rays are formed, while the thicker proximal or basal portion is that in which the basal elements of the fin are developed. Just at the point where the basal portion of the fin joins on to the body there is a decided fold extending up and down obliquely on the sides of the embryo and continuous with the fin; this may be called the oblique or vertical pectoral fold; just at the base of the fin and in this fold the coraco-scapular cartilage makes its appearance as a somewhat L-shaped plate, with its anterior coracoid limb extending forwards and downwards and its upper and scapular limb extending upwards. This is the first rudiment of the shoulder-girdle; the membrane bones which develop around it afterwards ectosteally, appear much later than in the stages so far described. The coraco-scapular cartilage I have studied most successfully in entire embryos of *Alosa* hardened in picric acid, cleared in oil of cloves, and mounted entire in Canada balsam.

As regards the detailed history of the development of the various ossicles of the pectoral fin I have little or nothing to record, further than to say that there is no evidence of a type of development like that seen in Elasmobranchs; the evolution of the breast fin of *Teleostei* being influenced by the specialized character of the limb-skeleton of the adult. The muscles are developed as in the Elasmobranch fin from mesoblastic strata of cells internal and external to the median plate, from which the cartilaginous axial portions of the fin are evolved. Sections through the pectorals of the larvæ of *Gambusia* show the details of muscular development to be very similar to that represented by Balfour in Fig. 346 (Comp. Embryol., II), as obtaining in *Scyllium*.

The next points of interest in this connection are the changes of position which the pectorals undergo in relation to the surrounding structures and their rotation upon their bases, by which they acquire an upright position so as to become mechanically effective as organs of propulsion or locomotion. At first quite longitudinal in direction, as shown in Figs. 32, 34, 40, and 42, the anterior portion tends to be gradually elevated as development proceeds, its base becoming more or less oblique in position as viewed from the side. Finally this process of the rotation of the base is carried so far that the fin acquires a nearly or altogether vertical position on the side of the body. The face of the fold

which was at first outermost is now anterior, and the face of the same which was innermost is now posterior. The displacement of the whole fin forward is not as real as would at first appear from our figures. The growth of the head and the elevation of the body have effected such changes in the relations of all the surrounding structures that the breast fins have not escaped its influence, and while it is unquestionably true that the breast fin has rotated on its base for an extent of almost ninety degrees, part of the apparent change of position is undoubtedly due to the concurrent development and increase in bulk of adjacent structures. The great gains in bulk which have taken place in the brain and body have had much to do with this alteration of the relative positions of adjacent organs.

The comparative embryology of the breast fins is very interesting, in that some variation in its relative position is evident upon studying a number of genera belonging to different families. In *Cybius* and *Parephippus* the primitive pectoral folds appear very far back or behind the vertical of the middle of the yelk-sack; in every other form with which I am familiar they appear farther forwards. In *Cybius* as many as twelve muscular somites may intervene between the point of origin of the breast fin and the auditory vesicle; in other forms the number of intervening muscular somites is usually less, being sometimes reduced to two or three (*Alosa* and *Pomolobus*). The homodynamic relations of the pectorals would therefore seem to vary greatly in the larval stages of *Teleostei*, and their serial relations to the gill arches are therefore also very variable. The unusual posterior origin of the pectoral rudiments of *Cybius* and *Parephippus* is also an indication that we may expect to find other anomalous modes of development, as indeed has been the case with some of the forms studied by Prof. Agassiz.—*Lophius*, for example.

As to the development of the ventral or pelvic pair of fins I have observed little that is new, and can only call attention to the contrast in the development of the organs as observed in *Gambusia* and *Salmo*. In the latter the ventral fin-fold appears on either side about the time of hatching, a little way behind the yelk-sack, with its base horizontal, like the pectoral at first, and on a level with the lower wall of the intestine and just above the origin of the pre-anal\* median natatory fold. In *Gambusia* it grows out as a little papilla, and not as a fold, where the body walls join the hinder upper portion of the yelk-sack a very little way in front of the vent. These two modes of origin are therefore in striking contrast and well calculated to impress us with a sense of

\* Under the head of the median fins I find that inadvertently nothing has been said of the pre-anal. It is, however, developed in many embryo fishes, as in *Alosa* and *Pomolobus*, to the greatest extent, less so in *Salmo* and *Coregonus*, slightly in the later stages of *Cybius*, *Morone*, and *Parephippus*; it is wanting in *Gambusia*, *Cottus*, *Apeltes*, *Idus*, *Carassius*, *Tylosurus*, *Siphostoma*, and *Hippocampus*, and is absent in *Gadus* on account of the peculiar mode of termination of the intestine. It is also present in the larva of *Lepidosteus*, according to Agassiz.

the protean character of the means at the disposal of Nature to achieve one and the same end.

#### 19.—THE DEVELOPMENT OF THE LATERAL MUSCLE PLATES AND SOMITES.

The lateral muscle-segments or somites of the body of the cod are developed, as in other fishes, by the transverse segmentation of the lateral muscle plates or somatopleures lying on either side of the neurula. The first evidence of muscle plates in the embryos investigated by me appeared about the tenth day, as represented in Fig. 23, *pv*. They appear mainly in succession from before backwards, the first pair developing a little way behind the auditory vesicles or the solid rudiments of the latter. As development proceeds, however, the most anterior pairs of muscular segments are differentiated later than those which first make their appearance on the sides of the body. As a rule, however, it may be said that they are segmented off in succession from before backwards towards the end of the tail, in which they last appear. A little while after the blastoderm of the cod's egg has closed, there are about eighteen to twenty pairs of muscle-segments distinguishable in the body of the embryo. In vertical transverse section across the body they are at first triangular, with the inner concave face applied to the side of the neurula. Like so many other portions of the embryonic fish during these stages, they are quite solid, and in sections the only evidence of a very well defined structure is their columnar stratum or external wall of cells. They are not all developed in the end of the tail until that part of the embryo has been fully formed. In the progress of the growth of the tail the muscular segments first appear in the proximal portion or that with which the body is continuous. But in the caudal region, after it has budded out, they have at first a different form from that observable in the first muscular segments of the body. They are here crescentic in transverse section, and not triangular as they at first were in the body. They clasp the chorda, neurula, and a ventral mesoblastic strand of cells, thus (§), on either side, the neuralá being uppermost, the chorda in the center, and the mesoblastic strand of cells alluded to lowermost. At the tip of the tail, however, in its early stages of outgrowth, the whole of these structures are absolutely continuous; that is, blended and lost in an apical mass of cells in which no lines of demarkation can be made out. A little way forward the lines of separation between these structures become apparent, as may be seen in the tails of the embryos viewed from the side in Figs. 31 and 32. After the outgrowth of the tail the embryo's body has grown very notably in vertical thickness, upon which the muscle-segments of the body begin to assume the crescentic form seen on either side of the tail, except that in sections of the anterior regions the ventral limb of the crescentic muscular segments are truncated, resting with their blunt ends upon the splanchnopleure. With the growth of the body the mus-

le-segments also increase in volume, and a perceptible increase in their length and width also takes place, as may be seen upon comparing their dimensions as shown at *pv* in Fig. 31 with those represented in Fig. 32. A very remarkable metamorphosis of their cells now begins to take place, by which they become stretched out as muscle-cells which correspond in length with the segments themselves. These muscle-cells also soon become transversely striated like voluntary muscle fibers generally, and have a distinct oval nucleus imbedded in their medullary substance, which may be very nicely demonstrated by the use of borax carmine. The primitive fibers also soon split up by processes of division into fibrils which are arranged in bundles, the fibrils themselves appearing in transverse sections as if they were arranged around a central empty space. With the progress of development, however, still other changes of form of the muscle-plates as wholes occur, and the first of these to be apparent is the >-shaped form they assume when viewed from the side of the body. They are then arranged thus >>>> in succession on either side of the body. The development of this last feature also proceeds from before backwards, being more marked at the anterior end of the body than at the posterior at an early stage. Still another point may be alluded to here relating to their arrangement: with the advance of development the anterior and posterior edges of the muscle-segments also become more and more beveled, the bevel trending backwards. On this account they finally overlap each other; that is, the hinder beveled margin of one segment covers the anterior margin of the succeeding one, which has its front edge beveled in the opposite direction. During the later embryonic stages still other changes occur in the relation and form of the segments themselves, when a smaller <-shaped portion is developed at the upper and lower margins of the individual plates, which open forwards instead of backwards, the reverse of the middle >, but which fit into each other in the same manner. The foregoing constitute the main features of the metamorphosis of the lateral muscle plates of the larval fish into those of the adult, and relate altogether to changes of form and histological constitution.

Another series of changes also occur, which effect the arrangement of the muscular plates into a dorso-ventral, lateral system. This is the division of the muscle-plates into two superimposed masses on either side, by the development of a horizontal ligamentous septum along the middle of the sides, and extending inward almost from the skin to the vertebral column. This lateral septum is continuous on its upper and lower sides with the intermuscular ligaments placed between the single pairs of muscular somites, producing the remarkable appearance of systems of rings of muscular tissue, arranged in a ventral and dorsal position on either side of the vertebral column, and well seen in a frozen section of the tail of an adult fish. The lateral and intermuscular septa produce, together with the peculiar bending and beveling of the mus-

cle-plates during development, the appearance of muscular cones in two lateral series, one above and one below the middle line of the side. These constitute the dorso-lateral and ventro-lateral systems of muscular plates. External to the dorso-lateral and ventro-lateral plates, differentiated as above described, on the middle of the sides, there is developed a thin strip of muscle, which in some adult fishes is quite distinct, especially when they are boiled, when it appears as a dark muscular band, in striking contrast with the white substance of the muscular plates or cones. These outermost plates of dark-colored muscle are developed during a late larval stage, and appear to be derived by delamination from the same somites from which the dorso-lateral and ventro-lateral plates have been differentiated. These dark lateral bands of muscle in the adult are segmented the same as the deeper plates, and their segments correspond in number and segmental position to the latter.

A very great difference in the downward extent of the muscle plates over the sides of the yelk-sack, is manifested in the embryos of different species of the same age. In the salmon and white-fish, the muscle segments extend for a considerable distance over the yelk-sack at the time of hatching, and after a variable time they completely inclose what remains of it. This is due partially to the collapse of the yelk, as it is absorbed, and partially to a downgrowth of the muscle-segments over the sides of the sack, between the epiblastic and splanchnopleural layers, the latter being carried along with the growth of the muscular layer. In other forms the whole of the sack may be absorbed before there is the least tendency of the muscle-segments to grow down and inclose it by their ventral borders. This is a noteworthy characteristic of the embryos of the shad, cod, Spanish mackerel, and other species, and again illustrates the singular way in which the young of a relatively homogeneous group may differ from one another. In the salmon the ventral development of the muscle-segments seems to be hastened; in the other cases it is evidently retarded. In the cod, for example, the edges of the muscle-plates do not even reach down so far as to cover even the upper lateral portion of the remains of the yelk-sack, as may be noticed in Fig. 49.

The material for the development of the posterior muscle segments of the embryo is also supplied in a singular way. In *Tylosurus* there is evidence of the concrescence of the rim of the blastoderm at the tail end of the developing axis of the embryo as may be surmised from an inspection of Fig. 6, pl. XIX, which accompanies my essay on the development of that form. The rim in this case does leave the embryonic axis at right angles on either side, as in the cod, as shown in Figs. 19 and 20 of this memoir, but at an obtuse angle after the time for the closure of the blastoderm is approaching. While there is a veritable caudal swelling it is also manifest that a veritable concrescence of the rim of the blastoderm is taking place by intussusception or gradual

appropriation of its cells to form the caudal end of the embryonic axis. When on the eve of closure, the limbs of the rim of the blastoderm in *Tylosurus* form an acute angle with each other, and the yolk blastopore has the form of a wide oval with the narrow end next to the caudal swelling. In *Elacate* the evidence in relation to the concrescence of the rim of the blastoderm in the middle line is very striking. Here, the limbs of the rim of the blastoderm on the eve of closure, where their substance is continuous with that of the muscle plates anteriorly, form an acute angle with each other, and there is no caudal swelling intervening between them as in *Tylosurus*. Not only is it evident in this case that an actual concrescence of the limbs of the rim of the blastoderm occurs, but it is also plainly evident that a transverse segmentation into segments has occurred in the lower layer of the limbs before their concrescence. The segmentation affects only the lower or somatic layer of the blastodermic rim and extends some distance behind the caudal end of the embryonic axis already formed. This is the only instance in which I have found evidence of a normal process of concrescence of the rim of the blastoderm along the median line in embryos of osseous fishes before the formation of the caudal plate. The concrescence, therefore, takes place also in the plane of the nervous axis as well as in the enteric. It would appear as if the yolk blastopore in such cases might be the true blastopore of the gastrula stage of development. It is remarkable, however, that I should meet with such a state of affairs only in *Elacate* and not in other forms, as regards the fate of the inner edge of the blastodermic rim. Only in *Elacate* have I ever met with any evidence of direct marginal apposition, concrescence, and convergence of the blastodermic rim on the eve of the closure of the blastoderm; in other forms it closes as a round pore, as in *Gadus*, *Cybius*, and *Alosa*, and segmentation into muscular somites of its lower layer never occurs during its closure to form the caudal plate.

In these ways the rim of the blastoderm is completely used up in the different species to form the caudal end of the embryo, the most of its substance being finally converted into the muscle-segments of the tail. But the growth of the tail outward is a most remarkable phenomenon, in that there is as yet in some forms no vascular system whatever for the conveyance of nutrient matter, in spite of which the tail continues to elongate, evidently gaining bulk mainly to build up the lateral muscular masses the material for which must of necessity be transported outwards and backwards somehow from the yolk or other pre-existing tissue. The way in which this is accomplished is not clear to me except upon the theory of growth proposed by De Bary and Rauber. They regard cell-division as a consequence of growth, not growth a consequence of cell-division. Then, if we suppose with Rauber that cellular protoplasm has a structure consisting of vacuoles or lines radiating from a center, which favor intussusception of plasma from intercellular spaces, we may perhaps have an approximate explanation of

the process. I believe also that the segmentation cavity is a lymph space, and that, since the first blood-corpuscles are borne in it, the evolution of plasmin and fibrin may occur within it at an early stage and aid in such a process as the outgrowth of the tail, and thus indirectly in the development of its lateral muscles.

At the time of the closure of the blastoderm the number of muscle-segments developed in different species is also subject to considerable variation; so marked is this in extreme cases that it is proper to call attention to it in this connection. We find, for example, in many forms, not more than eighteen to twenty muscle-segments developed on either side of the body up to the time when the blastoderm closes over the yolk. In exceptional cases, as many as seventy-five may be developed, as we find in the instance of *Tylosurus*. This variation is doubtless due to the influence of heredity, the embryos which have the most segments at an early stage descending from adults which have a proportionally large number of muscular segments developed, while those embryos with but few are descended from parents with a less number.

The inclusion of the yolk-sack, or what remains of it at a late stage of development in young salmon, by the downgrowth of the ventral ends of the muscular segments overlying the sides of the abdomen, is a very interesting phenomenon. It recalls in some respects the process of inclusion of the yolk by the blastoderm at a much earlier stage. Unlike the latter, however, they do not coalesce at one point or come together as a round pore of gradually lessening diameter like the rim of the closing blastoderm, but they join on the median ventral line from the isthmus back to the pre-anal fin-fold; the opening which remains at this time between the ends of the down-growing episkeletal muscle plates has the form of a very elongate median, ventral cleft. The abdominal cavity in the young salmon is also relatively long in contrast with that of the young cod, but in larval Clupeoids it is of still greater relative length than in the salmon and proportionally longer than in any other forms known to me of the same stage. The downgrowth of the lateral muscle plates in all Teleostean types appears to take place in a somewhat similar manner to form the episkeletal muscular stratum external to the ribs.

#### 20.—DEVELOPMENT OF THE INTESTINE AND ITS APPENDAGES.

The development of the intestine of the *Teleostei* or true fishes is peculiar in a number of respects; these are, first, its primitively solid and depressed form, and secondly, the mode in which the oral end of it appears to be developed from behind forwards, there being apparently no clearly marked oral invagination of the epiblast or a stomodæum; thirdly, the mode of formation of the proctodæum or anus; fourthly, the appearance of a lumen in it not by a process of invagination from below or behind, but by a separation or retreat of its cells from its axis. Like the intestine of other vertebrates it is developed from the true



hypoblastic or nethermost embryonic layer, which is notably thickened at an early stage along the ventral side of the body of the embryo, but is still quite thin or almost wanting underneath the head; in fact it appears to be almost entirely undeveloped below the fore and mid brain at the time the blastoderm closes. Its condition shortly after the closure of the blastoderm in the embryo cod is shown in Fig. 31, at *i*, where its solid rudiment is visible as a band of cells underlying the notochord in the living egg. While its anterior extremity is not traceable to below the anterior end of the head, the posterior extremity is lost in the caudal mass at *i*, with which it is continuous. This relation of continuity of the hinder end of the intestine with the caudal mass shows that we must regard this condition as homologous with that observed in *Amphioxus*, *Elasmobranchs*, and other forms where the continuity of the neural canal at its posterior extremity with the intestine is effected through the intermediation of a short post-anal section of the latter or a neurenteric canal. This primitive continuity of the neural tube with the intestine has been so fully elucidated by Kowalewsky, Hatschek, and Kupffer that it is only necessary to refer to their memoirs on the subject and especially to the general treatise and the monographs of Professor Balfour. While I have found it impossible to convince myself by means of sections that there ever exists a neurenteric canal in embryos of osseous fishes, I feel assured that the solid nature of the posterior end of the neurula obscures this relation and prevents the development of it. This does not, however, permit us to deny the possibility of a primitive union of the enteric and neural tracts at the tail, and thus to realize a gastrula stage of development for the *Telostei*. Kupffer has placed some observations upon record in regard to the connection of the vesicle named after him with the hinder part of the neurula. I have already remarked of Kupffer's vesicle that it is an evanescent structure, and of uncertain significance in relation to any organs developed afterwards. It has been observed by me in the ova of *Gadus*, *Alosa*, *Cybius*, *Tylosurus*, *Coregonus*, *Apeltes*, and two undetermined forms, so that it seems to be pretty generally present. In the cod its relation to the yolk blastopore *bl* is shown in Figs. 26, 28*a*, 29*a*, 29*b*, 30*a*, 30*b*, 31, and 32, at *Kv*. In 30*b* it seems to be joined by a fine canal to the blastopore, and in 28*a* it appears to be provided with a cellular wall. Its relations are, not, however, constant, as may be inferred from an inspection of the different figures in which it is represented as present. After the stage shown in Fig. 32 had been passed, I was no longer able to identify it with any succeeding structure which it could be supposed was derived from it. I therefore reserve my decision as to its true nature. In some forms it appears long before the closure of the blastoderm, in others coincidentally with that phenomenon.

The great generalization, first distinctly formulated by Haeckel, that animals generally, pass, in the course of their development, through a gastrula stage, applies to the osseous fishes, and, notwithstanding the uncertain fate of Kupffer's vesicle, it is evident that the caudal plate,

with which the hind-gut and neurula are conterminous posteriorly, must in its mesial or axial portion represent the neurenteric canal, though an actual tubular intercommunication of the gut and neurula are never developed as in the embryos of *Amphioxus*. While, therefore, it is not yet possible to assert that there is a true gastrula mouth (prostoma) developed, in the embryos of *Teleostei*, we are in a position to say that, inasmuch as the rim of the blastoderm is used up in the formation of the caudal plate, which is taken up into the posterior portion of the body, the true blastopore probably coincides with the last portion of the solid neurula, to be formed at the anterior border of the yolk blastopore, and cannot be identified with the latter itself. The latter is not, therefore, homologous with the blastopore of the frog's ovum. I cannot accept the views of Zeigler in regard to the homologies which he has sought to establish between the whole of the Teleostean and amphibian ovum, for reasons relating partly to the history of the blastopore and partly on account of considerations which arise from a study of the fate of the yolk.

In embryos of the cod on the sixteenth day of development, Fig. 32, the intestine has made a very notable advance in differentiation as compared with the stage shown in Fig. 31. In the anterior portion it has barely acquired a lumen, and is still much depressed; but farther back from a little in front of the breast fin to the vent *v* it has gained in vertical thickness very notably, become more cylindrical, and has acquired a central cavity. Its anal end apparently terminates upon the yolk. Just opposite the pectoral fin-fold *ff* the ventral wall of the intestine is becoming quite thick in the vicinity of *lv*. This thickening represents the rudiment of the liver, which appears in the cod, as in other fishes, to be at first a solid outgrowth from the intestine. The condition of the intestine on the sixteenth day, as in Fig. 32, is gradually followed by a more advanced state, such as that shown in Fig. 34, taken from an embryo on the nineteenth day of incubation. It is shortly after or at about this stage that the anal end of the intestine is carried outwards to end in the ventral fin-fold, some distance above its margin, as shown in the just-hatched embryo represented in Fig. 40. The rudiment of the liver in Fig. 34 has been more fully developed, and now projects as a lateral, ventral and dorsal thickening of the intestinal wall at *lv*. It has not yet apparently acquired a lobulated structure, such as afterwards becomes apparent in more advanced stages. As the development of the liver proceeds it becomes gradually more conspicuous as a lobulated organ on the left side of the intestine, but is reflected around the latter above and below, as shown in Fig. 40. From the time of hatching onwards the intestine gradually acquires a spacious lumen, but no greenish biliary secretion was noticed in it, such as is commonly observed at this stage in embryos of *Cottus*, *Salmo*, etc., of the same age. It is singular that the secretion of bile in fish embryos should precede

the injection of food; this secretion in such embryos is probably analogous to the meconium discharged by recently born infants.

Meanwhile the lumen of the œsophagus, pharynx, and mouth are being differentiated. In sections at this stage the œsophagus has a lumen, and is not solid, as Balfour states (Comp. Embryol., II, 63), but is depressed or cylindrical at its hinder part, while beneath the head it rapidly widens, where its width exceeds its depth several times. In the cod, however, its anterior flattened portion is short, and is not so extended as the same part in embryo Clupeoids. This flattened anterior portion of the mesenteron is molded upon the lower face of the brain, and is concave from side to side on its upper surface and convex from side to side on its lower. Its walls are very thin in contrast with the more posterior portion of the intestine or mesenteron, and are hardly more than one layer of cells deep in places. In longitudinal sections of embryos of the Clupeoid *Alosa*, in which the mouth is just about to break through, the most anterior or hyomandibular cleft which intervenes between the hyoid and mandibular arches seems to be the most developed, but it does not appear to break through the skin. Behind this the six gill-clefts are developed on either side of the pharyngeal portion of the fore-gut. They appear to be of the nature of narrow lateral paired outgrowths from the sides of the depressed fore-gut, and have at first only a very narrow cleft like lumen. The gill-clefts are at first very much crowded together antero-posteriorly in the young just-hatched cod, as may be gathered from Figs. 40, seen from the side, and 46, viewed from below, where the gill-clefts are shown at *g*. Dohrn holds that the mouth is to be regarded as an anterior outgrowth of the mesenteron from behind forwards, that it is divided in the middle line, and that the two limbs of the larval mouth grow out laterally and separately. He also seems to regard these paired oral outgrowths as the first of the branchial clefts, counting the second as the hyomandibular. As already stated, I have not been able to fully convince myself that this is the fact, although I have seen evidence in a series of sections of embryo Clupeoids which have inclined me to think Dohrn's view the correct one.

The mouth breaks through in or near the angle formed by the lower fore part of the head and the anterior epiblastic wall of the yolk-sack at the point *m* in Fig. 40. In *Alosa* the point where the superficial external epiblast is continued into the oral hypoblast is exactly in the angle alluded to above, and, as far as I can make out from longitudinal sections, there is no clear evidence of a distinct epiblastic oral invagination or stomodæum, such as is found in *Petromyzon*, for example. As development advances, the upper lip grows forward in advance of the end of the lower jaw to a marked extent, exposing the roof of the larval mouth considerably. The lower jaw, after this, begins to elongate, and soon grows in length so as to regain what it had apparently lost in relative length as compared with the upper. It is during the early stages, before the outgrowth of the lower jaw, that the mouth gapes, the man-

dible being short and immobile. After the mandible has grown to be of the same length as the upper it first begins to show signs of mobility, though it is not until some days after incubation that the jaws of the embryo begins to move, and then mostly rhythmically in respiration, the water being sucked in through the mouth and passed through the gills, the same as in the adult. Only after the young fish have the jaws distinctly developed, as in Fig. 49, do we begin to note that there are voluntary snapping movements of the mandible manifested.

At the time of the birth of the young cod there is no circulation of the blood; there are no blood vessels, in fact; which accounts for the non-functional development of the branchial apparatus at this period. The function of respiration at the time of exclusion and for some time thereafter, as during development within the egg, is apparently performed by the skin, which presents a large amount of surface, as may be seen in Fig. 40. Not only is this true, but the skin itself in the living embryo of this stage is lifted off perceptibly from the underlying structures, as shown in Figs. 42 and 43. This subdermal space, filled with fluid, probably a serum, is of the nature of the serous space around the yolk, and doubtless has a respiratory in addition to an assimilative function. The existence and office of such spaces in embryos have hardly received the attention they merit; they probably represent the earliest and most unspecialized contrivances for the transfer of pabulum in solution, in the form of paraglobulins, fibrins, or other plastic matters, from one part to another of a nascent organism.

The intestine during the later stages of development is gradually separated from the notochord at its hinder extremity by the interposition between the former and latter of more and more tissue, mostly of a mesoblastic character, which can scarcely be accounted for except upon the supposition that in each and every cell of the embryo there inheres a power of growth dependent again upon the intussusceptive powers of the cells themselves, by which they are enabled to appropriate soluble plasma through their neighbors or by way of intercellular or the extensive serous spaces already alluded to. The gradual evolution of the embryo fish before there is the slightest evidence of a systemic circulation forces the foregoing conclusion upon the student. He sees, for example, a germinal disk, at the commencement of development, of a determinate form and size, but it is not long before he begins to discover that additional material from the yolk has been added to the embryo, the bulk of the embryo itself perceptibly surpassing in size the original bulk of the disk from which it took its origin. This has been accomplished, too, in all cases, before there is a trace of circulation; in fact, before even the heart has begun to pulsate. It is this gain in bulk of the embryonic structures above and beyond the original mass of the germinal disk which cannot be accounted for on any other hypothesis, as pointed out by Rauber. The segmentation cavities of the ova of various types accordingly acquire a profounder meaning than has

hitherto been generally ascribed to them. They are, in fact, the primal representatives of nutritive spaces—lymph cavities; perhaps even of the food and water vesicles of *Protozoa*.

On the twenty-seventh to the thirtieth day of development, as shown in Figs. 49 and 45, respectively, the regions of the intestine for the first time begin to be clearly mapped out. In Fig. 49 the depressed œsophageal portion of the alimentary canal ends just over the lower lobe of the liver *lv* and just in front of what appears to be its upper portion *y*. In front of *y* there lies a body, covered with large stellate pigment cells, which I have identified with the air or swim bladder. Its mode of origin I have not made out in the young cod, but in *Gambusia* and *Alosa* it is a distinct dorsal diverticulum of the intestine, which arises a little to one side of the median line. Its hinder end is prolonged backwards with the advance of development, and is at first a small and inconspicuous structure, with a thick wall, which, on its ventral face, may be lined by what appears to be glandular epithelium, as in *Gambusia*. The connection of the pneumatic diverticulum with the intestine is by a narrow open canal, which may remain open in the adult, as in physostomous forms, for example, where it forms a pneumatic duct, or it may be aborted during a post-larval stage, as in the physoclistous species.

Behind the liver and air-bladder the intestine becomes suddenly widened, as shown in Fig. 49, and has its internal surface elevated into low folds or papillæ, which are the rudiments of the gastric and intestinal follicles of a later stage. This widened portion of the intestine is continued backwards until a constriction is encountered at *ic*. From the liver back to the constriction alluded to, the middle portion of the intestine later becomes the stomach. The constriction is apparently the pylorus and pyloric valve, while the section of intestine from the constriction to the vent *v* becomes the hind-gut of the adult, with an almost uniform caliber throughout.

Peristaltic action of the intestinal wall shows itself very early in fish embryos, or about the time that the three regions are distinctly marked out as described above. I have frequently witnessed its manifestation in newly hatched shad, and also when they were a few days old and had begun to take small crustaceans as food. The peristaltic contractions of the intestinal wall would push back the food to about the point where the œsophagus ended and where the liver began, and where the intestine was considerably widened. This widened portion was then continued back to a similar pyloric constriction, beyond which I but rarely saw the food carried.

The histological features of the intestinal walls of embryo fishes are interesting in that it is the mucous or epithelial layer which is principally developed. The muscular layers, both the longitudinal and annular, are thin. The latter is pretty thick in embryos of *Salmo*. In the neighborhood of the commencement of the stomach in embryos of *Alosa* the mucous pits and folds of the enteric epithelium are most pronounced

and thickest. Here, probably, we have the first evidence of the development of true gastric follicles. Of the development of the pyloric appendages I can say nothing more than that they, like the liver, are undoubtedly diverticula of the intestine, but which evidently develop much later than that organ, for in no form studied by me had they made their appearance up to the time, and even as late as two weeks after, the yolk was absorbed.

The vent of young fishes at first ends blindly. At the very moment the tail begins to bud out as a little rounded knob-like prominence the anal end of the gut breaks its continuity with the caudal mass of cells, and its blind extremity is directed straight backwards. Meanwhile the rest of the tail continues to grow backwards in length, leaving the anal end of the gut in the angle formed between the lower border of the tail and the yolk-sack, as shown in Fig. 40. In transverse sections of the tail of embryos a little younger than that shown in Fig. 32, a ventral strand of cells may be seen which appear to have been continued backwards from the anal end of the gut into the caudal mass of cells, but it is difficult to assure one's self that they inclose a canal which would answer to a post-anal section of the intestine, the homologue of the neurenteric canal. At this stage the anal end of the gut is sometimes club-shaped, and may end apparently on the yolk, as in the young cod, or may soon be slightly prolonged backwards and ventralwards between the thin dermal and splanchnopleural layers to end in an emargination at the edge of the ventral median fin-fold. This mode of termination is the usual one, and, so far as I am aware, the embryo cod is the only exception to it. Here, instead of ending at the margin of the fin-fold, the vent does not grow out so far, but ends within the margin of the fin-fold and some way from it, as may be seen in Figs. 40, 45, and 49. Moreover, with the outgrowth of the tail there is no marked accompanying prolongation of the hind section of the gut, such as we may note in young of *Salmo*, *Coregonus*, *Alosa*, *Pomolobus*, and *Clupea*. In these forms as the growth of the tail proceeds the anal end of the intestine, on the contrary, makes an accompanying growth in length backwards, by which the vent is pushed farther and farther back from the posterior end of the yolk-sack. Another type of development of the hind-gut of the embryos of osseous fishes is met with in *Oybirum* and *Parephippus*, where the hind-gut grows out to the margin of the ventral fin fold, but is not prolonged backward behind the yolk-sack, in consequence of the subsequent growth in length of the tail. With the collapse of the yolk-sack, however, in these two genera, the pre-anal fin-fold lengthens; this lengthening of the latter fold is, however, wholly ascribable, to the collapse of the yolk-sack, and not to any backward growth in length of the tail as a whole.

Of the development of the spleen and pancreatic tissues I can add nothing to what is already known, which is very little, except that in *Gambusia* I have met what may possibly be a splenic rudiment behind

the liver, and partially enveloping the hind-gut, in embryos which had not yet absorbed the yelk-sack. The pancreatic tissues of fishes seem to be intimately bound up with the history of the pyloric appendages, and we may therefore expect to know more of them when the development of the cæca has been worked out.

## 21.—DEVELOPMENT OF THE RENAL ORGANS OR CORPORA WOLFFIANA.

The remarkable researches of Semper, Balfour, Sedgwick, Fürbringer, Rosenberg, Cellacher, and others, on the early history of the kidneys of vertebrate embryos has within a comparatively recent period thrown a flood of light upon what had previously been a most obscure and poorly understood subject. While it will not be possible for me to add much to the general principles of development of the renal organs, so ably worked out by my predecessors, and in many senses my monitors, I can add here what I have observed in the development of those organs in *Gadus*, *Alosa*, *Gambusia*, and *Salmo*, bringing out some singular peculiarities in the evolution of the mesonephros or wolffian body itself as manifested in the embryos of these different genera.

In the figures accompanying this memoir I have not represented the segmental ducts, except in Figs. 46, 49, *pnp*, and in a diagrammatic cross-section, Fig. 33, *sd*. The details, which I have mainly worked out by means of sections of the embryos of various other genera, I reserve for illustration and description in future special essays upon those types. The development of the renal organs in different genera of *Teleostei* differs greatly in detail, as we shall learn further on. The following general description of the development of the segmental ducts or pronephros seems to apply to osseous fishes generally:

At about the time the tail begins to bud out and the muscular somites of the body have been formed, the segmental ducts are folded off from the splanchnopleure or peritoneum as a pair of longitudinal canals on either side of the middle line, as shown at *sd*, Fig. 33. They lie in close contact with the peritoneal wall of the abdomen, and at their anterior ends they open freely into its cavity. They are also usually bent upon themselves more or less markedly, inwards and backwards, and then forwards again, as shown in Fig. 46, from below, at *pnp*, and in Fig. 49. These are the primitive open funnels or free anterior extremities of the pronephric or segmental ducts as we see them in the living cod embryo of a late stage. In the young cod, some days after hatching, their anterior ends are found to lie on either side of the front end of body, extending forwards to near the auditory vesicles, and as development advances they seem to approximate the latter more closely. Traced backwards, the segmental ducts pass over the peritoneum almost exactly parallel to each other till they converge and join the allantoic or urinary vesicle *al*, conspicuously shown in Figs. 40, 45, and 49. The exact mode of their union with this vesicle I have not learned in *Gadus*, but it prob-

ably occurs at the posterior dorsal part, as in *Cybium*. The allantoic or urinary vesicle opens either into a cloaca or into the extreme hinder and possibly cloacal portion of the anal end of the intestine, as in *Alosa*, *Salmo*, and *Hippocampus*; a cloaca is, however, probably fully developed at a later stage, into which the generative ducts, bladder, and intestine open. Upon the development of the cloaca I have made very insufficient observations, and whether it is developed from the anal end of the larval intestine and lower end of the allantoic sack I am not able to state. While in some forms there is an emargination of the ventral fin-fold where the intestine ends, there is as yet no common external depression in which the alimentary, genital, and urinary canals terminate; this structure must therefore be relatively late in developing.

The segmental ducts are simple, straight cylindrical canals throughout, except at the anterior extremity, the walls of which are composed of a single layer of cells. They constitute the simplest expression of the renal excretory system of the vertebrates, and are not provided with any Malpighian bodies or other accessory excretory organs at the stage of development now under discussion, as there is as yet no circulatory system to supply blood to any glomeruli, even if these were developed. The only place where it may be supposed that anything like a glandular character has been acquired by the organ, is at its anterior end, where it is bent upon itself in the peculiar manner already described. The diversity of manner, however, in which we find the accessory organs developed in the adults of different genera, as well as the relatively late or early development of these structures in different forms, is no less interesting than the fact that the glandular portion is at first formed in different regions in the embryos of dissimilar genera. For instance, *Salmo* is in marked contrast with *Gambusia* in that the mesonephric glomeruli are developed from a little behind the pectoral fins almost to the allantoic vesicle or urinary bladder even before hatching, while in the latter genus the mesonephric portion is quite anterior, and is crowded forward against the auditory vesicle. In still other instances the pronephros does not extend nearly as far forward as in either of these cases; of this we have an illustration in the embryos of *Alosa*, where the pronephros ends far short of the head, but its anterior termination is similar in form to that observed in other families. Balfour has noticed some of the conditions of the organ in the adults, and he observes (*Comp. Embryol.*, II, 579): "In some cases the cephalic portion of the kidneys is absent in the adult, which probably implies the atrophy of the pronephros; in other instances the cephalic portion of the kidneys is the only part developed." This has its significance, and it is important that the peculiarities of different genera in respect to the mode of origin of the renal organs be investigated. In *Alosa* there is no evidence of glomeruli on the inner side of the segmental ducts until long after hatching; such also appears to be the case with *Gadus*. In *Gambusia* and *Salmo*, on the other hand, the segmental tubes are already developed, in the first case



in the head region, and in the last along almost the whole length of the ducts at the time of hatching. In *Gambusia* the segmental tubules form a complex, convoluted mass just behind and partly below the ear, and is richly supplied with blood long before the young have absorbed the yelk-sack or have been discharged from the ovarian follicles of the parent. Along the segmental ducts or pronephric canals, behind this point, there is no evidence of tubules whatever, yet in the adults of *Gambusia* we find the Wolffian body or kidney extending dorsally along the whole length of the body cavity. No such complex head-kidney, as we may call the structure found in the embryos of *Gambusia*, is developed in the young of *Salmo*, even at the time of hatching, although segmental tubes have already been formed. An examination of *Alosa* of the same relative age shows that absolutely no segmental tubes or accessory glomeruli are developed. To what cause are we to assign this difference? The cause is apparently a physiological one, and is probably not due to any phylogenetic influences, except as these may be expressed in an accelerated or retarded state of development of the systemic circulation. Both in *Gambusia* and *Salmo*, of the stage of development here considered, the blood vascular system is already far advanced, while in *Alosa* and *Gadus* there is still no circulation; this seems to me in part at least to offer an explanation of the great differences observed in the development of these organs in the embryos of the same age of different genera. As regards the local or general development of the mesonephric structures along a part or the whole of the segmental ducts, that difference is of course probably to be ascribed to hereditary or phylogenetic, and not to physiological influences.

The value of the evidence regarding the opening of the glomeruli into the body cavity will depend altogether upon what is meant by the latter term. If it is held, as it is by me, that the body cavity of fish embryos is the same as, or is at first continuous with, the segmentation cavity, then the glomeruli, as far as I am able to interpret my sections, are shut off from the body cavity. This is the view also which I should take of the sections figured by Balfour, Zeigler, and Ellacher. The segmental tubes are probably developed, like the glomeruli, from mesoblast, which lies above the peritoneal layer and between it and the aortic and venous vascular tract. In sections through the pronephros of *Alosa* it has appeared to me as if it opened anteriorly into the body cavity, but I could see no evidence of a glomerulus; but this, it is to be remembered, was in embryos which had not yet developed a circulation. The peritoneal or splanchnopleural layer is well marked in embryo fishes, but it does not usually extend far out over the yelk in early stages, so that it is easy to see that it cannot include the yelk. The true hypoblast, after the development of the intestine in *Alosa*, seems to have vanished as a discernible layer, so that the gut lies directly upon the yelk, and is therefore bounded on either hand by the segmentation cavity, which is naught else but the body cavity itself, which diminishes in

size as the yolk is absorbed. This relation of parts is merely pointed out here in defense of the position which has been assumed in regard to the non-connection of the glomeruli with the body cavity, and will be again alluded to in its proper place.

## 22.—THE DEVELOPMENT OF THE HEART.

The development of the heart of the young cod is typical of that process in young osseous fishes generally, with this qualification only, that, like the history of other portions of the body, the details of it differ considerably in the embryos of different genera. The earliest evidence of its presence is represented in Fig. 30, showing the under side of the head of an embryo of fifteen days. Large cells have first arranged themselves in a transverse band-like layer below the fore-gut; the latter is not represented in the figure. This layer is continuous with a stratum which is continued forwards on either side of the fore-gut and mid-brain, and forwards in front of the eyes and below the fore-brain. It is mesoblastic, and the only portion of it which has crept around to below the fore-gut is the transverse band in which the heart develops. Sections through the heads of embryos in similar stages of other genera justify the foregoing description. This mesoblastic stratum is also continuous with the posterior pericardiac septum *pc*, just behind the heart, while at the extreme anterior end of the head it contributes to the formation of the trabeculæ cranii and rostral plate or cartilage, and behind and above the gut it shares in the development of the parachordal sheath. In the extremely early stage of heart development shown in Fig. 30 the cavity of the future heart is a circular opening *h* in the plate of cells, from which the organ is formed by growth in length. It now appears to be wider than deep, but it gradually elongates, and instead of its axis remaining vertical to that of the long axis of the head, it grows forward more or less horizontally, as seen in Fig. 29, where the head of a seventeen-days embryo is viewed from below. Its anterior extremity now widens into a funnel shape and begins to pulsate very slowly and irregularly—once or twice in a minute, perhaps. Its anterior, apparently open end is not free, however, as might be supposed from an inspection of the figure, but is continued into an exceedingly delicate, thin membrane, continuous behind with the pericardiac septum. It will also be noticed that in Fig. 29 its front end is bent to the right side. This lateral bending is continued during the progress of development, and finally when the embryo is hatched it opens backwards, as shown in Figs. 40, 42, and 46, and also in 41, as viewed from the side, where the primitively anterior end is shown at *sr*. What was the lower side of the cardiac plate in Fig. 30 has grown downwards, then forwards, then is bent to the right, as in Fig. 26, then still more in Fig. 42, till, as viewed from the side in Fig. 41, the heart tube forms a loop with what was formerly the front end directed backwards and upwards and joined to the pericardiac membrane. It has now passed through the following stages: First, it has the form of

a plate of cells; secondly, its cavity appears as a round opening in the plate; thirdly, it grows in length so as to form a nearly straight tube; fourthly, it bends upon itself so as to carry its venous end upwards and backwards; fifthly, it is differentiated into three distinct regions, as shown in Figs. 41, 42, and 46. In freeing itself and becoming tubular, a serous space is formed around it; the pericardiac space, which is continuous, at least by way of the open venous end of the heart with the segmentation cavity, from which also the fluid filling the heart and pericardiac cavity appears to be derived. Not unfrequently colorless blood cells or white corpuscles may be seen in both the segmentation and pericardiac cavities during this stage and later, which are moved or swayed in their bath of serum by the pulsatile action of the heart. These phenomena may be observed in numerous species, and seem to be a normal accompaniment of the development of the Teleostean heart. The presence of now and then a colorless blood cell in the pericardiac cavity during the early stages of *Gadus*, and even of colored ones in the pericardiac spaces of *Salmo* and *Tylosurus*, shows that this cavity is not wholly shut off from the yolk hypoblast ("couche hæmatogène"), and therefore not altogether discontinuous with the segmentation cavity. In *Elacate* the segmentation cavity extends under the head, back towards where the heart originates, and so close to it that a relation of continuity seems altogether probable at an early stage. The thin veil like fringe shown at the posterior end of the heart of the young cod in Fig. 41 I have found in longitudinal vertical sections of the recently-hatched embryos of *Alosa* to be actually continuous with the exceedingly thin pericardiac membrane below and behind the heart, the venous end of the organ actually opening through it into the segmentation cavity. The latter is regarded by me as synonymous with the body cavity; so that the body cavity itself is derived from the segmentation cavity. The body cavity is divided from the heart or pericardiac space by the posterior pericardiac membrane, which is developed concurrently with the heart itself. The pericardiac membrane incloses the heart space ventrally and posteriorly and is of splanchnopleural origin. In *Alosa* sections through it show it to be a membrane of filmy tenuity, with nuclei imbedded in it here and there. It is much thinner in that species than even the outer epiblastic covering of the yolk-sack. In *Gadus* it is a transverse fold of notable thickness, during the early condition at least, or off the middle line of the body, as shown in Figs. 30 and 46.

The differentiation of the heart tube into regions is a gradual process; the first portion to be marked off is the dilated anterior end or venous sinus, then the ventricle and bulbous aortæ. A glance at Figs. 29, 46, 42, and 41 will show the steps of the process; how the heart tube has been bent upon itself in shifting the venous end round to its final oblique position in the middle line. I have not been able to make out the double tube or one within the other, as represented by several investigators. Doubtless there is an outer pericardiac layer, but neither in

the living heart nor yet in sections of it have I succeeded in making it out; at most it must be very thin, like the pericardiac membrane itself. Moreover, the whole of the cardiac walls are contractile, as seen in the living embryo. The contractility of the heart at an early stage is a very remarkable phenomenon, in that it as yet contains no clearly marked, spindle-shaped muscular cells or fibers; the contractility manifested during its early phases is apparently almost an automatic process, the stimulus for which may or may not proceed from any splanchnic nerves. It would appear that the nervous system during this early stage was hardly well enough developed to take part in the stimulation of the organ. I never saw any knot of nervous matter on it, or in its vicinity, which I can identify as a cardiac plexus or ganglion, and traceable as an appendage of the tenth or vagus nerve.

With the advance of development the blood is also formed, but not until about ten days after hatching; meanwhile, the heart has also assumed its final position in the median line; the venous end is no longer swung slightly to the right side, but occupies an antero-posteriorly inclined position in the middle line, as shown in Fig. 49. Its walls, especially those of the ventricle *h*, have become thicker, but there are as yet no muscular pillars or partial septa developed in it, as we find in the ventricle of *Cottus* and *Alosa*. The valves, also, cannot be said to be developed as folds, such as we see in the adult. The office of the valves seems to be performed solely by the rhythmical constriction of the cardiac tube at definite points. First, the venous sinus fills with blood, which is still pale and comparatively rich in serum; then the atrio-ventricular valvular constriction opens, and the blood is forced, by the contraction of the walls of the sinus, into the ventricle, when the intervening constriction again closes, and confines the blood in the ventricle, from whence it passes, by a similar process, into the bulbus, and so into the branchial vessels. I do not mean to imply, however, that the contracted portions of the heart-tube do not mark the regions where valves will appear in future, nor to convey the impression that the regions where the rhythmical valvular collapse of the cardiac tube occurs during pulsation are not constant in the embryonic hearts of young fishes. All of the cardiac compartments appear to exhibit pulsations even to the bulbus, which, according to Huxley (*Anat. Vertebrates*, 140), is not rhythmically contractile in the adult. True, the walls of the embryonic bulbus are thin, like those of the sinus, and it may be that its dilatations and contractions are simply an effect of the distension produced by the rhythmical contractions of the ventricle. The effect of the pulsations of the ventricle upon the blood current are visible at a late stage, just as we see in the circulation of the gills of the salamander or the web of a frog's foot; what is here meant is that the blood flow is not at a uniform rate, but the current in the vessels moves slightly slower and faster alternately, owing to the alternate exertion and non-exertion of the propelling power of the heart during the diastole

and systole of the ventricle. These phenomena are well known to physiologists, and some of their effects are matters of every day class demonstration, upon man and the higher animals, by means of the sphymograph; in other words, the fish embryo has a pulse.

It has already been remarked that wide differences of cardiac development occur in different genera and families of osseous fishes. Take the case of *Idus melanotus*, the golden ide; in the embryo of this species the venous end of the heart grows down between the front end of the yolk and its epiblastic covering until the heart itself lies ventrad of the anterior part of the yolk-mass. The Cuvierian ducts, which collect the blood from the cardinal veins, actually pass around the front end of the yolk on either side, and join the venous sinus below it; besides these venous vascular arches there is no circulation over the yolk-sack in this species.

In *Tylosurus* the venous end of the heart is prolonged in front of the head of the embryo into an annular vessel which traverses the entire circumference of the yolk in a plane coinciding with the axis of the body. Later two vessels arise from the cardinal veins which carry the blood from the body over the yolk back to the outlying venous end of the heart. Then below the head a huge pericardiac space or chamber is gradually formed, which is roofed over entirely by the epiblastic covering of the yolk-sack into which the heart depends, having been disproportionately elongated in consequence. Its venous end is fixed to the extreme lower part of the huge heart chamber where it is continuous with the yolk hypoblast or blood-generating layer which overlies the yolk. Here at its point of attachment the three vitelline veins join the heart and pour their contents into it. The remarkable abundance of blood corpuscles in the heart cavity and their origin has already been described in my paper on this species, so that I will not here repeat what has already been well enough elaborated elsewhere.

In *Apeltes* the venous end of the heart is pushed out from the right side of the body and is at first joined to an asymmetrical system of vitelline vessels, which at a later stage become quite symmetrically arranged.

In *Salmo* the heart is never prolonged outwards anteriorly or laterally in the embryo, as in the foregoing species. The vitelline system of vessels develop somewhat asymmetrically, and the great venous vitelline trunk does not lie in the middle line but somewhat to the left side. A part of the blood which passes through the vitelline capillaries passes through the liver, and there are no greatly developed representatives of the Cuvierian ducts which traverse the yolk-sack, as in *Idus* and *Tylosurus*.

In the young gold-fish, *Carassius*, the Cuvierian ducts embrace the anterior extremity of the yolk as in *Idus*, in order to reach the heart, which is ventrad of the yolk in position.

In *Siphostoma* a subintestinal vein passes down behind the yolk-sack and traverses its ventral surface in the middle line to empty into the heart in front, which does not have its venous end prolonged but simply opens to this median vessel in a ventral direction.

The embryos of *Fundulus* have the heart somewhat prolonged over the yolk anteriorly.

In *Gambusia*, a diffuse, superficial, vitelline capillary system arises from a very short subintestinal vein and lateral venous trunks which are probably Cuvierian, but which are also assisted by the hepatic vein on one side. The capillaries so arising converge at the anterior end of the yolk, where the venous end of the heart is prolonged downwards between the epiblastic covering of the yolk and the yolk hypoblast.

In the cod, when the blood-vessels are developed, thirty days after hatching, the venous sinus opens upwards and backwards and receives three sets of vessels, viz, lateral and ventral intestinal and the cardinal veins, the latter by way of the Cuvierian ducts.

In *Cottus* a pair of anteriorly divergent veins, lying on the ventral face of the yolk-sack, pass upwards and forwards to empty into the venous sinus, just below where the cardinals debouch.

*Alosa*, *Cybius*, *Parephippus*, *Elacate*, *Osmerus*, and *Pomolobus* do not have a vitelline circulation at all, and here the heart soonest acquires its adult position, as in the cod. But in all of these forms it is in the highest degree probable that the heart opens directly into the segmentation or body cavity, as I have demonstrated in *Alosa* and *Pomolobus*. The mode of absorption of the yolk in these forms also becomes clear on the grounds already stated in my paper on that subject, namely, by direct gemmation of corpuscles from the yolk hypoblast into the segmentation cavity, from whence they are taken up into the circulation by the heart.

### 23.—DEVELOPMENT OF THE CIRCULATION AND THE FUNCTION OF THE YELK HYPOBLAST.

The various physiological adaptations of the circulatory system, if we may so speak, which we have described in the preceding chapter, show us clearly that one and the same function may be performed by the profound, almost radical, modification of the system of organs which is concerned in its manifestation. In no set of organs within a restricted group of types do we find any instance which presents more striking variations than those observable in the arrangement of the vessels upon the yelks of different species of embryo Teleosts. To trace the course of the vessels themselves in the different forms to be described is no easy task; this will therefore not be attempted with the less important ones, but only with the larger vascular trunks, which are also the first to be developed.

The development of the vessels themselves is so important for us to

understand that I shall here reproduce what Balfour has said of it in his Comparative Embryology, II, p. 519:

“The actual observations bearing on the origin of the vascular system, using the term to include the lymphatic system, are very scanty. It seems probable, mainly it must be admitted on *à priori* grounds, that vascular and lymphatic systems have originated from the conversion of indefinite spaces, primitively situated in the general connective tissue, into definite channels. It is quite certain that vascular systems have arisen independently in many types; a very striking case of the kind being the development in certain parasitic *Copepoda* of a closed system of vessels with a red non-corpusculated blood (E. Van Beneden, Heider), not found in any other Crustacea. Parts of vascular systems appear to have arisen in some cases by a canalization of cells.

“The blood systems may either be closed, or communicate with the body cavity. In cases where the primitive body cavity is atrophied or partially broken up into separate compartments (*Insecta*, *Mollusca*, *Discophora*, etc.), a free communication between the vascular system and the body cavity is usually present; but in these cases the communication is no doubt secondary. On the whole it would seem probable that the vascular system has in most instances arisen independently of the body cavity, at least in types where the body cavity is present in a well developed condition. As pointed out by the Hertwigs, a vascular system is always absent where there is not a considerable development of connective tissue.

“As to the ontogeny of the vascular channels there is still much to be made out both in vertebrates and invertebrates.

“The smaller channels often arise by a canalization of cells. This process has been satisfactorily studied by Lankester in the Leech,\* and may easily be observed in the blastoderm of the chick or in the epiploon of a newly-born rabbit (Schäfer, Ranvier). In either case the vessels arise from a network of cells, the superficial protoplasm and part of the nuclei giving rise to the walls, and the blood corpuscles being derived either from nucleated masses set free within the vessels (the chick), or from blood corpuscles directly differentiated in the axes of the cells (mammals).

“Larger vessels would seem to be formed from solid cords of cells, the central cells becoming converted into the corpuscles and the peripheral cells constituting the walls. This mode of formation has been observed by myself in the case of the spider's heart, and by other observers in other invertebrata. In the vertebrata a more or less similar mode of formation appears to hold good for the larger vessels, but further investigations are still required on this subject. Götte finds that in the frogs the larger vessels are formed as longitudinal spaces, and that the walls are derived from the indifferent cells bounding these spaces, which become flattened and united into a continuous layer.

"The early formation of vessels in the vertebrata takes place in the splanchnic mesoblast; but this appears to be due to the fact that the circulation is at first mainly confined to the vitelline region, which is covered by splanchnic mesoblast."

Has it, however, been proved that a splanchnic layer covers the yolk of fishes at a late stage, or after the inclusion of the yolk by the blastoderm? The reply to this is most positively in the negative in the case of those forms devoid of a vitelline vascular system. In those types, however, in which a vitelline system of capillaries is found, the answer is not so clear. Sections of the salmon, just after hatching, are very instructive, and we here find an arrangement which is most interesting, especially if those through the region of the liver be examined, from the ventral border of which it is evident that vessels are continued directly over the yolk, and that if they are not wholly channeled out of the thick plasmodium or yolk hypoblast they are at most covered on the external side only, by an exceedingly thin layer of cells. Inasmuch as we know that there are free nuclei imbedded in this plasmodium or yolk hypoblast, is it not possible that they may become the means of developing cells for the walls of the vitelline capillaries as well as blood corpuscles? As remarked some way back, I found that the external epiblastic somatopleural and outer peritoneal layers of the external yolk-sack of the young salmon might be entirely stripped off from the yolk and that they were nowhere adherent to it, and that this exposed the vascular layer covering the yolk. Moreover, the space which lies between the vascular and outer envelopes of the yolk has been derived from the segmentation cavity and becomes abnormally and greatly distended with water when salmon embryos are affected with what is known as "dropsy" amongst fish-culturists. In such cases, too, the space will often contain dead blood corpuscles, after some of the vitelline vessels have been ruptured and injured, which often leads to the partial or complete stoppage of the vitelline and hepatic circulations, which may of course be fatal to the life of the embryo. The hind portion of the outer sack is also sometimes abnormally distended backwards and is finally constricted and sloughed off, while the embryo, which has lost a part of the outer yolk covering in this curious manner, may go on developing normally if the place where the diseased part was broken off has healed promptly. From all of these facts, it may be inferred that whatever the significance of a splanchnopleural layer may be it cannot in any case be other than the inner or lower peritoneal part which has been reflected over the yolk and which is traversed by the vitelline blood-vessels. Now in sections through just-hatched salmon, its tenuity is very great and is present only as the thinnest kind of a film over the true yolk hypoblast, but, as already stated, whether it may be certainly identified with the innermost splanchnopleural layer is a question which I cannot certainly answer. On the inner side of the vessels, the blood-cells are seen to lie in immediate contact with the plasmodium or yolk-



hypoblast, and it is to be inferred that blood cells are budded off directly into them, the division of the free nuclei in the subjacent plasmodium probably multiplying and giving rise to these blood corpuscles. In this way it is conceivable that the yelk is gradually broken down, just as we know that by a similar process the yelk of *Alosa*, which has no vitelline circulation, is absorbed. The lumen of the vitelline vessels is also depressed or somewhat flattened upon the yelk surface, and not round as in other parts of the body, and in some cases (*Tylosurus* and *Apeltes*) they have at first the form of exceedingly irregular channels, which are evidently much more deeply excavated into the plasmodium layer at some points than at others. In *Apeltes* the first sign of any vessels is the appearance of a large irregular sinus on one side of the body between it and the yelk in which the blood corpuscles vibrate in unison with the pulsations of the heart, there being as yet no complete open channel or cyclical path for the passage of the blood back to and through the heart. The vessels end blindly at first and are also progressively lengthened, and possibly the rhythmical impulses given to the primitive blood during pulsation helps to open up the channels still farther. Such blind vascular terminations are found on the yelk of a number of species at an early stage of development of the blood system and usually end acutely but finally push towards and open into some pre-existing channel, when they at once become wider. In such blind vascular terminations the blood cells simply oscillate back and forth. In *Tylosurus* the early blood cells may form adherent masses in the great meridional vessel of very uneven caliber, which is the first to be formed and wherein these masses move fitfully or only oscillate with the pulsations of the heart. They soon acquire a reddish tinge, but the fact that they adhere together shows that possibly they are of the nature of confluent white corpuscles or even masses detached from the plasmodium layer which here evidently forms the floor of the vessel, in the act of segmenting and becoming blood corpuscles. This primitive blood of *Tylosurus* is also rich in serum and poor in blood-cells. In *Apeltes* the blood cells are more numerous at a similar stage.

In *Gambusia* the blood-vessels which traverse the yelk, like in *Salmo*, seem more or less deeply imbedded in the yelk-hypoblast layer, and I find it difficult to determine the nature of their outer coverings; internally they seem to lie in immediate contact with the yelk, so that the contained blood-cells in sections of hardened specimens are packed right against and impressed into the plasmodium or yelk hypoblast. The vascular network over the yelk of *Gambusia* is, however, much finer than in *Salmo*, and relatively thicker as seen in sections, but the external covering of the yelk-sack, unlike in the latter species, is not thick and two-layered, but exceedingly thin and formed solely of epiblast on the ventral and lateral portions.

In *Alosa* no cellular elements are distinguished in the yelk-hypoblast; it is a thick homogeneous coating over the yelk, with scattered free

nuclei imbedded in it, and in sections strands of its substance pass inwards to be insinuated between the coarse yelk masses in the interior, which are composed of a different kind of granular protoplasm. The free nuclei are most abundant in the dorsal and anterior portion of the yelk-hypoblast. No vessels or traces of any are ever found traversing it, and with the approach of the later stages of development it is not clear that the heart maintains its wide communication with the segmentation or body cavity as observed at an earlier stage. The yelk, as absorption proceeds and diminution of its bulk results, assumes a fusiform shape or becomes somewhat like an oat-grain in form. All this while, however, its anterior end continues to lie close to the heart and may even be drawn out into a conical process, directed towards the venous sinus. This conical process consists almost entirely of the yelk-hypoblast or outer rind of the yelk proper, which does not disappear with the collapse of the yelk, but is kept of about the same thickness until the whole of it with its contained granular protoplasm is absorbed. In this collapse we may also note another point of interest; it is that the yelk diminishes behind and below so that its anterior end maintains its close relation to the heart while the posterior end, as it recedes towards the head, uncovers more and more of the under side of the liver behind and above it.

The mode in which the yelk hypoblast is continually kept of the same thickness is very remarkable. While its substance is being removed externally by the gemmation of blood-cells from its surface into the segmentation cavity, as in *Alosa*, or into the vitelline vessels in *Salmo*, its thickness is maintained by the apposition of material to its under or inner surface from the underlying yelk, the internal granular matter of which is slowly transformed into the clearer and more homogeneous plasma of the yelk-hypoblast proper. This transformation goes on until the whole internal yelk mass is thus made transferable to the nascent organism of the young fish, by means of the blood-cell gemmation already spoken of. Yelk absorption is therefore a physiological process of the most far-reaching significance. The yelk itself may be compared to the endosperm of a large seed in which the stored proteinaceous matters are slowly broken down by the agency of an organic ferment and rendered soluble and diffusible through the cellulose walls of the component cells of the infant plant. The analogy does not stop here, however. If we look deeper, it is not improbable that we may hit upon the true significance of another set of phenomena which have not, as far as I am aware, been viewed in the light in which we propose to view them in a succeeding chapter.

The absorption of the yelk of the cod embryo is evidently similar to that of the shad. In Fig. 49 a yelk canal *yc* passes forward to the heart from what is left of the yelk *d*. This canal is evidently similar to the arrangement seen in *Alosa*, where there is an anterior conical process

from the yelk hypoblast; the layer *hy* in our figures is the homologue of the yelk hypoblast in *Alosa*.

In the study of the yelk circulation of *Coregonus albus*, or Lake whitefish, the vitelline vessels in optic section appear to have an inferior as well as outer wall and to be connected together by a thin membrane stretching between them. Can it be that this vascular membrane is continuous with the heart through the thin posterior splanchnopleural pericardiac membrane? It would seem as if this might be the state of affairs in this species, if not in all forms. The fact that the Cuvierian ducts develop in the upper lateral portions of this membrane is greatly in favor of such an interpretation. In *Coregonus* we may also observe that there is in the living embryos a very shallow fluid space between the yelk and the vascular layer between the courses of the vessels. - In hardened specimens of salmon embryos sections show the vessels depressed; this I now suspect may be due in part to the compression and shrinkage of the outer yelk-sack under the influence of chromic acid. While we can say positively that the posterior and ventral pericardiac wall does not include or cover more than a small portion of the upper surface of the yelk at the time of hatching in *Alosa*, it is probable that when the yelk is almost absorbed that it may entirely envelop it. It may also be said that a marked acceleration in the development of the vascular splanchnopleural yelk-layer continuous with the venous end of the heart may and does probably occur if we may be guided by the evidence supplied by the investigations of Cellacher on the trout. This view will account for the early development of a yelk vascular system in some forms and its absence in others. It will also explain why it is that in some forms an intercommunication exists between the heart and segmentation cavity while it is absent in others. The remarkable law of acceleration and retardation, which was first distinctly formulated by the eminent palaeontologist, Professor Cope, is exemplified on every hand in a study of the development of osseous fishes, and furnishes a clew and key to much that would otherwise be obscure.

The embryo salmon, immediately after hatching, has an arrangement of capillaries which is in the highest degree interesting. The main vessels now consist of a great median dorsal aorta which passes backwards just below the subnotochordal rod to the upturned hinder extremity of the chorda. Here intercommunication between the aorta and caudal vein is established by way of a singular caudal network of capillaries, which, first of all, empty into a sinus-like, non-contractile dilatation before they pour their contents into the caudal vein, which then passes forwards ventrad of the aorta towards the head, dividing into the two posterior cardinals above the intestine. These cardinals then give off capillaries again which pass around the intestine and unite into a subintestinal vein as large in diameter as one of the cardinals. The subintestinal vein then passes forwards over the yelk, and, bending a little to the right, ends under the liver, into which it pours its blood,

again breaking up into smaller vessels in the hepatic tissue, from which the blood again emerges to be conveyed in larger capillaries over the yolk-sack, and which pass outwards, downwards, and forwards, to empty into the great median vitelline vein on the ventral face of the yolk, a little to the left side of the middle line. The anterior and posterior cardinals together with the great vitelline vein empty their blood into the venous sinus, from whence it is passed into the heart, and from thence through the branchial vessels is sent through the carotids and aorta, the latter of which is supplied by the combined currents from the four hinder branchial vessels, which converge and meet in a common aortic trunk below the medulla oblongata. The subclavian artery of the pectoral arises from the vicinity where the branchial vessels unite into the aortic trunk. The origin and course of the submaxillary and cephalic vessels I have not made out. Supraocular and postcerebellar veins pass backwards on the head to empty into the jugulars.

The somatic capillaries are somewhat interesting in respect to their arrangement. They are given off from the aorta and pass outwards on either side through the muscular septa between the muscular somites on the middle line of the side, all of them traversing the common septum which divides the dorso-lateral from the ventro-lateral portions of the muscular plates. While these vessels are of a capillary character in the embryo, they become the segmental arteries and veins of the adult. The course of the blood current in them is not in the same direction in all of them, however; in some it is afferent and in others efferent in direction, so that it would appear that some of the segmental vessels were really venous and others arterial. After reaching the surface the arterial segmental vessels divide dorsally and ventrally into branches which follow the courses of the intermuscular septa to pass inwards at the dorsal and ventral borders to unite with the cardinal or caudal veins. The venous segmental vessels are supplied from two vessels which have exactly the same course as the intersegmental capillaries of the arterial segmental vessels, but the blood-flow is outwards. They bend over the upper and lower edges of the muscle plates, follow the septa, and at the middle line of the side, at the level of the horizontal septum, between the dorso-lateral and ventro-lateral plates, converge into an incurrent segmental vein. These intersegmental veins and arteries do not alternate regularly; there may be two arterial vessels in succession followed by a vein between the next two following segments. The dorso-ventral intersegmental capillary loops convey the blood from the aorta and to the great veins, so that in the case of a true intersegmental vessel it may have either a single or a double origin from the aorta, according as the outgoing vessel passes directly to the surface at the middle of the side or by way of dorsal and ventral arterial loops. The mode in which these vessels are channeled out in the body I have not been able to make out.

In *Coregonus* the vitelline vascular system is not so complex, but,

as in *Salmo*, there is a subintestinal vein, which, unlike the sub-intestinal vein of *Salmo*, is bent upwards just at the hinder extremity of the yolk sack to end in the liver, in which it breaks up into a hepatic plexus to emerge in the form of vitelline capillaries.

In *Gambusia* the subintestinal vein is short or absent. My reason for thinking it absent is the fact that there is no vessel in this form which has the same origin and termination in the liver as in the salmon. What might be regarded as a subintestinal vein is the anterior end of the caudal, which is bent downwards abruptly and traverses the posterior portion of the abdominal cavity obliquely to divide on either hand into a posterior vitelline vessel or vitelline canal on either side of the yolk, which passes forwards to join and pass somewhat beyond the outgoing Cuvierian vessels into which the liver also pours its blood at one side. From these lateral vascular arcs the vitelline capillaries take their origin; they have a generally downward and forward direction.

About the twenty-fifth day after impregnation, and five days after hatching, in the series of cod embryos studied by the writer, there was a complete circulation apparent in the branchial vessels, the aorta, and cardinal veins, but only for a short way back. This primitive circulation did not extend much beyond the extremity of the intestine or abdominal cavity at this stage. In Fig. 40, twenty-two days after impregnation no sign of circulation could be detected. In three days more, however, blood corpuscles began to be more abundant and the vessels could be seen to be slowly and progressively forming from before backwards in the strand of vacuolating mesoblast underneath the notochord. With the progressive lengthening backwards of the aorta and caudal vein, the point of union between them was also pushed backward, but by what histological process was not made out. The point of union between the caudal end of the aorta and the caudal vein is shown at *p*, Fig. 45, representing an embryo ten days after hatching. A subintestinal and lateral venous intestinal trunks were also developed at this stage, which were joined together by short vertical vessels.

#### 24.—DEVELOPMENT OF THE PIGMENT CELLS OF EMBRYO FISHES.

In the embryo cod, as in young fishes generally, pigment cells begin to be differentiated just under the epithelial layer of the epiblast at an early period. In the cod they appear as small rounded scattered cells of a slightly darker color than the surrounding tissues about the time of the closure of the blastoderm. From that time forward, however, they become progressively darker and more densely loaded with granules of melanin. They also soon lose their primitively rounded or biscuit like form and become depressed and manifest a tendency to throw out flattened pseudopods or prolongations in all directions. When far advanced in development, as in the later stages, the dark melanin granules do not entirely obscure the nucleus of the pigment-cell which may be noticed in its center as a very refringent body entirely devoid of

pigment granules. The pigment-cells of the body are the first to be developed and are the first to become stellate, as shown in Fig. 32, where those on the tail are still approximately round. Up to the time of hatching they are pretty uniformly scattered over the body and less abundantly on the head, as shown in Fig. 34.

Beyond this stage a marked change in their distribution occurs which cannot be explained without supposing them to possess to a certain extent a power of migration or means of changing their original position beneath the dermal epithelium. They aggregate in unerring regularity in every embryo at about the same places after hatching, as may be seen in Figs. 40, 42, 45, and 49. Two clusters of them are uniformly aggregated on the dorsal and ventral surface of the tail, as shown in Figs. 40 and 45 at *pi*. In the first figure they are less densely aggregated than in the last; this is due to a spreading of the pseudopodal prolongations of the pigmented protoplasm composing them. Some of them in the cod now have, when highly magnified, a striking resemblance to a flower, the corolla of which is represented by the radially arranged and flattened black protoplasmic processes of the cell with the clear nucleus in the dark center. On the head region they remain isolated and even without marked processes, as may be seen upon looking at those represented on the brain and jaws in Fig. 49.

A second and internal layer of pigment cells is developed in the embryo cod. These are confined to the dorsal parietes of the abdominal cavity and seem to underlie the peritoneum. These appear later than those found in the skin, and whether there is any genetic relation between the dermal pigment cells and those of the abdominal cavity would perhaps be hard to say, though it is to be remembered that since we know them to possess migratory powers in other situations, it may not be impossible for the pigment cells of the abdomen to have primarily originated from the same layer as those of the skin. In Fig. 49 they are shown as especially well developed just over the intestine, liver and air-bladder as far forward as the base of the breast fins.

In the young four-spined stickle-back, *Apeltes quadracus*, a second kind of pigment cells are developed, forming a row on the median dorsal line and a row on each side of the body. These are of a dirty yellow color, but they develop precisely like the more numerous black ones which surround them and which give the very dark color to the young of this species, which are as dark as young tadpoles, two or three days after hatching.

The young goldfish (*Carassius*) has only black pigment cells developed after hatching, no sign of the bright red color being apparent in just-hatched embryos known to have been spawned by red-colored parents. The same remark applies to the young of *Idus melanotus*, another cyprinoid, in which the skin of the adult is brilliantly colored with red or orange-red pigment.

To show that light has probably very little to do with inducing the

development of pigment in an embryo fish, we have the very remarkable case of *Gambusia*, in which pigment-cells are developed in the skin, especially on the head, to a remarkable degree, or almost as densely as in the young of *Apeltes*, while the young fish is still inclosed in the ovarian follicle of the mother. The conditions by which it is surrounded in the follicle being especially unfavorable to the accession of light, inclosed as it is within the more or less extensively pigmented walls of the abdomen of the parent, and we are driven to the extremity of supposing that this prenatal pigmentation of the embryos of *Gambusia* is due to the unsuppressed influence of heredity.

In conclusion, it may be of interest to note that the young of *Parephippus*, which in other respects develop almost exactly like the Spanish mackerel during the early stages, soon show a tendency to form a reddish pigment over the abdomen and remains of the yelk-sack, on the third day after hatching. The reddish pigment-cells of this form are often confluent and have long and complex interjoined processes, much flattened, like pigment-cells generally. In the young of the same species an inch in length, the future vertical bands of the adult are already outlined in black. The red pigment seems therefore to have a larval significance, and to be useful probably during an early period of development only.

#### 25.—THE LAW OF DISPLACEMENT OF THE GERMINATIVE VESICLE.

It is well known that in the large-yelked or meroblastic eggs of many vertebrates and invertebrates there is a migration of the nucleus of the egg at a late stage of ovarian development towards the surface before the nascent ovum has left its follicle. It is noteworthy that, on the other hand, it is only the small holoblastic or evenly segmenting ova without a yelk which retain their nucleus nearly in the center to the time and condition of emission from the ovary. Examples of this type are presented to us in the mature ova of mammals, of *Amphioxus*, and many invertebrates. In the egg of the oyster only a slight eccentricity of the nucleus is notable in the mature egg, and we find that its eggs depart but slightly from the holoblastic or even type of segmentation. In *Nassa*, a gasteropod studied by Bobretsky, the segmentation is more unequal, and therefore approximates the meroblastic type more nearly than the egg of *Ostrea*. The observed facts with regard to the displacement of the nucleus or germinative vesicle before impregnation, lead us to enunciate the following general principle: *The nucleus or germinative vesicle is permanently displaced from the center of the ovum in proportion to the amount of food-yelk which is developed, the amount of its eccentricity, or the distance through which it is displaced from the original center of the ovum is governed entirely by the amount of food-yelk which is stored in an egg during its intraovarian growth.* This appears to be a fundamental law of ovular development in general, and one which is far-reaching in its significance in relation to the later phenomena of

cleavage. In fact, it gives us a clew to why it is that there is such a distinction between ova as the evenly and unevenly segmenting or the holoblastic and meroblastic types. It will also be seen that it is probable that the two types pass into each other by insensible gradations, which we find is truly the fact when we come to institute a large series of comparisons between observed types of development. Moreover, the food-yolk has simply a physiological significance; it is merely a store of food, which has been superadded during the intraovarian growth and maturation of the egg, yet the effect of this superadded yolk is to modify the process of segmentation profoundly. That store of deutoplasm which is added to the egg to nourish the embryo and carry it to that condition of development when it can in a measure take care of itself, is also of profound significance in relation to natural selection, by the operation of which it can alone be supposed that yolks were evolved. This all-comprehending Darwinian law is therefore seen to have influenced the mode of segmentation of ova by and through the minor and secondary law of nuclear displacement which has been indicated above.

The degree also to which the nucleus is transported from its primitively central position determines the degree of inequality of the first segmentation. It is now in the highest degree probable that in the formation of the germinal disks of meroblastic ova the process is one and the same throughout the animal kingdom, viz, that its development is accomplished by the migration and concentration of the germinal matter of the egg at its animal pole. We have evidence in superabundance in favor of such a view of the matter, in a great many departments of embryology. There is an evident tendency on the part of the germinal protoplasm of ova to separate itself spontaneously from the food yolk or deutoplasm and aggregate itself either superficially over the whole surface of the ovum, as in the case of centrolecithal, or at one pole mainly, as in meroblastic or telolecithal types. We will find, however, that the distinction between these two forms is primarily less important than might be supposed, for the meroblastic type passes through a distinct centrolecithal stage prior to the development of the germinal disk in *Gadus*, while in other forms the mode of disk development is complicated by a network passing down into and between the deutoplasm masses from the external stratum of germinal protoplasm, as in the ova of Clupeoids and *Leuciscus*. The remarkable centrolecithal segmentation and arrangement of the protoplasm of the eggs of the arthropods is, therefore, found not to be so radically different from the usual type as might at first be supposed. Its arrangement, under slightly different laws of segmentation, is referable to the same fundamental principle governing the dissociation and aggregation of the protoplasm and deutoplasm into separate masses. I would also regard the deutoplasm as almost entirely passive in the process of its absorption during the later stages, for we have seen that it is actually appropriated by a remnant of the original



germinal protoplasm, which takes it up by intussusception and apposition. The coarser granules of the deutoplasm are slowly broken down, as we saw in the case of *Alosa*, and converted into the more homogeneous and much more finely granular and more highly vitalized protoplasm of the yolk hypoblast.

In the very act of the mechanical dissociation of the protoplasm of the egg from its deutoplasm, we have an explanation of why the nuclei are attracted to the former and repelled from the latter. The first is the portion of the egg which is dynamic in character, the portion in which developmental potentiality inheres; the second is in the static condition of what Beale might perhaps call "formed material." This attraction of the nucleus or germinative vesicle for the protoplasm of the egg points to its true nature, and must be of a directive or trophic character, as insisted upon by Rauber; its office, in short, appears to be to direct those phenomena of protoplasmic rearrangement and contractility, and perhaps of metabolism, which transpire during segmentation. The rhythmical metamorphosis of the nuclear bodies into complex "asters" or caryokinetic figures, with granular lines radiating in all directions through the surrounding plasma, like the pseudopods of a heliozoön, would seem to indicate that something of this sort is the function of a part, at least, of the nucleus.

The first segmentation furrow, or that usually described as such, which divides the germinal disk of the Teleostean ovum into two halves, is, according to Hoffmann's investigations, not the first, but, on the contrary, must be considered as the second to be developed in the order of time. His researches have shown that a cleavage spindle is developed, when the germinal disk is finally marked off from the yolk hypoblast, just after impregnation. The axis of this spindle also coincides with the diameter of the egg. We therefore have, in this fact, the final proof of the law of nuclear displacement, which has been pointed out a little way back, and also why it is that there may be a great dissimilarity in the size of the deutoplasmic, as compared with the protoplasmic mass of germinal matter, dependent as this must be upon the amount of food yolk which has been stored in the ovum during its intrafollicular development.

Inasmuch as the yolk of some ova has the form of coarse ovoidal bodies, involved in a matrix of soft plasma, as in those of *Lepidosteus* and *Amia*, for example, an approach is evidently made towards the condition of the stored nutrient materials in the cells of seeds. As in the latter, we may call these bodies, which are said to be composed of *ichthyine* by chemists, *globoids*. Upon making sections of the mature ovarian ova of *Lepidosteus*, I find that the germinative vesicle has left the center of the egg and passed outwards almost into contact with the egg-membrane. Here the nucleus is surrounded by a mass of germinal matter evidently destined to form the germinal pole of the egg. The coarse globoids of the central and lower portions of the egg gradually

grow smaller as they approach the nucleus, around which they form a discoidal mass with the nuclear body imbedded in the center. The globoids of this incipient germinal disk are very small but ovoidal in form like the larger ones of the deutoplasm. They therefore simulate the granules or microsomata of other protoplasm in general. The nucleus still maintains its spherical form in the disk. It may be that in some such way the protoplasm of the germinal disk of the eggs of osseous fishes is developed. We find, in fact, that an opaque granular stage precedes the clear stage of maturity, by which time also the nucleus has migrated from the center and disappeared in the rind of protoplasm which now envelops the deutoplasm; this protoplasmic envelope now constitutes the true hollow egg cell with the nucleus in a very eccentric position imbedded in it or intimately blended with its substance, while the deutoplasm may be looked upon as stored material or added material, in short, rather as the cell contents than as an active part of the cell itself. According to this view it also becomes of interest to note that the position of the oil spheres or drops in fish ova is due to the same cause as that of the deutoplasm or food yolk. They are, in fact, always more or less deeply imbedded in the deutoplasm itself, and not to any extent in the protoplasmic envelope. The fish-egg is, moreover, directly comparable to a fat cell, in which large oil-drops have been formed internally, the presence of which has compelled the nucleus to assume a parietal position at some point in the superficial enveloping protoplasm; just as in the fat cell the nucleus has been repelled from its central position by the encroachment of the stored fat, the nucleus of the Teleostean ovum has been displaced by the encroachment of the stored deutoplasm.

In the true first segmentation we also saw that the protoplasm was differentiated into two parts, viz, a true germ disk capable of segmenting and developing the embryo, and a protoplasmic yolk envelope, the function of which appeared to be entirely that of an appropriative and transformative membrane, histologically a syncytium, concerned mainly in the elaboration of blood from the deutoplasm. The workings of the general law which we have been tracing is evident wherever we meet with meroblastic or centrolecithal ova, and is, as we have seen, of great physiological importance and invariably determines the plane of the true first cleavage and consequently the relative dimensions of the germ disk and yolk. It may be said, however, that the proportional bulk of germ and yolk is probably determined by the higher laws of the struggle for existence. It is also true that the bulk of the yolk has not the slightest value in classification, as great variations occur in respect to size within the limits of the same family of fishes. Again, it is singular that the eggs of some amphibians should approach much more nearly than those of the fishes the holoblastic or evenly segmenting type, while the much smaller ova of many of the latter should present us with an extreme form of the meroblastic type. The only explanation

applicable to these cases seems to lie in the physiological relation pointed out as subsisting between the germinal and nutritive matter of the egg, which determines the plane of the first cleavage, but this does not dispose of the question why one form with a much smaller egg should be supplied with a much smaller amount of germinal matter than another with a much larger egg. The consequences of the comparison just instituted between the ova of the frog and fish do not stop here, however, for we actually find that the amount of germinal and nutritive matter in proportion to each other is not the same even in different genera and species of fishes. I now call to mind illustrations of this from amongst the Salmonoids. In *Osmerus* the germ is much larger in proportion to the yolk than in *Salmo*. Of two forms belonging to different families, *Alosa* has a relatively much larger germ than *Tylosurus*, in the latter of which the germinal disk is reduced to the extreme of relative diminutiveness amongst the fish ova which I have studied.

The remarkable phenomena which have been discussed in the preceding paragraphs are due altogether to the inherent motility of the protoplasm of the germ and nucleus. What the nature of the force is which impels the protoplasm of the fish ovum to migrate towards the germinal pole or to aggregate into a germ at all we cannot say. While it exhibits actual contractility and a self-moving power resident in and manifested by its own substance, science is not yet ready to assert that it knows anything of its efficient cause. This is a phenomenon as inscrutable in its essential nature as the movements of the living amœba. The movements of the protoplasm of the egg of the fish in the act of forming the germinal disk only resemble to a certain extent those of the amœba; there is no exact parallelism. The amœba, in the active stage, moves about continually in its own peculiar way like any other perfect animal; practically, the protoplasm layer of the fish ovum stops moving in a distinctly amœbal manner after it has aggregated itself into the germinal disk. In these respects the perfect organism of the amœba differs from the germinal matter of the fish no less than in its want of any power of segmentation and metamorphosis into a determinate species of fish embryo. The current ridiculous and unscientific statement that the germs of animals may be likened to an amœba has no foundation in observed fact. From their earliest incipency fish ova differ radically from an amœba in appearance, and would not be mistaken for one by the merest tyro.

The nucleus of the amœba after the ingestion of food is usually displaced to a marked extent from the central position. This eccentric position of the nucleus of the amœboid *Protoplasta* seems to be dependent upon essentially the same cause as the displacement of the nucleus in the meroblastic egg, viz, in consequence of the absorption of materials by the endosarc, which require to be raised to the condition of the living protoplasm of the rest of the animal by metabolic processes. The

ingested food-materials taken up voluntarily by the amœba are analogous to the deutoplasm stored in the protoplasmic envelope of the meroblastic egg, and carried to it by the blood vascular network which traverses the ovarian follicles; with this difference again, that whereas the transformation of ingested material by the amœba is probably carried on by the help of organic ferments developed during digestion, the addition of new material to the growing egg is probably effected by a cumulative process without the help of a ferment, the stored proteids undergoing an actual retrogressive metamorphosis into non-contractile globoids, or granular, globular, or ovoidal vitelline bodies in a condition of stasis or quiescence.

It also appears to be true in general that, whenever the layers of cells comprising the whole or part of the yelk begin to segment, those of them containing yelk material or granules have the nucleus more or less extensively displaced from its central position, which is in conformity with the general principle stated. For convenience we may name those forms of cells and ova which do not have the nucleus permanently and notably displaced, as homogeneous or *homoplastic*, and those which exhibit marked permanent nuclear displacement as heterogeneous in composition, or as being *heteroplastic*.

This scheme does not exclude such types of cells as those of the notochord or the yelk-sack of a fish egg with its contained oil drops. We find, indeed, that a large oil sphere may be the last part of the yelk to be gradually broken down during yelk absorption, as in *Cybium*. The reasons for regarding the yelk as a cell have already been stated, and it will be needless to vindicate the claim of the chorda cells with their large fluid cavities to that designation. The true first cleavage of the Teleostean egg occurs when the germ disk is finally differentiated at the time the first segmentation nucleus divides, leaving one half of the latter in the plasmodium or yelk hypoblast, the other in the germ disk. At this stage, therefore, the germ is a cell and the plasmodium envelope covering the yelk another. The germ cell is the active animal cell; the lower or yelk-containing cell is the passive and negative one, the contents of which are mostly broken down during development by the metabolic agency of the plasmodium envelope.

A set of phenomena are, however, to be considered in this connection which must qualify the preceding general statements. I have been careful to say that the nucleus of the meroblastic egg is *permanently displaced* when maturation is complete; that is, even after the extrusion of the polar cells. In holoblastic ova there is a marked recession of the remains of the nucleus concerned in the first segmentation towards the center of the egg after the extrusion of the polar cells; in fact after being shoved to the periphery to form the amphiasters and polar cells its remains return to a more nearly central position as the first segmentation nucleus than that occupied by the germinative vesicle at the time the egg was freed from the ovary. This is also a fundamental distinction be-

tween the meroblastic and the holoblastic types of eggs. The return of the segmentation nucleus towards the center of the egg is apparently prevented by the presence of yolk or deutoplasm in the egg. The second or subsidiary principle qualifying the first in that *the degree of recession of the segmentation nucleus towards the center of the egg is determined by the amount of food yolk which is present in its center or at its vegetative pole.*

We must not, however, forget to mention that in the meroblastic eggs of the frog, and *Clepsine*, according to Whitman, the first segmentation nucleus may return to the center of the egg after impregnation and segment twice, forming four nearly equal cells, before the four new nuclei are finally repelled to the animal pole to establish a meroblastic form of segmentation. These appear to be cases in which the final displacement of the nuclei has been retarded. The principle involved is the same, however.

## 26.—ON SOME OF THE PHENOMENA OF SEGMENTATION AND GROWTH.

Recently the study of the processes of segmentation has been pursued very successfully by a large number of investigators upon a considerable number of forms. One of the most recent papers on the subject is by Prof. A. Rauber,\* who has taken special pains to investigate the successive development of the segmentation furrows and their relations of direction to each other and to the axis of the nucleus in the act of division, as well as the direction of the cleavage planes of segmenting cells in relation to the growth in extent of a flat membrane or other structure. He also considers the phenomena of contractility or movement of the protoplasm during segmentation and has arrived at what appear to the writer to be some very important conclusions respecting what may be regarded as evidence of its structure and the relations which this bears to processes of growth and metabolism or waste and repair. Without pretending to review Rauber's important contribution to the subject of the morphology of protoplasm, if we may so speak, the writer has for a considerable time past been approaching somewhat similar conclusions regarding the nature of the physical substratum of vital phenomena. Many of our most able investigators have been striving to represent it in an altogether too simple and homogeneous form, until the idea is widely prevalent amongst otherwise well-informed persons that there is really some such thing as an homogeneous primordial living jelly out of which all living beings are formed. It is stated by some to be a colloid, like gelatin or gum, but if we study it narrowly we find that it differs physically in a good many respects from these substances, one of the most striking of which is, that unlike the not-living colloids it will not mix in any given proportion in the living

\* *Neue Grundlegungen zur Kenntniss der Zelle. Morphologisches Jahrbuch, VIII, pp. 233-338, pls. XI-XIV. Leipzig, 1882.*

state with water as they do. That it has colloidal properties of a kind which are conditioned by its living state no one would perhaps deny, but to treat living matter with the same terms and with the same implications as not-living diffusible substances is manifestly an abuse of terms. Nor does the implication stop here, for if we look into the processes of secretion in living bodies, there is apparently a tendency on the part of the living membranes to act somewhat like dead ones, yet it will require little reflection to satisfy the most ordinary mind that there is not only a difference of degree, but of kind, when the two are compared. The not-living membrane depends upon purely physical principles for its workings, while the living membrane is an apparatus in the true sense of the word, often comprised of many parts with diverse functions, such as columnar or ciliated pavement epithelium, with connective fibers, muscle, nerves, and vessels composed of cellular units, each of which may have a specific share in the processes of transudation carried on in follicles or cavities of glands or other organs. It is true the differentiation of these complex structures disappears as we descend in the scale of life, yet it is also true that we have almost as constantly developed in the lowest types, as well as in the highest, certain bodies in the interior of cells, which, with a few unimportant exceptions, seem to have some sort of a vital relation to the plasma in which they are imbedded; we refer to nuclei. These bodies, if we may place any reliance upon what is manifested during embryonic development, seem to be very intimately, and even physiologically, related to the phenomena of cleavage during development, and not improbably with nutrition and the metabolic processes occurring in the interior of the cell. If something of the sort is not their function, the apparently more fluid contents, and even trabecular network sometimes found in their interiors, are without significance. Leaving out of the question any radial, or, more exactly, any heliozoöidal\* arrangement of the granular matter around the nucleus as argued by Rauber, it is at least evident that the process of impregnation is almost always, if not invariably, accompanied by nuclear metamorphosis and the development of asters, or single and double heliozoöidal figures, imbedded in the protoplasm of the egg. These phenomena seem to be more or less constant accompaniments of later growth, of which impregnation seems to be in reality only a particular phase, bridging the vital continuity between sexual parents and their offspring. The phenomena of indirect cell division or that accompanied by caryokinesis or the development of cleavage spindles or a double heliozoöidal arrangement of the granules of the medullary or inner cell substance, which may extend even to the peripheral cell surface or wall, probably have a similar significance and seem intimately bound up with the primary phenomena of growth or segmentation, of which they are a pretty constant attendant in the early stages of most forms which have been

\*A word which has suggested itself from the resemblance of some nuclear figures to a Heliozoön with its radiating pseudopods.

closely enough investigated. In fishes, these caryokinetic or nuclear figures are found by me in the segmenting blastodermic cells at a late stage, or after the blastoderm has already covered half of the yolk sphere. Similar facts are recorded of the germinal area of the blastoderm of higher types by Rauber.

Hardly any one would attempt to dispute the ground taken by Rauber that cell-division is the result of growth, addition, or further differentiation of plasma, and not the reverse, else we should very soon be brought to the absurd position of assuming that cell-division might go on indefinitely without the addition of new matter, which we know is not the case. In the preceding section on nuclear displacement, which is very commonly manifested, so commonly, indeed, that perhaps relatively very few perfectly holoblastic or evenly segmenting ova are known, we have an extensive piece of evidence in favor of the doctrine that growth must precede segmentation. Just exactly how the passive deutoplasma is broken down and raised to the grade of protoplasm we do not know in detail in many cases, but we do know enough of the process in others at least to infer what may be its general type or mode in all. In general terms, it may be stated that the deutoplasm or yolk is more or less closely invested or inclosed by the germinal protoplasm, the function of which is clearly appropriative either by direct contact or by the agency of fluid ferments acting by means of what Foster would call "vascular bonds," or even through mere intercellular or segmentation cavities.

Let us look and see if we have any evidence for this theory of growth in the later phenomena of maturation of the fish ovum. We know that in the later stages of egg-development the protoplasm is to a great extent superficial or peripheral in position. In the process of germ-formation at one pole we in reality behold neither more nor less than a growth, upbuilding, or aggregation of the protoplasm from the surface of the vitellus, or even from its interior, to form a germ-cell which will be segmented off at the time of the first true cleavage. That cleavage will, however, leave behind a modicum of germinal protoplasm surrounding or even insinuated into the yolk in strands, which will be the efficient cause of the transformation of what remains of the deutoplasm into a form fitted to enter into the further development of the embryo as a plasmine-like substance or as veritable nucleated protoplasmic bodies. These later phenomena, after development has progressed to a definite stage, are every whit as much to be considered phenomena of growth as if the embryo were already feeding. The incorporation of a food yolk seems to a certain extent almost like the process of digestion in an amœba, with only this difference, that the deutoplasm cannot properly be called dead matter, like the food of the amœba, but protoplasm in an inert or quiescent state. The parallel does not stop here, however, for we actually have fluid spaces formed in many embryos around or partially around the yolk, or in closed cavities surrounded by cells, just

as in protozoa and amœboid forms generally the food particles are taken into spherical cavities and surrounded with a watery fluid to which ferments are probably added during digestion from the surrounding plasma, so as to dissolve and incorporate by diffusion and intussusception that which was not into that which so becomes part of the amœba. The presence of clots of amorphous matter in the cavities of fish embryos when sections are prepared has often struck me as evidence of something of the sort described above. These may, however, be artificial products, and the effect of the extractive and solvent action of chromic acid and the precipitating action of alcohol.

Not less interesting than the phenomena just described are some of the irregularities of cleavage. These irregularities were formerly not much noticed, or if noticed, investigators were not in a position to assign to them their true significance. It is probably true that most embryos will be found to vary more or less notably, if the segmentation of large numbers of ova be carefully studied and compared. We know, for example, that two well-marked types of segmentation are found to obtain in *Ostrea virginica*, as shown by Brooks. Those of other types also are known to vary, often greatly, in the details of the relations of the segmentation furrows, especially at their points of meeting—that is, where new furrows run towards and join preceding ones. From the investigations of Rauber, we may infer that in some forms this irregularity is very marked—so much so, that outline diagrams of the cleavage furrows of, say, the morula stage of any two ova of the same species would nowhere exactly coincide if superimposed. This irregularity is found to obtain extensively in meroblastic ova, and is, perhaps, almost constant in them, so that we discover that a tendency toward individual variation exists in part at the very beginning of development, without, however, interfering with a well-marked or characteristic plan of development in the case of each form.

The irregularities in the cleavage of the germinal disk of fishes become evident at a very early stage, but most conspicuously after the germ has been divided into four cells, as shown in Figs. 33*a*, 33*b*, and 39. Slight differences in size may also be noticed at the time the germ is divided into two segments, as in Fig. 44. In Fig. 33*b*, giving an outline representation of the germ of the shad's egg, the whole has an oblong, sub-square form, as seen from above. The first transverse furrow *ii*, *a*, *ii*, is bent obtusely at the two points where the furrows of the second cleavage *i*, *i*, meet it. This is actually an effect of the second segmentation, because we do not find a short section *a* of the first cleavage furrow having a course different from that of the outer limbs *ii ii* prior to the second cleavage. At the time the first segmentation furrow is fully formed in fish ova, generally, it divides the germ disk straight across, as in Figs. 9, 35, 37, and 44. The inequalities observable in the arrangement of the furrows seem to be mainly due to the displacement of the cleavage planes, or, perhaps, more properly, to a slight angular shifting



of the nuclear plates developed during the second cleavage. Rauber seems to lay stress upon a more or less extensive swinging round of the axis of the cleavage spindles, after the second and especially the third series of cleavages begin in the frog's ovum, by which he very clearly and beautifully accounts for the variations of segmentation in that form. This he thinks may be due to what he calls *segmental attraction* between blastomere and blastomere. The contractions of the protoplasm of the blastomeres during segmentation, by which certain ones are displaced, repelled or attracted by others, is also considered. He likewise thinks that the poles of nuclear spindles or new centers of adjacent cells may have an attraction for each other. The cleavage planes or the furrows between homologous cells of similar ova may differ from one another in direction as much as  $90^\circ$ ; ordinarily the variation is much less, for a variation of  $3^\circ$  to  $5^\circ$  would be sufficient to produce the most striking variations.

For my own part I would be inclined to ascribe the dislocation of the cleavage planes in such germs as are shown in Figs. 33*a* and *b* mainly to the contractions of the protoplasmic mass of the blastomeres during segmentation. It is quite certain that at the beginning of cleavage the germs of fish ova are discoidal, and that during the development of the first cleavage furrow the disk elongates remarkably in a direction at right angles to the first furrow, as may be seen in Fig. 9. Not only does this occur, but the two new segments are also usually produced on the upper surface into pretty acute obconical points when viewed from the side. This occurs when the cleavage furrow is developing, and implies a heaping up of the protoplasm of the blastomere by its own powers of movement. Sometimes after the two blastomeres have assumed the conical form spoken of they slowly subside, in consequence of which they again assume a depressed form with the cleavage furrow very much less distinct. All of these phenomena signify an active movement of the substance of the blastomeres, in which the nuclear spindles undoubtedly have an important office to perform. That contractions of the outer substance of the blastomeres do occur we have evidence in the development of the superficial wrinkles in the cleavage furrows shown in Figs. 33*a*, 35, and 44.

The effects of the dislocation of the cleavage furrows by the contractility, or perhaps reciprocal attractions and repulsions of the blastomeres, is further shown when we glance at Figs. 12 and 13. The heavy dotted lines of Fig. 33*b* also show how the third series of cleavage furrows may be displaced so as not to meet each other exactly where they join those of the second *i, i*. This displacement of the cleavage furrows seems to be the rule rather than the exception in almost all forms of development. This disjointing or dislocation of furrows, too, does not seem to arise from mutual pressure during the early stages so much as from actual contractions, as already pointed out, but the pressure of the cells upon each other, which takes the place of the contractions after

the latter have subsided, tends to compel them to maintain their irregular forms and thus afford the starting points for still further irregularities of cleavage.

After a while also the series of successive segmentations are no longer synchronous—that is, after the third or fourth series of cleavage furrows have developed, it will be found that if sections of germinal disks are examined there will be evidence of division in some cells and not in others. In some cells caryokinetic or nuclear figures are present; in others they are wanting. Not only is this true, but where the disk contains upwards of 1,000 cells the nuclear figures of different cells are also found in various stages of metamorphosis, while a much larger number are quiescent or resting. This tendency to heterochronous division of the nuclei of the germ is another very striking illustration of the law of acceleration and retardation of development.

The acceleration and retardation of the metamorphosis of the nuclei again probably depends upon the nutritive processes and metabolism occurring reciprocally between the component cells of the disk, and by way of intercellular spaces as well as the segmentation cavity. It is certain that respiratory processes go on during segmentation, and it may not be impossible for the segmentation cavity with its thin roof to be partially respiratory in the ovum of osseous fishes. It is also quite certain that Newport and Ransom were justified in regarding the water space around the vitellus and germ or the cavity between the ovum proper and the egg-membrane as respiratory in function. One of these authorities, I do not recall which, first named this cavity the *breathing chamber*. It is developed, as already described, at the time of fertilization. There can also be little doubt that respiration goes on in young fishes, which are without a circulation at the time of hatching. If, as we have seen, there is evidence of the existence of respiratory processes in embryos long before any spontaneous movement of the body is visible, it is fair to infer that such a process can and probably does influence the rate of segmentation.

The final proof, however, that respiration occurs in fish embryos is that it is positively necessary in the construction of hatching apparatus to have it so arranged as to constantly change the water upon the eggs. This is done simply to supply the developing embryos with fresh, oxygenated water. Still another proof that respiration must occur in fish embryos before a circulation is developed is the fact that young shad move by contorting the tail some time before they leave the egg-membrane. The muscular contractions of the lateral muscular plates so manifested must be accompanied by the evolution of carbonic acid, which must be carried off and replaced by oxygen. This can manifestly not be accomplished more directly than by way of the water in the so-called breathing chamber surrounding the embryo fish.

It will be noticed upon comparing the outline of the disk represented by Fig. 10 with Fig. 33, that the latter is more nearly circular than the former. The latter has passed farther into the resting stage,

and is therefore more depressed. This elevation and depression of the upper surface of blastomeres in the course of segmentation is often a very marked feature, and gives rise to the most singular superficial irregularity of the whole disk up to the time of the completion of the morula stage of development. These changes are doubtless due to internal movements of the substance of the blastomeres, dependent upon an internal radial contractile structure.

The radial and reticulated structure of protoplasm is to Rauber of the most profound significance in relation to the phenomena of growth and development. His conclusions are here, in part, reproduced :

“1. Radial and trabecular structures of protoplasm are not essentially distinct but are manifestations of the same principle, in that the latter is developed as a result of vacuolization, the former in the direction of readiest division (*spaltbarkeit*).

“2. The radial and trabecular structure of animal as well as vegetable protoplasm is a factor with which every investigation into the history of the growth of an animal or a vegetable body has to do; such structure is vitally related to the beginning of development.

“3. The radial and trabecular arrangement of protoplasm grows both by the addition of new material from without, at its peripheral ends, as well as by the incorporation of such material within the pre-existing substance, or both by apposition and intussusception. The protoplasmic streaming necessary for this purpose is facilitated by the interradiial passages and the corresponding series of spaces in vacuolate protoplasm.”

Of the nucleus he says :

“The finer phenomena of caryokinesis display to us, in a manner such as does scarcely any other process, working nature at her loom. Groups of granules are the raw material which she next arranges in rows of threads. In astonishment we observe the most manifold looping and splitting of the granular threads and the completion of the most delicate chromatin structures.” \* \* \* “The structure of the nucleus is variable only during the periods of division. In the condition of rest its structure \* \* \* is monotonous.

“The function of the nucleus can only be such an one as is entirely independent of the differentiation of protoplasm [in different species of living forms], such an one, indeed, as is needed by the most diverse protoplasmic structures. This function can only be trophic. \* \* \*

“Whether this trophic or directive function relates to the metabolic processes, to the formation of centers, or to the regulation of diffusion-streams of protoplasm, must remain undecided, as much as the probabilities are in favor of the latter.

“The essential nature of the structure of the nucleus is difficult to make out; the fundamental form seems to be a radial one, and in such cases may usually be referred to a radial structure, which is not directly manifest externally.

"The nucleus is neither inevitably present in the cell nor yet in protoplasm. Plasmodium on the one hand, and enuclear protoplasm on the other, prove this.

"The surface growth in extent of cellular membranes is conditioned as a rule by two cleavage planes of the cells, perpendicular or at right angles to each other. Attending growth in thickness, as in the epidermis, for example, there is, besides the above, a cleavage plane of the cells parallel to the surface."

The laws of growth and cleavage, which are touched upon in the foregoing extracts, are to some extent a realization of what must have coursed through the mind of almost every student who has busied himself with embryological investigations, or who has watched the recent advances of histology without himself taking an active part in the work. For my own part I believe that we have arrived at a new era in embryological and physiological research. We shall hereafter not only be obliged to figure and study the changes of external form which transpire during development, and the contours of the cells which are concerned in bringing this about in an embryo, but also the phenomena manifested and the changes suffered by the whole contents of the individual cells in the process of embryonic evolution. Here is where anatomy and physiology converge; and upon a comparative embryology as exhaustive in its methods and results as that here contemplated, will it alone be possible to found a comparative physiology equally exhaustive, but infinitely more valuable in its practical application to the needs of every-day life than the physiology of the present. The masterly monographs of Strasburger, Flemming, Fol, the Hertwigs, Whitman, Leidy, and Mark, besides many others, have brought us face to face with a series of facts and phenomena, the significance of which has hardly yet been fully appreciated.

#### 27.—THE GASTRULA AND COELOMA OF TELEOSTS.

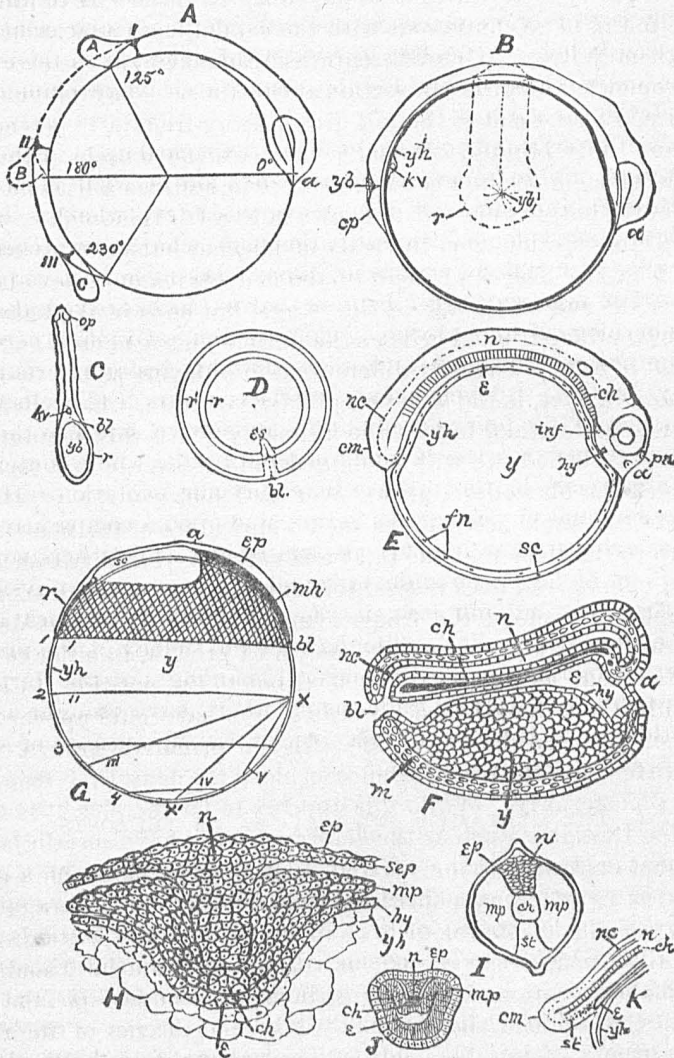
The epibolic growth of the blastoderm over the yolk of the osseous fish ovum, as in other meroblastic ova, has given rise to not a little discussion amongst embryologists as to the true nature of its gastrula stage. As Whitman\* has pointed out, there is a fundamental similarity in the mode of formation of the neurula in embryos of *Clepsine*, the frog, sturgeon, salmon, chick, and saw-fish. The conerescence of the rim of the blastoderm to form the neurula seems in reality to be the key to the interpretation of the development of the gastrula of the embryos of bony fishes and some other meroblastic types, as well as the development of the coeloma and lateral musculature of the body.

In order to make it easier to understand the gastrula of *Teleostei*, a series of diagrammatic and semidiagrammatic figures are introduced here.

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\* *Embryology of Clepsine*, pp. 86-95.

Fig. E, in longitudinal vertical section, represents, for instance, the relations of the neurula *n* and the mesenteron *e*, showing them to be continuous by way of the solid caudal mass *cm*, in such forms as *Alosa* and



*Gadus*. The dotted line *nc* can be regarded as indicating an open neural canal only at the middle and anterior end of the body of the embryo; nevertheless, the hinder end of the neurula, with the further growth of the tail, actually acquires a lumen, as in Fig. K, but by this time the tail, having grown out some distance, the continuity between the hind gut *e* and caudal mass has been torn asunder, and all that is left to mark their original continuity is a strand of cells *st*, shown in Figs. I and K. This strand of cells was, however, only in part continuous with the gut,

as a portion, perhaps the most of it, is concerned in the development of the subnotochordal vascular tract of the tail. In Fig. I, which is a section through the caudal knob *cm* of an embryo of the same age as that shown partly in K, the muscle plates *mp* on either side of the notochord are found to be continuous with the caudal mass and to embrace between them the swollen, undifferentiated, caudal end of the chorda *ch*, which is somewhat quadrate in section at this time. The hind gut when finally formed as the end of the tail grows away from it, is perceptibly swollen and ends blindly. Dorsally, between the muscle plates, the caudal end of the neurula *n* is quite distinct in sections a little forward of the tip of the outgrowing tail, as shown in Fig. I. It is solid, however, like in the cross-section from the body of a somewhat younger embryo shown in Fig. H. The caudal end of the neurula cannot, however, be traced over the end of the tail at this stage, but ends in the same apical mass of undifferentiated cells as the muscle plates, chorda and post-anal strand of cells. The only differentiation of layers at the tip of the caudal knob, in fact, is the skin or epiblastic stratum which covers it.

In order to understand in part the means by which this arrangement of parts is established, we will be obliged to look closely into the manner in which the blastoderm grows over and incloses the yolk. The large size of the latter necessitates such a spreading of the blastoderm, because up to the time that the tail buds out, the yolk diminishes but slightly in volume and does not exhibit any signs of segmentation like the deutoplasmic pole of the frog's ovum. In Fig. D a blastoderm is shown in outline at two consecutive stages of growth, in order to illustrate the fact that as the rim *r* is pushed out to the position *r'* the embryonic shield *es* is lengthened towards *bl* by what would appear to be a concrescence of the rim *r* in a line with the primitive groove. This progressive fusion of the blastodermic rim along the neural axis lengthens the embryo posteriorly. While this appears to be the fact, it is not to be forgotten that, inasmuch as the embryonic cells have certain powers of movement or translation conferred upon them in virtue of a power of more rapid growth in one direction than another, which is again dependent upon the operation of certain hereditary and fixed laws of cleavage, all of which is to be considered, no less than the histological forces which make the concrescence spoken of possible. It is at first hard to understand in what manner the whole of the rim of the blastoderm is incorporated into the body of the embryo fish, but of this we have such overwhelming proof in observed fact that it will be unnecessary to appeal to other evidence. His and Rauber, who were the first to clearly describe this process of concrescence or precession of the rim of the blastoderm, have been criticised by Balfour (*Comp. Embryol.* II, p. 254). From what the latter remarks it is evident that he never witnessed the closure of the blastodermic rim at the vegetative or caudal pole of the living Teleostean ovum, in some of the rapidly developing forms of which it may actually be seen in the process of transformation

into the caudal plate *cp*, Fig. B, which is wholly converted into the caudal mass *cm* of Fig. E. To urge the history of the closure of the blastoderm in Elasmobranchs as evidence against the process as it occurs in *Teleostei* is unfair, because a considerable part of the blastodermic rim in the embryos of cartilaginous fishes has evidently nothing to do with the development of the body of the embryo, but closes after the latter has been elevated above the yelk upon a stalk.

An actual crater-like depression *yb*, Fig. B, with a fine canal ending upon the yelk, is seen at the time of closure of the blastoderm in *Teleostei*. Viewed from above, as in the dotted outline in B, *yb* is seen to have radiating wrinkles extending out over the concreting rim *r*. This yelk blastopore so formed is not homologous with the similar pore or cleft in the frog's ovum, because it cannot be shown to have anything more than an indirect connection with the intestine in the embryo fish. This is illustrated in Fig. C, showing an embryo of *Tylosurus* in outline, unrolled from the yelk, with the oval blastodermic rim *r* attached. The latter is concreting; its contents are flowing together, as it were, where its two limbs join the hind end of the body at *bl*. The opening *yb* is the yelk blastopore, and is distinct from the place where the actual concrecence is occurring viz, at *bl*. In fact, while there is no actual opening into the neural or neurenteric canal at *bl*, neither existing at this stage of development, only the extreme anterior part of the yelk blastopore coincides ultimately with that of the blastopore of the frog, but only momentarily, in that the lumen of both intestine and neurula, contrary to what obtains with the frog's embryo, are wanting at this stage. The sides of the primitive groove coalesce so quickly in *Teleostei*—if, indeed, it can be said that there is such a groove in those forms as we know it in the frog—that evidence of its presence even is evanescent, or it is at most very feebly developed. In the frog the margins of the groove are free, and the neural furrow in the medullary plate is most conspicuous even at the time the blastopore closes, but the latter marks approximately the position of the permanent anus. In *Teleostei* there is no such relation between the yelk blastopore and the vent, which arises in them on the ventral side of the caudal end of the embryo, as shown in Fig. K. An exact comparison of the parts of the frog's ovum with those of the osseous fish is not possible, as Ziegler has endeavored to show; his failure to make out the true state of the case was his want of the appreciation of the true nature of the yelk. In fact, the continuity of the epiblast with the mesenteron, as in the embryo frog, is broken in the embryo fish by the time the yelk blastopore has closed and the caudal plate has formed.

The frog's ovum undergoes total but unequal segmentation; the fish ovum, on the other hand, undergoes equal segmentation of its germ; partial segmentation, as regards its whole mass, while the final segmentation—gemination—of the yelk substance as leucocytes into the segmentation cavity or blood vessels is carried on after the embryo is far

developed and the heart and vitelline vessels have been formed. There is also present around the yolk of fish embryos an homogeneous envelope, *yh*, Fig. E, containing free nuclei, which is not present in the frog's ovum, nor has it any exact homologue in the latter, because it incloses a large mass of deutoplasmic matter often optically and physiologically different in character from the protoplasm of the germ or the envelope itself.

Van Bambeke and E. Van Beneden, who first described some of the most important phenomena presented by what I have called the *yelk-hypoblast*, named it the intermediary layer. In consequence of the presence of a large amount of deutoplasm in the yolk of the fish ovum, yolk-segmentation has been retarded, in fact has been wholly interrupted, so that nuclear multiplication alone, unattended by actual segmentation, occurs in the yolk envelope *yh*, shown in Figs. B and G. The distribution in it of the free nuclei *fn* is shown in Fig. E.

In the frog the liver develops as a ventral diverticulum of the fore part of the mesenteron, which grows into and at once appropriates a part of the segmented yolk; the latter in fact becomes fused with and forms the exceedingly thick ventral wall of the mesenteron. In the embryo fish this fusion of the yolk mass with the mesenteron or primitive gut never takes place; the liver arises as an independent diverticulum or thickening of the ventral wall of the mesenteron. There is no evidence of continuity between the intestine and yolk of the osseous fish at any stage of development. The appropriation of the yolk by the budding of leucocytes from the yolk hypoblast is effected in some types by direct gemination into the segmentation cavity, from which the white blood-cells are sucked up by the heart (*Alosa*, *Pomolobus*), or where the pericardiac septum between the heart chamber and body cavity is so accelerated in development as to extend over the yolk as a veil in which the vitelline vessels are developed (*Salmo*) and which are concerned in breaking down the yolk. In the first case the body and segmentation cavities remain connected; in the latter they are soon separated by the development of the vascular veil which grows over and around the vitellus. In all forms of osseous fishes at an early stage the two seem to be continuous. This is shown in the cross-section of the body of a young fish, younger than E, in Fig. H, where the epiblast *ep* and *sep* and the hypoblast *hy* include the muscle and splanchnopleural plate which ends bluntly at *mp*, and to the right of which the segmentation cavity extends, so that in the event of the splitting of *mp* to form the splanchnopleure and muscle plate proper there would be a continuity established between the two; none of the lower layers in fact extend far out on either side of the body between the epiblast and yolk hypoblast. That this view is the true one is proven by the mode in which *Amphioxus* develops its muscle plates as outgrowths into the blastocœl or segmentation cavity of the blastula after the invagination and development of the gastrula, as seen in the cross-section J of an embryo of *Amphioxus*, modified



from Kowalewsky. In fact there is abundant evidence of the truth of the cœloma theory, proposed by the Hertwigs, presented in the mode of development of the muscle plates of *Teleostei*, as lateral outgrowths of the lower layer before the differentiation of the mesoblast from the hypoblast by delamination, as indicated in Fig. H. Ziegler has in fact reached the conclusion that the chorda is of hypoblastic origin, so that embryologists are almost unanimous in regard to the origin of the primitive axis of the body of the chordata. Such an origin is indicated in the cod by the position of the caudal end of the chorda below the level of the upper half of the thickness of the caudal end of the body of the embryo shown in Fig. 31.

The evidence favoring the marginal ingrowth of the lower layer is to me not as strong as that in favor of delamination. My reasons for this opinion may be stated somewhat more clearly by referring to Figs. 14 and G. In the first, and in both optical and actual sections of a similar stage in other forms, the lenticular germ is shown to be composed of a mass of equal sized cells; in fact a morula condition is developed and no differentiation of layers is perceptible except the single outermost and epithelial stratum of the epiblast. In flattening and spreading there is not enough lateral movement to account for the formation of the wide rim in Figs. 15 and 16, by an infolding of the edge of the disk as it spreads so as to bend the separate lower layer inwards all round the margin. In fact, the segmentation cavity is at first smaller and deeper in proportion to its width than shown in Fig. 15, and the marginal infolding, as it might appear, does not continue with the spreading of the disk, but afterwards takes place only at one point, viz, where the rim is continued into the sides of the embryo. The weight of the evidence is therefore in favor of delamination as the means by which the primary diploblastic condition of the germ is developed and not by gastrulation. The first diploblastic phase of the germ of the fish egg is therefore apparently a planula, and neither its upper nor its lower layers are but one cell deep, but consist in both cases of three or more rows of cells, which can scarcely be said to be arranged in regular layers except those which limit the upper and lower surfaces of two the primary ones.

In Fig. G the upper or epiblastic stratum in vertical section is left white, while the lower combining the mesoblast and hypoblast is shown black in section with its superficial extent in the blastoderm indicated by the hatched lines. The embryonic axis of this blastoderm is cut through from *a* to *bl*. At first one would suppose that there was clear evidence of invagination from behind forwards from the point *bl* in the earlier stages, but, as it has already been remarked, this does probably not begin much before the stage represented in figure G has been attained, and then by the peculiar mode of concrescence previously described.

With the growth of the blastoderm over the yolk the lower layer of the rim *r* does not increase in width, as required by the invagination

theory, but tends rather to become narrower vertically as it advances over the yelk globe toward the level of the line 2, Fig. G, while it is gradually constricted in diameter as it grows past the level of lines 3 and 4, beyond which it closes at  $x$ . In ova with a very large yelk, like those of the salmon and silver-gar, the blastoderm does not close at a point opposite to that where the germ was developed. This has given rise to some dispute amongst embryologists, a disagreement which may be explained by the diagrams A and G. If we suppose A to represent a very large yelked osseous fish ovum, with  $a$  as its germinal pole, the body of the embryo will then grow, say, to the point A, the blastopore closing at I. The rim will then cease to advance at the tail of the embryo or at the point  $x$  in Fig. G, when, as shown in the latter, the portion of the rim on the opposite side of the egg will have to advance faster from the point II onward towards III, IV and V, closing at  $x$  instead of  $x'$ . When the egg is a medium sized one, like that of *Alosa* in Fig. B, the embryo stops growing in length only when the tail reaches the opposite pole, as shown in Fig. A; if the egg is still smaller, the embryonic axis may continue to grow beyond the opposite pole, so that the blastoderm does not close till it reaches the point marked III beyond which the tail buds out at c. This third form also requires another mode of closure of the blastoderm, differing from the two preceding types.

In a large ovum, according to Fig. A, the embryo ceases to grow in length when it has extended itself over an arc of the yelk globe of, say,  $125^\circ$ ; in *Alosa*, or the second form, it extends its growth through an arc of  $180^\circ$ ; in the third and smaller type of ovum (*Carassius*), it may grow to a greater length and embrace an arc of  $230^\circ$  on the surface of the yelk sphere. In the first type the rim of the blastoderm is sometimes drawn out into an oval prior to closing, as shown in Fig. C taken from *Tylosurus*. These different modes of growth in length of the bodies of the embryos of different species of fishes are matters of observation with me and go far toward reconciling the differences of opinion which have been expressed by different observers as to the growth of the blastoderm over the yelk. It is evident, at any rate, that what may be observed on this point in one type may not apply to another.

The segmentation cavity *sc*, Fig. G, extends laterally, with the advance of the rim of the blastoderm, towards the opposite pole of the egg, and does not disappear, as held by Haeckel and Balfour. In Fig. E it extends between the yelk hypoblast and its epiblastic outer coverings from  $a$  clear to the tail  $cm$ , and from one side of the body down, over, and around the yelk to the other side. It may be seen developed to a remarkable extent in some forms, as in *Cybiium*, *Coregonus*, and *Alosa*. Figs. 47 and 48 show it in two stages, under the blastoderm of the cod.

In *Coregonus*, the oil-drops, by their buoyancy, bulge the yelk hypoblast upwards into the cavity, so that immediately over each considerable droplet there is a perceptible rounded elevation of its floor.

The various cavities which different observers claim to have seen dur-

ing the early stages of development I believe, in some cases, at any rate, to have been purely the products of reagents. The separation of the cells during cleavage is a very probable occurrence, and originates by the cells pushing and displacing each other somewhat during this process, as suggested by Whitman. The evidence which I have been able to gather, both from the living eggs and sections, leads me to the conclusion that the *Keimhöhle* of Stricker and Cellacher is the true blastocœl of the Teleostean ovum, as Ziegler has more recently urged. The yelk hypoblast is its floor, and the at first two-layered epiblast its roof. Its development is constantly the same in all of the forms studied by me, and I have not yet found any evidence of the existence of species without the epiblastic or outer covering of the yelk-sack, as asserted by Von Baer.

I see no valid reason for not regarding the yelk as an active part of the ovum, through the intermediation of the yelk hypoblast, and it seems evident that the segmentation cavity is simply a space filled with fluid which facilitates the gliding of the blastoderm over the yelk during growth, and that it is placed between the blastoderm and the yelk, with its free nuclei peripherally displaced to a remarkable degree. In other words, I would regard the yelk as an integral part of the egg, taking a share in segmentation only at a very late period. In consequence of the almost entirely passive condition of the yelk during the earlier stages, the blastoderm is obliged to spread to an extreme degree, and in parts becomes remarkably attenuated. On this account I would still hold to the view first expressed in my paper on the development of *Tylosurus*, that the germ-disk alone is practically the homologue of the whole Batrachian or Marsipobranch ovum, since we actually do not find any intimate connection of the yelk with the embryo, except by way of the vascular system, which develops late in most forms. In *Alosa* the yelk might be removed at any stage without taking away any essential part of the embryo except the floor of the segmentation cavity. The mode of development of the gastrula and cœloma, is, we find, greatly modified by the presence of the yelk, but it is not an active factor in the development of either by means of any process of segmentation.

The free nuclei of the yelk hypoblast apparently proliferate as the blastoderm spreads. They are, at any rate, at first confined to the germinal pole of the ovum, and are only found at the opposite pole after the yelk-globe has been included by the blastoderm. The inference, therefore, is that they spread and multiply with the lateral growth of the blastoderm. It is these nuclei possibly which are the centers of certain free cells around the margin of the germinal disk when the latter has attained the morula stage, as in *Cybbium* and *Tylosurus*, as shown in Fig. 3, Pl. XIX, of my essay on the latter form. If such is the case, it is possible that the germinal wall (*Keimicall*) at the edge of the blastoderm of the chick is homologous with the yelk hypoblast of the fish ovum. In fact, it is highly probable that there is a yelk hypo-

blast inclosing the true vitelline matter of the eggs of birds, reptiles, and Elasmobranchs which is altogether homologous with the same layer in the Teleostean ovum. It is also likely that it has a similar origin in all of these truly meroblastic forms, in all of which an extreme permanent displacement of the germinative vesicle also occurs.

In Fig. E, at *hy*, the hypophysis or pituitary body is shown as a dorsal diverticulum from the fore part of the mesenteron. The latter is much depressed from above and expanded laterally at this stage. The origin of the hypophysis from the fore part of the mesenteron in *Teleostei* seems to be pretty well established.

I see no difficulty in referring the development of the muscle plates of *Teleostei* to a process essentially similar to that seen in *Amphioxus*, viz, as outgrowths from the primitive hypoblast. A comparison of Figs. H and J may make this clearer.

#### 28.—THE DEVELOPMENT OF THE EGGS OF THE CODFISH.

When the ova of the cod have been in the hatching apparatus for some time, various organisms will be found to have attached themselves externally to the vitelline membrane covering the eggs. Monads, infusoria, and algæ avail themselves of the surface of the egg membrane as a nidus upon which to fix themselves, as shown in Fig. 34, where the most conspicuous of these protégés is a bell-animalcule of the genus *Vorticella*. The monads belong for the most part to the subdivision originally characterized by the late Prof. H. J. Clark, and known as the collared flagellates. There were a few free forms observed which were not identified, however. When these organisms become attached in considerable numbers to the eggs dirt tends to accumulate on their surfaces, giving the eggs a soiled, bad appearance. The most important of these adhering organisms is a one-celled algaous plant or protophyte, club-shaped, with its narrow end fixed to the egg; these are most numerous, and they contain brownish protoplasm (phycoxanthine) corpuscles which are adherent to the cell wall. Eggs kept in the liveliest motion were soonest covered with these unbidden guests. Apparatus in which there was least active movement of the water did not pollute the surface of the ova as quickly. It may be laid down as a rule that the more violent the motion of the eggs the sooner were they loaded with foreign organic growths, which no doubt has a tendency to interfere with the respiration of the embryo through the egg membrane, as well as to weight the egg so as to make it sink and smother. It is very probable that the accumulation of sediment and organisms upon the eggs may have had a great deal to do with the excessive mortality of the cod ova in the experiments at Wood's Holl. It caused least trouble in the apparatus used by Colonel MacDonald, operated by the gentle alternate rise and fall of the water by means of siphons acting intermittently.

On the twentieth day, with the water at 38° Fahr., the young fish

frees itself from the bondage of the vitelline membrane, but it has been known to hatch in thirteen days with the water at 45° Fahr., according to the observations of Mr. R. E. Earll. The time of hatching depends very much on the temperature of the sea water, according to the investigator just referred to, where he records the fact that it required as many as fifty days for the eggs to hatch with the water at 31° Fahr., or a little above the freezing point of salt water. Sars says the ova which he had caused to hatch came out in sixteen days. From what we know of the times of hatching of various species, it appears to be the rule that the greatest variation in the time of hatching is found to occur in cases where the spawning takes place with the water at a low temperature; the least variation, on the other hand, appears to occur with those species which spawn when the temperature of the water is comparatively high. An increase of temperature seems to disproportionately accelerate and abbreviate the rate and time of development, while a decrease appears to disproportionately retard and prolong the rate and time.

When the young codfish first leaves the egg membrane its tail remains crooked for some time, but soon straightens out, as noted by Sars and Earll. The embryo appears to rupture the egg membrane by spasmodic movements of the tail, which already exerts considerable force as it strikes the membrane, which tends to break open where the head lies, which is most frequently the extremity which first becomes free. This is the fact, too, with the mackerel and moonfish, but in all of these cases the tail is sometimes the first to become free. The buoyancy of the yolk will for some time tend to keep the young fish turned upon its back, but as soon as the tail has become straight they begin to right themselves.

Professor Sars has not recognized the presence of the germinal layer described by me, and his theory of impregnation is highly improbable, in the face of recent facts. Another point remains to be noticed, where he says, "One can discover, with the aid of a strong microscope, numerous oil bladders of different sizes, and scattered irregularly over the whole surface of the yolk." He alludes here to what I have denominated vesicles, since they are not stained by hæmatoxylin or carmine. As remarked in the introduction, they disappear entirely. It is certain that they coalesce, as he also observes. Are they protoplasmic corpuscles? If they are, they should stain; and if fragments of the germinal vesicle, they should be still more liable to be tinged by carmine; but they do not. Their disappearance and superficial position is positive proof that they are not oil spheres. I hold to my original interpretation, viz, that they have watery contents. To the Norwegian naturalist, however, belongs the credit of having called attention to the fact that certain fish ova float and develop at the surface of the water.

All of the species of floating ova yet investigated by the United States Fish Commission exhibit great mortality in the hatching apparatus, no

matter what may be the form of the latter. Buoyant eggs seem, in fact, to be much more sensitive to slightly unfavorable conditions than heavy or adhesive ova with thicker membranes. The floating eggs will not stand stagnant sea water for any great length of time, even at a temperature of 38°Fahr. Buoyant ova die in the latter eventually, just as certainly as heavy eggs in standing fresh water at 75°. A few ova to a large amount of water abstract the oxygen much less quickly than where the proportion of eggs is in excess of the water. The buoyancy of the cod's egg is undoubtedly due to the diminished specific gravity of the protoplasmic matter of the vitellus, and not to the presence of any oils. In this respect it represents a unique type of the buoyant ovum.

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## EXPLANATION OF THE REFERENCE LETTERS USED IN THE PLATES.

- A indicates the position of the embryo's head.
- a. auricle.
  - ab. air bladder.
  - al. allantois, median vesicle into which the segmental ducts open.
  - ar. dorsal aorta.
  - as. asterisk.
  - au. auditory canal.
- B. indicating the position of the tail of the embryo.
- ba. bulbus arteriosus.
  - bf. pectoral or breast fins.
  - bl. blastopore.
  - c. caudal vein.
  - co. pericardiac cavity.
  - ce. cerebellum.
  - ch. notochord or chorda dorsalis.
  - chs. chorda sheath.
  - c. pl. cell plate.
  - cv. first cerebral vesicle or neural sinus.
  - d. yolk or deutoplasm.
  - e. mesenteron or primitive gut.
  - ep. epiblast, cuticular layer mostly.
  - ff. primitive lateral fin-folds.
  - fb. cerebrum or fore-brain.
  - fc. choroidal fissure.
  - h. heart.
  - hy. hypoblast; hypophysis in Fig. E.
  - inf. infundibulum.
  - g. gills, and inferior cranial arches.
  - gd. germinal disk.
  - i. intestine.
  - io. intestinal constriction, pylorus. (†)
  - Kv. Kupffer's vesicle.
  - l. lens.
  - liv. or lv. liver.
  - m. mouth.
  - mo. medulla oblongata.
  - mc. medullary canal.
  - ms. medulla spinal; spinal cord.
  - mes. caudal mesoblast.
  - mi and mi<sup>1</sup>. micropyle.
  - mk. Meckel's cartilage.
  - mp. muscle-plate.
  - n. nucleus; neurula in Figs. E, F, H, I, J, K.
  - na. nasal pit.
  - no. neurenteric canal or strand of cells.
  - nf. nerve filament.
  - œ. œsophagus.
  - op. optic vesicles.
  - p. point where the dorsal aorta joins the caudal vein.
  - pp. polar protoplasmic prominence.



- pc.* post-cardiac membrane.
- pv.* muscle plates; protovertebræ.
- pn.* pineal gland.
- pr.* protoplasm; protolencite; germinal pellicle.
- prn.* nodose protoplasmic processes from the edge of the germinal disk.
- pnf.* pronephros.
- pi.* pigment cells.
- pst.* medullary plate.
- pf.* pectoral fold.
  - r.* rim of blastoderm.
- sv.* sinus venosus.
- s. ep.* sensory layer of epiblast.
- sh.* sensory hillocks or papillæ of lateral line.
- sg.* segmentation cavity.
- sc.* segmenting corpuscles †
- st.* subnotochordal strand of mesoblastic cells; in part the postanal gut.
  - s.* sagitta.
  - t.* tongue.
  - v.* vent.
- ve.* ventricle.
- vt.* vitelline membrane; egg capsule.
- vs.* vesicles imbedded in the germinal pellicle.
- w.* posterior wall of pericardiac cavity.
- y.* rudiment of air bladder and dorsal lobe of the liver.
- yb.* yolk blastopore.
- yc.* yolk canal.