A Contribution *to the* History *of the* Embryonic Development *of the* Teleosteans. By Edouard Van Beneden, Professor in the University of Liège. (With Plate IV.)

During my stay at Villafranca, in August and September, 1874, I had occasion to make some observations on the development of an osseous fish. I applied myself chiefly to the study of the segmentation and endeavoured to elucidate the much discussed question of the origin and mode of formation of the embryonic layers. I found daily, in the midst of the produce of my fishing with Müller's net small pearl-like bodies, colourless and perfectly transparent, floating freely near the surface of the water; the diameter of these little hyaline spheres scarcely exceeded that of a large Noctiluca, and it was only after I had examined them with a lens that I discovered that I had before me the embryos of an osseous fish.

Several times the fishermen brought me masses of a gelatinous appearance, taken on the surface of the sea and formed by hundreds or thousands of agglutinated eggs. These eggs presented all the characters of those of which mention has just been made; they had the same transparency, and the same composition. All the eggs in the same mass were always found to be in the same stage of development. This circumstance greatly facilitates the study of the successive phases of development. The eggs die very rapidly on the microscope-stage and consequently one cannot witness the progress of the development under one's eyes in one and the same egg. But since all the eggs of a given mass develope simultaneously, it is always possible to determine the time which has been necessary for the production of modifications which have arisen since the moment when the last eggs examined were detached from the agglomeration.

In all the masses of eggs which were brought to me the eggs were always in course of cleavage or else they contained very young embryos. I have never found embryos on the point of hatching nor even embryos provided with primordial vertebræ. On the other hand, I have never found in the free isolated condition eggs in course of segmentation nor even embryos young enough to be utilised in the study of the formation of the germ-layers.

It seems therefore probable that the agglutinated eggs come from the same fish as those which were taken isolated. It appears that the eggs laid in mass remain for some time adherent one to another and afterwards separate and then float free from all adhesion on the surface of the sea.

I ought, however, to add that having preserved some groups of eggs in jars in order to follow step by step the modifications which are produced, I never saw the eggs become detached from one another. But it is not possible to keep them indefinitely in a living state under these conditions. Although I took care to renew the water several times a day, I found it impossible to preserve my embryos alive for more than from twenty-four to thirty-six hours.

Although I had no intention, when I went to Villafranca, of occupying myself with the embryology of fishes and the desire of arriving at a solution of the various questions relative to the organisation and development of the Dicyemida left me but little leisure, yet I could not resist the temptation of utilising the beautiful material for study which I found placed in my hands. None of the difficulties which are usually met with in the study of the development of fishes present themselves in this case. The capsule of the eggs is very thin and of perfect transparency. The deutoplasm is constituted by an albuminoid globule which is perfectly homogeneous, hyaline, free from all granulation and limited by a very sharp and perfectly regular contour. In the protoplasm of the egg, whether of the germ or of the protoplasmic mantle which clothes a part of the vitelline globe, there exists neither fat-globule, nor vesicle, nor formed element of any kind; nothing, in a word, which one could confuse with a cell or a cell-nucleus.

Very similar pelagic eggs, belonging probably to a closely-allied species, were observed by Haeckel (1) during his last visit to the coast of Corsica. He has published his researches on the history of their development in the second part of his work 'Die Gastrula und die Eifurchung der Thiere.' He found the same eggs at Nice in 1876. Haeckel did not succeed any better than I have done in determining with precision the species to which the eggs which he had under observation are to be attributed. Basing it on the description given by Retzius of the eggs of *Gadus lota*, Haeckel puts forward the opinion that the fish, the development of which he studied, is a Gadoid allied to the Burbot, perhaps a *Motella*.

The eggs which have furnished me with the observations of which I am about to give an account present a very great resemblance to those studied by Haeckel. Found under the same conditions, at the same part of the Mediterranean coast, they have very nearly the same dimensions, the same appearance, and the same composition. At the period of deposition they are agglutinated in masses of various volume and form. The quantity of matter which holds them together is very small indeed, so that one cannot say of my eggs as Haeckel says of

his, that they are embedded in a gelatinous substance. It is not possible to isolate fragments of the cementing substance —scarcely possible to see it between the eggs.

My eggs have a diameter of 0·80 to 0·85 millimètres. They are colourless, and present the transparency of crystal. The membrane, which is very thin, shows neither canalicular pores nor punctation of any kind; it is homogeneous, of considerable resistency, and very elastic. It is very difficult to tear it without injuring the contained egg.

The youngest eggs which I had under observation showed the disc segmented into two spheres. They were brought to me one morning about seven o'clock. I received two other masses, the eggs of which were at the end of their segmentation; they had also been collected very early in the morning. It is probable that the deposition of the eggs takes place during the night or in the morning about sunrise. In all these eggs the form was that of an ellipsoid, nearly a sphere, the major axis being scarcely a sixth longer than the minor. At one of the extremities of the major axis (animal pole) is situated the germinal disc or germ. This rests on the vitelline globe, which has the same form as the egg itself, only the ellipsoid is truncated at one of the extremities of its major axis along a surface, concave at its centre, convex at its margin. From this results the formation of a polar chamber, limited externally by the membrane of the egg, internally by the vitelline globe. It is in this space that the segmented germ is lodged.

The vitelline globe is formed by a hyaline substance, which is perfectly homogeneous, colourless, little refringent, and devoid of all structure. It holds in suspension a single solitary structural element. This is a brilliant spherical mass, with very dark contour, and occupies constantly the same position in the globe. Excepting for this, the deutoplasm is absolutely devoid of any granulation, of any vesicle or element which could be mistaken for cell or nucleus. The substance which composes it is an albuminoid matter; it is coagulated by alcohol or osmic acid, and is rendered turbid by acetic acid. The refringent sphere held in suspension in the deutoplasmic globe is a drop of oil or of fat. It is coloured black by osmic acid, and dissolves in ether. Haeckel observed this same "Oelkügel" in the eggs which he studied, but the drop instead of being suspended in the vitelline globe occupied the vegetative pole of the egg, and was simply embedded in a spheroidal depression of the surface of the vitelline globe. This is a characteristic which separates the eggs studied by Haeckel from those observed by me. Owing to the circumstance that the specific gravity of the oily drop is less than that of the substances which compose the other parts of the egg, the

eggs studied by Haeckel took in the water the same position invariably. The animal pole was always directed downwards, the vegetative pole upwards. I ascertained that in my eggs the position of the oil-drop was quite constant. It is always placed eccentrically and invariably occupies a position in the vegetative hemisphere, but is immersed in the albuminoid substance which surrounds it on all sides. I have in vain endeavoured to explain to myself this fact by some peculiarity of structure in the deutoplasm. I entirely failed to discover any trace of filaments connecting the oil-drop either with the surface of the vitellus or with the germinal disc. Van Bambeke (2) has made out in the fecundated egg of the Tench, the presence of pseudopodia which penetrate the vitelline sphere radiating from the base of the germinal disc. These pseudopodia which are visible before the commencement of segmentation have the function of bringing up to the disc certain elements previously disseminated. Ransom (3) had already seen granular currents comparable to those to which Van Bambeke attributes the characters of pseudopodia. I have not observed anything comparable to these.

The difference between my eggs, then, and those which Haeckel had under observation, has reference (1) to their mode of aggregation. Haeckel says, "Diese Laich bildet kleine weiche Gallertklumpen in welche zahlreiche, kleine, vollkommen durchsichtige Eier eingebettet sind." I cannot say so much of my eggs, which adhered one to the other, but were certainly not embedded in a sort of jelly. (2) The eggs of Haeckel were spherical, and measured 0·64 to 0·65 mm. in diameter. (3) The position of the oil-drop constitutes a third differential character.

It seems to me, then, certain that we have not studied the eggs of the same species; but the differences are so trivial that I think one must ascribe the eggs studied by me and by Haeckel to allied species, if in any case the affinities of Teleosteans can be judged by the characters presented by their eggs.

As I have stated above, the youngest eggs which I observed had the germ segmented into two. I have represented one of these eggs in Plate IV, fig. 1. The segment spheres are convex externally, adherent one to another by a nearly plane surface, and terminated on the side in contiguity with the nutritive vitellus by a convex, well-marked line, which is, however, less obvious than the lines which mark their lateral limits. They are formed of a very clear and perfectly homogeneous protoplasm; it is not possible to discover in it any trace of a nucleus. The segment spheres do not rest immediately on the vitellus; they are separated from it by a layer of a substance which is finely

granular, but devoid of any globule. I took the very greatest pains to ascertain whether there exists in this layer any structure which might be considered as a cell-nucleus. Neither on examination of the fresh, living eggs, nor any more after having treated them with osmic acid and colouring matters (picro-carmine, Beale's carmine, hæmatoxylin), did I find any trace of anything of the kind. Acetic acid of 1 per cent. dilution gave me no better results. This layer forms for the deutoplasmic globe a continuous investment of such a sort that at no point do the segmentation spheres rest directly on the deutoplasm. It extends in every direction beyond the margin of the segmented disc, and everywhere is seen to be closely spread upon the vitellus. It is not easy to see the limit of this protoplasmic mantle, so fine does it become at its margin. It does not present the same thickness in every part. At the centre beneath the segmented germ it forms a biconvex lenticular body which occupies all the space between the vitelline globe, which is depressed at this point and the deep face of the segmentation spheres. In addition it presents a considerably increased thickness beneath the margins of the germ. This thickening forms all round the germ a circular wall, triangular in section. One side of the triangle is adjacent to a segmentation sphere; it faces upwards and inwards towards the animal pole of the egg; the second side is adherent to the nutritive vitellus, and concave. It faces downwards towards the vegetative pole of the egg. The third side is free; it faces directly outwards, and is slightly convex. It is separated from the membrane of the egg by a space filled with a clear and hyaline liquid. This layer is homologous, as I shall show further on, with that which Van Bambeke has described in the Roach by the name of *intermediate layer*. This name is appropriate on account of its interposition between the germ and the deutoplasmic globe, and has the great advantage of not in any way prejudging its morphological value. I shall then designate it by this name: I shall call the thickening which occurs at its centre beneath the germ the *median lens* (lentille); with Van Bambeke I shall call the circular thickening subjacent to the margins of the segmentation disc the *peripheral welt* (bourrelet).

I was able to see the successive phases of the segmentation by examining at small intervals new eggs removed from the mass which was brought to me at seven o'clock in the morning, and in which the eggs were at that hour provided with two segmentation spheres. About half-past eight all the eggs exhibited the disc segmented into four; so that at least an hour and a half, and probably more, must elapse between the appearance of the first groove and the moment when the two first spheres

divide in their turn. I have ascertained that the time which elapses between two successive phases of cleavage is shorter and shorter as the cells diminish in volume and in consequence of such diminution. I pointed out the same fact after having studied the segmentation of the egg of *Gammarus locusta*, and it is remarkably evident in the Rabbit. I will not delay over a detailed description of the segmentation. This phenomenon has been often described and figured, and, moreover, I was not able to study either the order or mode of appearance of the successive grooves with sufficient completeness to be able to add anything to what is already known. All my attention was concentrated on the *intermediate layer*, and I endeavoured to see as exactly as possible the modifications which it underwent during the earliest period of embryonic development. I have represented in the Plate two phases of the segmentation properly so called. Figure 2 shows the stage at which the development had arrived about eleven o'clock. At this moment I could not discover any trace of nuclei in the cells of the germ whilst still living; but on treating the eggs with osmic acid, then by weak alcohol, colouring them subsequently by means of picro-carmine, I was able to demonstrate the existence in each of the segmentation spheres of a fine spherical nucleus, homogeneous and devoid of nucleoli. When the eggs are allowed to die on the object-slide the protoplasm of the spheres becomes cloudy and finely granular, taking at the same time a slightly brownish tint. There appears then at the centre of each sphere a large clear spot, ill-defined and homogeneous in appearance. These spots are simply the nuclei. Acetic acid of 1 per cent. also renders them very obvious. At this phase of development a very sharp line separates the segmented germ from the "intermediate layer." The latter has retained precisely the same contour as that seen in the phase previously described. It shows clearly its median lens and its peripheral welt. I could not discover in any part of this layer the least trace of a nucleus. Neither during life nor after the death of the egg, when the intermediate layer shrinks somewhat and when nuclei make their appearance in the segmentation spheres, nor indeed by means of osmic acid, nor by picro-carmine, nor by acetic acid, nor by hematoxylin, could I succeed in causing nuclei to appear in this layer. I think myself, then, entitled to affirm that at this phase of the development of the egg there exists no trace of nucleus in the intermediate layer.

At five o'clock in the afternoon the eggs had arrived at the stage which I have represented in fig. 3. The cleavage-disc presents, taken as a whole, the form of a plano-convex lens; it

rests by its plane face on the intermediate layer with which it is seen to be everywhere in immediate contact. It is composed of polyhedric cells which are very clear and in each of which it is easy to distinguish, even in the living state, a large, spherical or slightly ellipsoidal nucleus. The cells are not, however, pressed one against the other in every part: here and there among them small spaces of varying form and dimensions can be observed. But neither at this stage of development nor at any moment during segmentation does there exist within the thickness of the disc any 'segmentation cavity.' It is well known that this cavity, discovered first of all by Lereboullet (4), in the Perch and the Pike, was re-discovered by Van Bambeke in the common Roach. The superficial cells are polyhedric as are the subjacent ones and also those which limit the blastodisc inferiorly. The cleavage mass or blastodisc does not therefore at this moment exhibit any trace of delamination. The only difference which the cells of the disc present is relative to their dimensions; the volume of the cells increases somewhat in passing from the surface to the deeper parts.

The intermediate layer has undergone important modifications. It is possible now to ascertain the existence, throughout the extent of this layer, of a great number of nuclei, generally oval, with very sharp contours and provided with one sometimes with two punctiform nucleoli. All these nuclei have very nearly the same dimensions, they are a little smaller than those of the cells of the blastodisc. If the focus of the microscope is arranged so as to give a surface-view of the deutoplasmic globe and if the region which immediately fringes the blastodisc be examined, a finely granular zone is distinguished there in which nuclei disposed regularly and at equal distances are scattered. It is impossible to make out any delimitation of cell-areas, but around each nucleus a small zone of a more granular character is seen in which a very manifest radial striation is apparent. The latter becomes much more obvious after the action of 1 per cent. solution of acetic acid. The perinuclear zones are separated one from another by clear spaces devoid of all granulation. These spaces together form a network in the meshes of which the nuclei with their radial haloes are disposed. It is clear that it is this part of the intermediate layer, namely, the part situated outside the blastodisc, which has been observed by Kupffer (5) in *Spinachia* and *Gasterosteus*, and which has received from this excellent observer the name of "Körnerzone" (nuclear zone). I have also been struck with the remarkable resemblance between the objects which I have just described and that cell-layer which appears in the Cephalopoda at the surface of the deutoplasm and is formed by the elements discovered and de-

scribed by Ray Lankester, from whom they have received the name of "autoplasts" (6).

In that part of the intermediate layer which extends beyond the blastodisc the nuclei form a single layer and are arranged with very great regularity. But if the focus of the microscope is so altered as to give an optical section of the egg, it is discovered that in the median lens and also in the peripheral welt the nuclei occur in various planes and that they appear to be disseminated without order in the protoplasm of the intermediate layer.

The simultaneous apparition of a great number of nuclei, surrounded each from the first with a granular radiated zone, in a layer which up to that moment had presented no trace of nuclear elements, can only be explained by admitting an endogenous generation of cells in the protoplasm. The regular grouping of the granules of the protoplasm around each of the nuclei as soon as they make their appearance indicates a subdivision of the protoplasm into so many cell-territories. It is not to be concluded from the fact that we cannot distinguish the limits of the cells, that there is no individualisation of the elements. The radial striation of the protoplasm around the nuclei proves that we have not here to do merely with a genesis of nuclei, but, in fact, with a formation of cells; nuclei and cell-bodies appear simultaneously.

I was unable to follow further the development of the eggs whose history I had watched from the morning onwards; the following morning most of them were dead. In others the blastodisc, considerably extended, covered in a great part of the deutoplasmic globe, and already exhibited the first traces of the embryonic rudiments in the widened portion of the marginal welt. A few hours later all my embryos had ceased to live, in spite of the care which I had taken to renew the water frequently in the course of the day.

But a short time before this, I had been led to study a stage of development very close to that which I have just described. Figures 4 and 5 represent an egg in this stage, one seen in optical section (fig. 4), the other seen from the surface (fig. 5). The blastodisc, a little more flattened than in the previous stage, is also more extended. It is in immediate contact throughout its inferior surface with the intermediate layer. There exists no trace whatever either of segmentation cavity or of germinal cavity (Keimhöhle).

The disc is formed of clear and nucleated polyhedric cells. The only important character in which it differs from the pre-

ceding stage consists in the differentiation of the superficial cells. These have become flattened, and form a sort of simple pavement epithelium, which limits the blastodisc externally. In section these cells appear lenticular, their external face is nearly plane, their deep face is regularly convex or presents facettes, by which these cells are moulded to the subjacent elements. The deepest cells present nothing particular.

The blastodisc has then divided itself by delamination into an enveloping lamella (Umhüllungshaut of Reichert (7), epidermoid layer of Vogt (8) and Lereboullet, Deckschicht of Götte (9), and a deeper mass, which is *the primitive external layer*.

The intermediate layer has not changed; the only peculiarity which I observed, was that in optical section, there may be distinguished, both in the peripheral welt and in the median lens, certain rounded cells which appear to be definitely individualised, though immersed in the protoplasm of the intermediate layer (fig. 7). I believe that these cells form the origin of a layer, which in the next stage will be found to have made its appearance between the blastodisc and the intermediate layer.

To the stage just described succeeds that represented in fig. 6. The eggs of this mass, which in the morning about ten o'clock presented the characters above indicated (figs. 4 and 5), had arrived in the afternoon about three o'clock at the stage which I am about to describe.

The blastodisc has extended considerably, and forms now a cap fitted to the truncated portion of the deutoplasmic globe, which has become slightly convex. The blastodisc has become flattened out and notably thinner. Between it and the intermediate layer has appeared an eccentrically placed cavity, and in the disc itself we can distinguish two regions: the one, central and thinner, forms the roof of the germinal cavity; the other, peripheral and thicker, forms a marginal welt to the blastodisc. This welt is in the form of a ring, broader at one side than the other, and thus brings about the eccentricity of the cavity.

The cellular constitution of the blastodisc has become notably modified, and first of all it is observable that the cells have multiplied and become much more voluminous. We may distinguish in the disc (assigning to it all which is placed out of the region of the intermediate layer, properly so-called):

1st. An enveloping lamella formed by a single layer of flat cells.

2nd. A layer constituted by polyhedric cells, pressed one against the other, clear and transparent, with a nearly homogeneous protoplasm and nuclei devoid of nucleoli. This layer forms the greater part of the blastodisc; it has its maximum

thickness in the broadest part of the marginal welt, so that in optical section it presents, taken altogether, the form of a comma, the head of the latter corresponding to the widened part of the peripheral thickening.

3rd. A layer formed by round cells, but little adherent to one another, finely granular, and provided with nuclei having punctiform nucleoli. This layer delimits the blastodisc inferiorly, but it does not exist throughout the area of the blastodisc; it is absent from the vault of the roof of the germinal cavity, or, at any rate, is only represented in this region by a few isolated cells. It is, on the other hand, fairly thick within the limits of the marginal welt of the blastodisc, which rests by its intermediation on the intermediate layer.

The intermediate layer has also undergone some modifications of importance. The median lenticular thickening no longer exists, but in its place are found on the floor of the germinal cavity certain cells which resemble in every respect the cells which line the inferior face of the blastodisc, and which form what we have called its third layer. It is manifest that the cells which rest on the floor of the germinal cavity are derived from the "median lens" of the intermediate layer; for we find by the side of completely isolated cells other cells, which, whilst projecting into the germinal cavity, are still partly implicated in the intermediate layer.

The peripheral welt has preserved very nearly the same condition as in the preceding phases, and its form is scarcely at all modified. Rounded cells, very like those which rest on the floor of the germinal cavity, are found embedded in the welt. Throughout the extent of the intermediate layer can be distinguished in contact with the deutoplasm a very regular range of flattened oval nuclei. The limits of the cells are too indistinct to justify one in saying that they form a simple pavement epithelium; but it appears as though an epithelial layer, formed by a single range of flat cells, were in the course of detaching itself from the deeper part of the intermediate layer. This epithelium rests directly on the surface of the deutoplasm.

Such is the series of the phases of this development, which I am able to describe with sufficient completeness to render it possible to draw positive conclusions from them The observations which I have made on the ulterior stages are too imperfect for publication. It now remains for me to compare the facts which I have above set forth with what is actually known relative to the formation of the germ-layers in Teleostean Fishes.

I. What comes out most strikingly in the first place from my

observations, with the fullest evidence, is this:—That the germ-layers do not proceed exclusively from the segmented germ, that part which at the conclusion of the cleavage-process forms what Lereboullet calls the *blastoderm*, and what I have denominated, with Haeckel, the *blastodisc*. A part of the germ-layers is derived directly from a protoplasmic layer which covers in the deuto-plasmic globe, and takes no part whatever in segmentation. It is this layer which Lereboullet designated under the name of "*membrane sousjacente au germe*," or at other times "*feuillet muqueux*." It was called "*vitelline membrane*" by Œllacher (10), "*Rinden-schicht*" by His (11), "*membrane intermediaire*" by Van Bambeke. It does not contain before the segmentation of the blastodisc any trace of cellular elements nor of nuclei; there develops in it, towards the conclusion of the segmentation period, by endogenous generation, a very large number of cells, and their mode of formation recalls in every respect the well-known phenomena of the formation of the blastoderm in Insects.

This intermediate layer was not recognised by Haeckel, which leads him to declare, "so können nur die Furchungszellen einzig und allein die Grundlage des enstehenden Fischkörpers bilden," and he attributes the formation of the endoderm to the invagination of the margin of the blastodisc. Is it possible to admit that this layer is absent in the eggs which Haeckel had under his eyes, and that the development of certain layers is brought about by invagination of the margin of the blastodisc in some Osseous Fish, and, on the other hand, in others at the expense of a special layer, which takes no part in the segmentation? I think not, when the identity of the constitution of the eggs is admitted, and I imagine that Haeckel himself will not feel tempted to adopt such a supposition. There are, moreover, several particulars in the drawings published by Haeckel which appear to me to prove that the intermediate layer existed in his eggs. In figures 55 and 56 of his pl. iv Haeckel figures the segmentation-spheres prolonging themselves outwards by means of a sort of tail applied to the surface of the albuminous globe (Eiweiss-kügel). If these figures are compared with figure 1 of my plate, it will be recognised that this tail corresponds to that part of the intermediate layer which extends outwards beyond the margin of the blastodisc.

This first conclusion from my observations gives support to the results arrived at and maintained in reference to various Osseous Fishes by Lereboullet, by Kupffer, by Owsjannikow (12), by Klein (13), by His, and by Van Bambeke.

I am, however very far from wishing to give any support to the famous "Parablast-theory" of Professor His, which is defended in so far as Osseous Fish are concerned by Owsjannikow.

The fundamental idea of this theory is that a part of the tissues of the embryo are not derived from the egg-cell but proceed from maternal cells, which immigrate into the vitellus and are destined to give rise to connective and vascular tissues. The embryo on this theory would be related to the maternal organism by a double connection; through its archiblastic tissues derived from the egg-cell it would be descended from parental archiblastic tissues; through its parablastic tissues it would be derived directly from the mother's connective and vascular tissues. Not only has this theory not been demonstrated by Mr. His, but the idea appears to me to be in formal contradiction to the facts which are the outcome of comparative embryology. But it is equally erroneously that Rathke (14), von Baer (15), Stricker (16), Reineck (17), Weil (18), and Œllacher have maintained that the various germ-layers are formed in the Osseous Fishes by a process of delamination at the expense of that part of the egg alone which takes part in the cleavage process. Whilst I cannot admit the view adopted by Götte and Haeckel—according to which the internal layer is formed by invagination of the margin of the blastodisc, and is accordingly derived like the external layer from the segmented disc—it by no means follows that I should deny that the phenomenon of invagination does take place in the Teleosteans. The following is the mode in which it seems to me necessary to interpret the phenomena which characterise the commencement of development. Directly after fœcundation the egg of the Osseous Fish divides into two very unequal cells, very dissimilar, differing in constitution and significance; the one is the germ which segments and from which the blastodisc is derived; the other is formed by the deutoplasmic globe—clothed, at least partially, by a thin layer of protoplasm forming "the intermediate layer." This cell is the origin of the endodermic layer of the future embryo; it has a constitution analogous to that of a fat-cell. This never proceeds to segmentation, but there appears in it at the conclusion of the segmentation of the other cell (the germ) a great lot of cells, which take their rise by "free-cell formation." Hence results the apparition of a cellular layer subjacent to the blastodisc. The latter is the homologue of the ectoderm of other Vertebrates, the former is the homologue of the endoderm. It is ascertained that the blastodisc extends its area little by little and tends to cover in by "epiboly"—the deutoplasmic globe. The observations of Van Bambeke have rendered it very probable that the intermediate layer makes a simultaneous progressive movement. The upshot of this is that the development of the Teleosteans commences by the total cleavage of the vitellus into two cells; one of the two continues to segment whilst the other

retains its characters and remains undivided. The fact of a greater indisposition to segment on the part of the cells destined to give rise to the endoderm is very frequent, not only among the Vertebrates but also in the other divisions of the animal kingdom. It is exhibited always in the case of the formation of a gastrula by epiboly ; and, indeed, it is after this mode that the development of the gastrula of the Teleosteans is carried out. The " discogastrula" has, then, no existence as the result of a special mode of gastrulation ; " discoidal segmentation" differs in no essential respect from the "inequal segmentation"(Inequale Fürchung) of Haeckel.

An objection to these views may perhaps be made on the score of the peculiar mode of formation of the cells of the intermediate layer, which do not result from segmentation. I may, in reply, call to mind that Strasburger (19) has demonstrated that the endogenous generation of cells is not a primordial form of cell-generation but a secondary mode derived from division pure and simple. It is connected to division by a complete series of intermediate forms. The simultaneous formation by the endogenous mode of a great number of cells in a single cell is an abbreviation or condensation of successive divisions which have become less and less complete. It is clear that the formation of the blastoderm of Insects must be interpreted in this manner, as well as the existence in the group of the Arthropods, chiefly among the Crustacea, of a series of transitional forms. This view receives in the present case a new application ; the formation of the endoderm in the Osseous Fish has the same relation to the development of the layers by regular segmentation, such as is presented, for instance, by the Acrania, by the Cyclostoma and Batrachia, which the formation of the blastoderm in insects has to the total cleavage observed in many Crustacea.

II. On comparing my observations on the constitution and development of the intermediate layer with those of my predecessors I find that Kupffer is the only one who has recognised and described in Osseous Fishes the development of cells on the surface of the deutoplasmic globe, beyond the area of the segmented germ. The "nuclear zone" of Kupffer, observed in the genera *Gasteroseus* and *Spinachia* evidently corresponds to that part of my intermediate layer which lies outside the blastodisc. The part of this layer situated under the blastodisc corresponds to the "*membrane formed of vitelline granules*," to the "*membrane underlying the blastoderm*," or to the "*mucous layer*" of Lereboullet. I find in it the essential characters attributed by Van Bambeke to his intermediate layer. The peripheral welt, triangular in section, on which rests the margin of the blastodisc, was perfectly described by Van Bambeke. As to the

"median lens" of my eggs, it appears to be absent in other Osseous Fishes. It even appears, according to some observers, that only the peripheral welt exists at first, and that secondarily by growth from the periphery towards the centre the intermediate layer becomes complete beneath the blastodisc.

Kupffer has put forward the opinion that his "nuclear zone" is something quite distinct from the "feuillet muqueux" of Lereboullet. It is clear from my observations that the two authors have had under their observations simply different parts of one and the same layer, the extent and characters of which vary in all probability from one genus to another. It is this same layer which Œllacher described under the name of 'vitelline' membrane. This excellent observer has been unfortunate in the choice of a name for the layer and has been led into error in assuming that the cells which he found therein at the conclusion of segmentation are cells derived from the blastoderm, having fallen from the roof of the germinal cavity.

Klein not only recognised this same layer in the Trout, but he demonstrated that cell-nuclei appear in it in great quantity towards the end of the period of segmentation. He gives to this layer the name of parablast. His, who has seen the same objects in the Salmon, gives to this intermediate layer the name of *Rindenschicht*, the peripheral welt is called by him *Keimwall*, and the cells observed therein at the conclusion of cleavage are designated by him *Parablastisch* or *Nebenkeim Zellen*.

III. At no period of development have I found any trace of that cavity which Van Bambeke calls the "*cavité de segmentation*." Kupffer, who was not able to demonstrate any such cavity in living eggs, found it in eggs of *Gobius niger*, which had been previously hardened in a solution of sulphuric acid. Lereboullet found, when he made the eggs of the Perch and the Pike coagulate, that the blastoderm, is at a given moment, "a hollow vesicle." Van Bambeke himself could only persuade himself of the existence of his "segmentation cavity" by the examination of sections cut from hardened eggs. This cavity has not been asserted to exist in the Trout by Reineck, nor by Weil, nor by Stricker, nor by Klein, nor by Œllacher, nor by His. No more did Haeckel find any trace of it. I may then venture to affirm, on the testimony of these various observers and on the strength of my own researches, that the presence of a cavity in the substance of the blastodisc is not a constant fact; even more than this, no author has been able to prove its existence in a living egg; it is therefore possible that in the cases where it has been asserted to exist it is an artificial product. The absence of any regular disposition of the cleavage spheres

around this cavity tends to confirm this view, as Kupffer has pointed out. I am anxious to insist, moreover, upon the fact that the name of "segmentation cavity" given to this cavity is not at all appropriate to it.

The segmentation cavity, as known in Amphioxus, the Cyclostomes, the Batrachians, and a host of invertebrates, is invariably situated between the ectoderm and the endoderm. It is limited on one side by the concavity of the ectoderm, on the other by that part of the primitive vesicle which will, after its invagination is accomplished, constitute the endoderm. If we once admit that the intermediate layer of Osseous Fishes is the homologue of the endoderm of the other Metazoa, it is clear that the cavity which develops between the blastodisc and the intermediate layer alone deserves this name. This cavity, which existed in my eggs, and which the majority of embryologists have observed in Teleosteans, is generally designated by the name of germinal cavity (Keimhöhle). It is indispensable to modify our terminology in accordance with the preceding observations. I propose, then, to designate under the name of *cavity of Lereboullet* the cavity pointed out by this author in the midst of the blastodisc in the Perch and the Pike, by Van Bambeke in the Roach, and by Kupffer in *Gobius niger*. Balfour (20) has discovered a homologous cavity in the Elasmobranchs.

It is necessary, on the other hand, to give the name of *cavity of von Baer*, *segmentation cavity*, or *blastocœl*, to the space which appears at the conclusion of egg-cleavage, between the blastodisc (ectoderm) and the intermediate layer (endoderm). It is this cavity which was described in the Trout by Stricker, by Reineck, by Weil, by Œllacher, by Klein, by His, and by Götte, and which has been called sometimes *Fürchungshöhle*, sometimes *Keimhöhle*. In common with Œllacher I consider this cavity as the homologue of the germinal cavity of the chick. It is for this reason that I think the name of Keimhöhle may be retained equally with the other names for this cavity. The existence of this cavity appears doubtful in the Roach and in the genera Gasterosteus and Spinachia, if we may judge by the observations of Van Bambeke and Kupffer.

IV. A final question which I wish to enquire into relates to the ultimate destination of the two primordial layers of the embryo of osseous fishes—the blastoderm on the one hand, and the intermediate layer on the other. At the phase represented in figure 6 of my plate the embryo is composed, if we except the enveloping lamella, of three cellular layers well defined in the marginal welt. The external one, limited by the enveloping lamella, is evidently derived from the blastodisc; the internal

one is only the intermediate layer, in the deepest part of which the cells are disposed as a simple pavement epithelium. Between the two exists an incomplete layer of cells which are very different. This layer is absent from the vault of the roof of the segmentation cavity, and its thickness diminishes progressively from without inwards. It is evidently this layer which Haeckel considered to be formed by the invagination of the margin of the blastodisc and as representing the endoderm. What is the origin of this layer? Is it derived from the blastodisc, or, on the other hand, is it composed of cells derived from the intermediate layer? I believe that it is composed of cells which originate in the peripheral welt of the intermediate layer. I base this opinion on the following considerations :

1st. The cells of this layer have the same dimensions, the same form, the granular texture, the same oval nuclei, sometimes irregular and always nucleolated, which we observe in the cells found in the substance of the peripheral welt, and which are, without doubt, of endodermic origin. They are, on the other hand, very different from the cells of the blastodisc.

2ndly. The cells which rest on the floor of the segmentation cavity, and which are formed at the expense of the "median lens" of the intermediate layer, possess the same characters as those which form the middle layer.

3rdly. The blastodisc remains all this time very sharply delimited inferiorly, and in no part is there a passage from one to the other. In no part have I found the slightest indication in favour of invagination.

According to my view, the intermediate layer not only furnishes the epithelium of the digestive canal, but it intervenes largely in the formation of the middle layer, to which it probably furnishes the connective and vascular elements. In the phase represented in fig. 6 we must then distinguish—

1st. The enveloping lamella.

2nd. An ectodermic layer derived from the blastodisc, and destined to subdivide subsequently into sensorial lamella and external mid layer (first and second secondary blastodermic layers of Haeckel).

3rd. Of an internal mid layer, of endodermic origin, destined to furnish the vessels and the connective tissues (third secondary blastodermic layer of Haeckel).

4th. Of an internal layer, destined to furnish new cells to the internal mid layer and to give rise to the epithelium of the digestive tube (fourth secondary layer of Haeckel).

Bibliography.

1. *E. Haeckel.* Die Gastrula und die Eifurchung der Thiere.

2. *Van Bambeke.* Recherches sur l'embryologie des poissons osseux. Mém. Cour. de l'Acad. Roy. de Belgique, tom. xl.

3. *H. Ransom.* Observations on the Ovum of Osseous Fishes. Philosophical Transactions, vol. 157, 1867.

4. *Lereboullet.* Recherches d'embryologie comparée sur la développement du Brochet de la Perche et de l'Ecrevisse. Ann. Sc. Nat., 4e ser., t. i, 1854.

5. *Kupffer.* Beobachtungen über die Entwickelung der Knochenfische. M. Schultze, Archiv f. Mikr. Anat., Bd. iv, 1868.

6. *Ray Lankester.* Observations on the Development of the Cephalopoda. Quart. Journ. of Micr. Science, vol. xv, New Ser., 1875.

7. *Reichert.* Müller's Archiv, 1857.

8. *C. Vogt.* Embryologie des Saumons, 1842.

9. *Götte.* Beiträge zur Entwickelungsgeschichte der Wirbelthiere. Archiv f. Mikr. Anat., Bd. ix, 1873.

10. *Œllacher.* Beiträge zur Entwickelungsgeschichte der Knochenfische. Zeitsch. f. Wiss. Zool., Bd. xxii.

11. *W. His.* Untersuchungen über das Ei und die Entwickelung bei Knochenfischen, 1873.

Untersuchungen über die Entwickelung von Knochenfischen, besonders über diejenige des Salmons. Zeitsch. für Anat. und Entwick., 1tes Bd., 1876.

12. *Owsjannikow.* Ueber die ersten Vorgänge der Entwickelung in den Eiern des Coregonus larvatus. Bull Ac. Imp. de St. Petersbourg, t. xix.

13. *Klein.* Observations on the early Development of the Common Trout (*Salmo fario*). Quart. J. of Micros. Sc., New Ser., No. lxii, 1876.

14. *Rathke.* Bildungs und Entwickelungsgeschichte der Blennius viviparus. Abhand. zur Entwick., Bd. ii, 1833.

15. *K. E. von Bär.* Untersuchungen über die Entwickelungsgeschichte der Fische, 1839.

16. *Stricker.* Untersuchungen über die Entwickelung der Bachforellen. Wiener Sitzungsber., Bd. li, 1869.

17. *Reineck.* Ueber die Schichtung des Forellenkeims: Max Schultze's Archiv, Bd. v, 1869.

18. *Weil.* Beiträge zur Kenntniss der Entwickelungsgeschichte der Knochenfische. Wiener Sitzungsber., Bd. lxv, 1872.

19. *Strasburger.* Ueber Zellbildung und Zelltheilung, Jena, 1876.

20. *Balfour.* On the Development of Elasmobranch Fishes. Journal of Anat. and Physiology, vol. x.

EXPLANATION OF PLATE IV,

Illustrating Prof. Ed. Van Beneden's Memoir on the Development of Osseous Fish.

FIG. 1.—Egg of Gadoid fish, observed at Villafranca at seven a.m., showing the blastodisc divided into two enucleate spheres—the granular "intermediate layer" and the homogeneous "deutoplasmic globe" with eccentric oil-drop.

FIG. 2.—A similar egg later in the day (eleven o'clock). The blastodisc consists of many large nucleated cells; the intermediate layer is still granular.

FIG. 3.—Optical section of a similar egg at a still later hour of the same day (five o'clock). The superficial cells of the blastodisc are hexagonal. In the intermediate layer are seen numerous "free-formed" oval nuclei.

FIG. 4.—Egg from the same mass on the following day, in optical section.

FIG. 5.—The same egg focussed, so as to give a surface view of the intermediate layer with its free-formed nuclei and the radiating granular striæ around them.

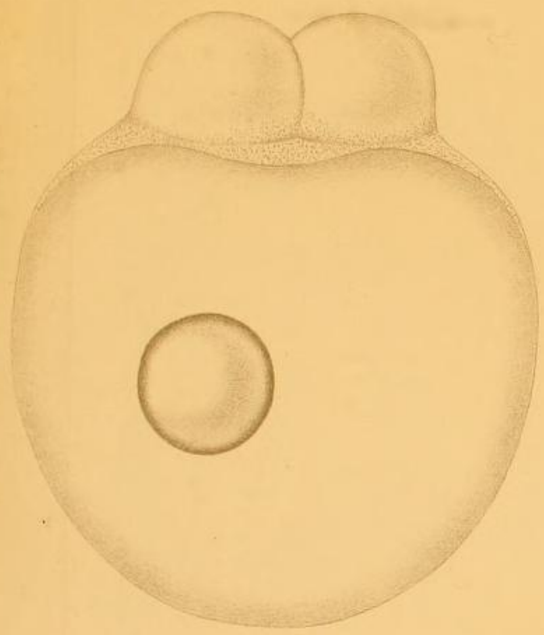
FIG. 6.—Optical section of a considerably later stage, showing the blastodisc, subjacent layer of large cells, peripheral welt of the intermediate layer and cells on the floor and on the roof of the segmentation cavity.

FIG. 7.—A portion of the egg drawn in fig. 4, showing the flattening form of the enveloping cells of the blastodisc, and the complete delimitation of cells in the intermediate layer together with the presence of free oval nuclei.

FIG. 8.—A portion of the intermediate layer as seen in fig. 5, in order to show the radiating striæ around the nuclei and the presence of nucleoli.

FIG. 9.—A portion of the view given in fig. 6 more highly magnified, showing on the one hand the similarity of the cells of the layer subjacent to the blastodisc to those of the intermediate layer, from which they are probably derived; and on the other hand the very different character of the cells of the blastodisc.

FIG. 10.—A portion of the floor of the segmentation cavity of fig. 6, showing the pinching off of cells.



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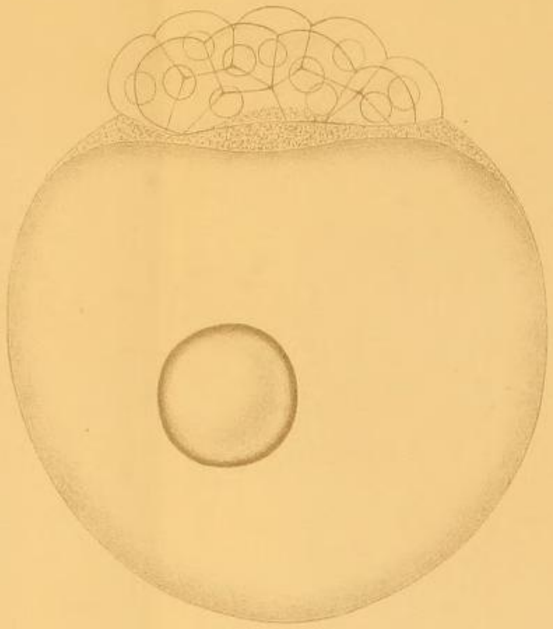

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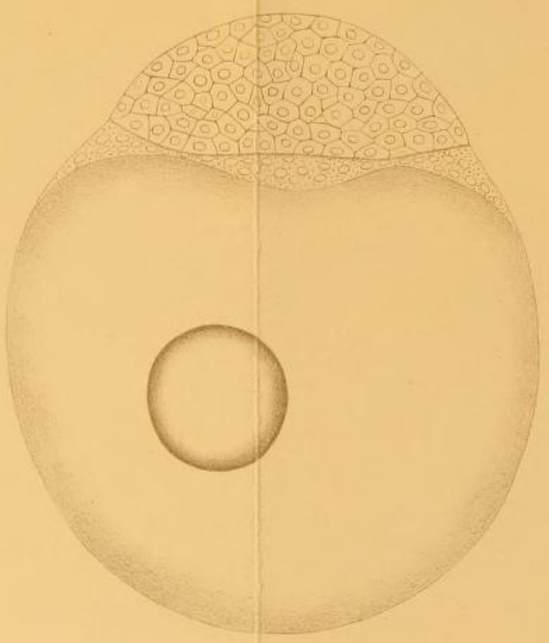

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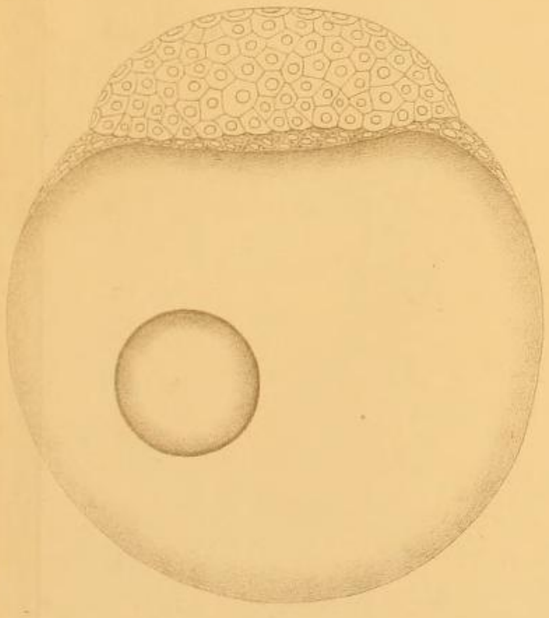

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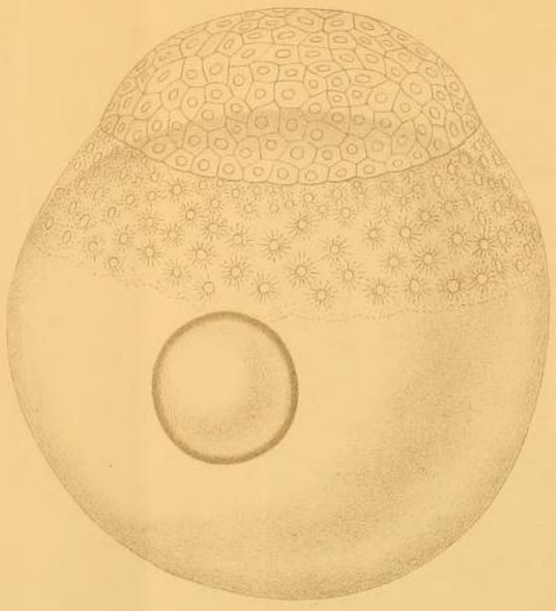

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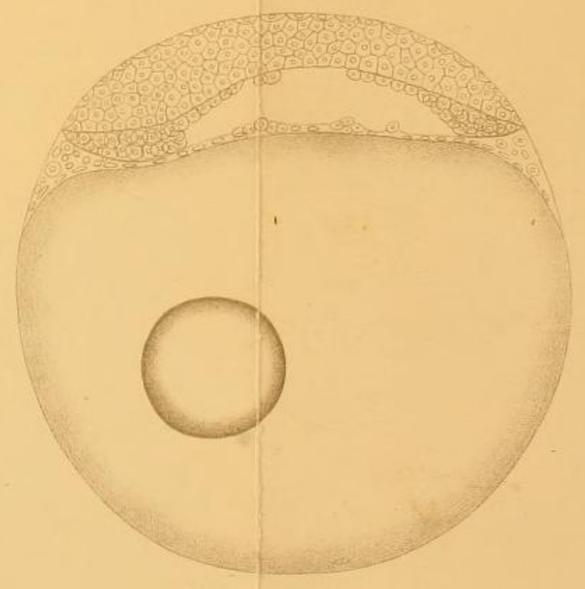

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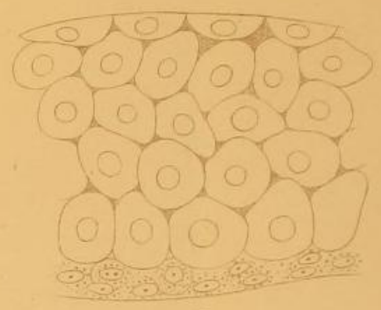

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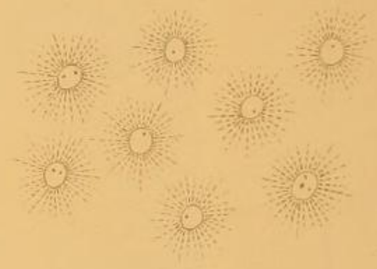

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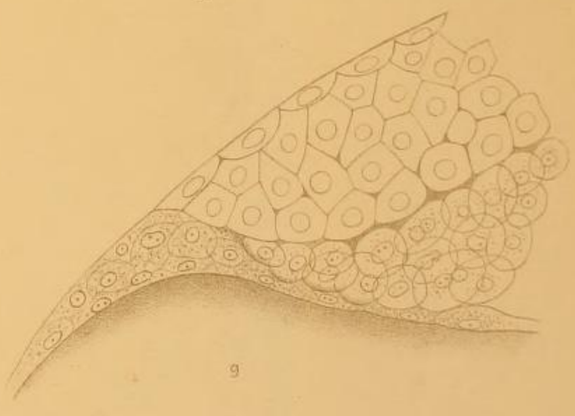

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