Q L

959

S8
1960

YF006169

UC-NRLF

5C 36 735

KUPFFER'S VESICLE

AND ITS

RELATION TO GASTRULATION AND CONCRESCENCE.

FRANCIS BERTODY SUMNER.

[Reprinted from Memoirs New York Academy of Sciences, Volume II, Part II, 1900.]

Submitted in partial fulfillment of the requirements for
the Degree of Doctor of Philosophy in the Faculty of
Pure Science, Columbia University

EXCHANGE

EX LIBRIS

Zoology Dept.

BIOLOGY
LIBRARY
G

NEW YORK ACADEMY OF SCIENCES.

MEMOIRS, VOLUME II, PART II, 1900.

ARTICLE III.

KUPFFER'S VESICLE

AND ITS

RELATION TO GASTRULATION AND CONCRESCENCE.

FRANCIS BERTODY SUMNER.

TEXT FIGURES 1-34.

UNIV. OF
CALIFORNIA

NEW YORK :

PUBLISHED BY THE ACADEMY.

Submitted in partial fulfillment of the requirements for
the Degree of Doctor of Philosophy in the Faculty of
Pure Science, Columbia University

58
1900
BIOLOGY
LIBRARY
G

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

Main Lib.
Zool Dept.

UNIV. OF
CALIFORNIA

FRANCIS BERTODY SUMNER

BORN AUGUST 1, 1874.

MINNEAPOLIS ACADEMY, 1887-1890.

UNIVERSITY OF MINNESOTA, B. S., 1894.

Scholar in Animal Biology, 1893-1894.

State Natural History Survey, 1892-1893.

University Fellow, 1894.

COLUMBIA UNIVERSITY, 1895--1901.

University Scholar, 1897-1898.

John D. Jones Scholar, 1898.

Senff Zoological Expedition to Egypt, 1899.

University Fellow, 1899, (resigned.)

University Table at Naples Zoological Station, 1898, 1899, 1900

Tutor in Natural History in the College of the City of New
York, 1899.

Member of New York Academy of Sciences.

X. NOTE ON METHODS.....	78
XI. SUPPLEMENT.....	79
BIBLIOGRAPHY.....	80

QZ 107
58
1900
BIOLOGY
LIBRARY
G

KUPFFER'S VESICLE AND ITS RELATION TO GASTRULATION AND CONCRESCENCE.

FRANCIS BERTODY SUMNER.

(Read February 12, 1900.)

[Text figures 1-34]

CONTENTS.

	PAGE
INTRODUCTION	48
I. THE TELEOST GASTRULA.....	48
Götte's view	48
A new factor.....	50
II. KUPFFER'S VESICLE	52
The prevailing view.....	52
Relation of Kupffer's vesicle to the "Prostomal thickening".....	53
Kowalewski's account of Kupffer's vesicle.....	53
Conclusive evidence of the preceding view.....	54
Previous observations of a similar nature.....	55
Condition in <i>Amia</i>	57
III. RELATION OF PROSTOMA TO GERM LAYERS.....	59
Prostomal thickening and gut-hypoblast	59
The relation of the gut epithelium to the notochord.....	60
Historical.....	61
IV. SIGNIFICANCE OF PROSTOMAL INVAGINATION.....	62
V. CONCRESCENCE AND CONFLUENCE.....	68
Problem stated.....	68
Experiments of Morgan and Kopsch.....	70
Conclusions.....	72
VI. FURTHER MORPHOLOGICAL CONSIDERATIONS.....	73
VII. YOLK VESICLES.....	74
VIII. THE FUNCTION OF KUPFFER'S VESICLE.....	76
IX. RECAPITULATION	77
X. NOTE ON METHODS.....	78
XI. SUPPLEMENT.....	79
BIBLIOGRAPHY.....	80

INTRODUCTION.

This paper is the outcome of observations carried on during the past few years in the Zoölogical Laboratory of Columbia University, in the Marine Biological Laboratory at Wood's Holl, in the John D. Jones Laboratory at Cold Spring Harbor,¹ in the Stazione Zoologica at Naples, and finally in the Department of Natural History in the College of the City of New York. I owe much to the help of those in charge of each of the laboratories named, being especially grateful to Professor BASHFORD DEAN for his constant interest in my work, his ever-ready assistance, and his many valuable suggestions. I also take pleasure in acknowledging the generous aid given me by Professor ULRIC DAHLGREN, of Princeton University, to whom I owe my success in obtaining the eggs of *Noturus*; and in expressing my appreciation of the help and advice given me by Doctor PAUL MAYER at Naples, as well as of the valuable material procured through the efforts of Doctor LO BIANCO. The eggs of *Salvelinus* I owe to the kindness of Mr. CHARLES WALTERS, of the New York State Fish Hatchery at Cold Spring Harbor, and to the courtesy of the U. S. Fish Commission.

I. THE TELEOST GASTRULA.

Götte's View.—The account of gastrula formation in the teleost advanced by GÖTTE ('73—his main idea had been briefly presented four years before) has been accepted by the majority of investigators in this field. Although it is now a commonplace to embryologists, I shall introduce this discussion by a brief statement of GÖTTE's view, since it is my object to prove that this view must be amended in one very important particular.

FIGURE 1.

Figures 1 to 4 (A and B) illustrate the orthodox account of gastrula formation. They show the teleost blastoderm, in surface view and in section, at four different

¹ As John D. Jones Scholar of Columbia University.

stages of development. **Figure 1** represents the early segmented germ-disc. In the stage shown in **figure 2** the blastoderm has increased in area but diminished in thickness, and a thin superficial layer of cells ("Deckschicht," GÖTTE) has become differentiated. In the stage shown in **figure 3** an important change has taken place in the relative thickness of the parts. The central region has become thinner, both relatively and absolutely, while the marginal region is now thicker. The natural

FIGURE 2.

inference is that a part of the central cells have migrated peripherad, and this is the inference drawn by GÖTTE. This thickened marginal region is called by GÖTTE "Randwulst" (von Baer). It is seen in the figure that the superficial layer ("Deckschicht") extends a trifle beyond the cells of the marginal wall and that its border rests directly on the yolk, leaving a small space, triangular in section, around the margin. Finally in **figure 4**, gastrulation is shown in progress. From the marginal wall a projecting ledge of cells is growing inward over the yolk. This process begins on one side of the blastoderm (the future posterior end of the embryo)

FIGURE 3.

but soon extends to the whole circumference, although remaining most conspicuous on the embryonic side. **Figure 4, A**, represents a blastoderm of this stage as seen in surface view. The invaginate layer ("Secundäre Keimschicht," GÖTTE) is seen to take the form of a diaphragm with an excentric and not quite circular aperture. The "Deckschicht," as before, ends freely on the yolk. **Figure 4, B**, is a section cut in the plane of the dotted line in **A**.

As to the mechanics of the process, GÖTTE maintained that the same peripheral movement of the cells which produced the "Randwulst" produced an involution of the margin to form the "Secundäre Keimschicht," and led to the growth of the latter centrad.

Although other writers have held, and probably with truth, that at least in some cases, the secondary layer is formed wholly or in part by *delamination* from the germ-wall, nearly all embryologists are agreed that the end result is as shown in **figure 4**. A circular sheet of cells, inflected at its margin, and covered by a pavement layer which does not share in this inflection—such is their teleost gastrula. According to this view the germ-ring or inflected layer represents the entire hypoblast and mesoblast of the embryo.

A New Factor.—One object of the present paper is to show that the above conception of gastrulation in the teleost, although in large degree true, fails to

FIGURE 4.

recognize a highly important factor. The fish gastrula presents a far more complex structure than the scheme of Götte contemplated.

More than a year ago, in a paper before the American Morphological Society (December 28, 1898) the writer described for the cat-fish blastoderm a pronounced thickening of the pavement layer ("Deckschicht") on the embryonic (posterior) margin of the blastoderm. Although stating that this structure was of constant occurrence, I was at that time unable to offer an opinion as to its significance. I have later devoted a great deal of attention to this question and have discovered this same problematic cell-mass in a considerable number of fishes belonging to widely different families. In all these cases, the origin and fate of this cell-mass appears to be the same. It is perhaps most readily observed in the trout; but although this fish has long been a classical object for embryological study, the appearances which I am about to describe have been noticed by only one previous writer (Berent, '96).¹

¹ BERENT'S observations were quite incomplete and his interpretations entirely incorrect. Even GREGORY ('99) the latest of those who have discussed the early development of the trout, has missed the point as completely as any of his predecessors.

Figure 5 represents a sagittal section through an early blastoderm of the brook trout (*Salvelinus fontinalis*),¹ absolute age unknown. One of the most obvious and striking facts is the condition of the pavement layer at the posterior (embryonic) margin. Its cells, instead of being thin and flattened as elsewhere, are here elongated in a vertical direction. The surface of the blastoderm at this point is notice-

FIGURE 5.

After camera lucida drawing. Pr, thickening of pavement layer at posterior border.

ably indented, and the arrangement of the cells with reference to the indentation is such as to strongly suggest an invagination occurring here. The line separating the "**Prostomal Thickening**," as I shall henceforth call this proliferation of the superficial layer, and the underlying cells of the blastoderm is not at all clear in this

FIGURE 6.

Sagittal section (after camera lucida drawing)—showing condition in *Salvelinus* blastoderm considerably more advanced than that shown in Figure 5.

section. The deeper stain of the superficial cells nearly disappears as we pass inward from the surface.

At a somewhat later period (a day or less older) the germ-ring is well established. It will now be seen (**figure 6**) that the "prostomal" mass of cells is continued forward into a thin layer underlying the cells of the germ-ring proper.

¹ It cannot be objected that the difference may be due to my having studied a different species of trout, since, as we shall see, the condition described seems to be a universal one among the *Teleostei*.

II. KUPFFER'S VESICLE.

The Prevailing View.—In the present paper I cannot enter into a complete historical review of this subject. KUPFFER's own conception of this structure we shall consider later. In the meantime, I shall state briefly the accounts given by AGASSIZ and WHITMAN ('84) who first satisfactorily described the condition in the pelagic type of egg and by HENNEGUY ('88) who was a pioneer worker in this line upon the trout. AGASSIZ and WHITMAN observed in the pelagic egg of *Ctenolabrus*, a number of small vacuoles, appearing in the periblast beneath this region of the embryo. These vacuoles soon united into one (so much had already been observed by KINGSLEY and CONN, '83) and above it the cells of the embryo began to arrange themselves as a columnar epithelium which arched over this cavity. In the case of the trout embryo, HENNEGUY states that the formation of the vesicle is preceded by

FIGURE 7.

Section showing fully formed Kupffer's Vesicle in the trout (after Henneguy).

FIGURE 8.

Embryo of *Ctenolabrus*, showing fully formed Kupffer's Vesicle. *Kv*, Kupffer's Vesicle, *GHy*, gut-hypoblast.

a radial arrangement of certain cells in the posterior undifferentiated region of the embryo. A cavity then forms in the midst of these cells, thus giving rise to the vesicle.

Figure 8 is drawn from one of my own preparations of *Ctenolabrus*. AGASSIZ and WHITMAN give no satisfactory figures, although their description is clear. **Figure 7** is taken from HENNEGUY's paper on the trout. Both of these figures represent the vesicle in its fully developed condition. They differ in that the vesicle of the trout is from the first completely bounded by cells, while in the pelagic egg the cellular wall is not complete. But the accounts agree in representing the wall of the vesicle as differentiating *in situ* from a homogeneous mass of cells without reference to any preëxisting (cellular) structure.

It is now well established that both types of Kupffer's Vesicle as above figured actually occur: the question as to which type is more primitive will be considered later. Both of the above accounts, although true as far as they go, are incomplete

in so far as they neglect the first steps in the formation of this interesting structure and leave out of consideration certain phenomena of high theoretical importance.

Relation of Kupffer's Vesicle to the "Prostomal Thickening."—To return to *Salvelinus*—at a stage considerably later than that shown in figure 6, we have the condition represented in figure 9, which, like the preceding figures, is drawn from a median longitudinal section of the embryo. In the anterior portion of the section there are three layers, not counting the overlying pavement layer, viz. :—dorsally, the neural axis, ventrally the single layer of gut-hypoblast, and between the two, the disc-shaped cells on the notochord. The neural and chordal portions lose their identity caudad in a homogeneous cell-mass. The ventral layer is continued backward into one or both walls of Kupffer's Vesicle. The condition caudad to the vesicle is less clear. It seems certain, however, that at this stage there is no distinct ventral layer extending to the posterior margin.

The position of this vesicle strongly suggests that it bears some relation to the mass of cells which we have discussed under the designation of "prostomal thickening." Its walls are one or both continued cephalad into the thin layer of gut hypoblast underlying the chorda. Moreover, this prostomal cell-mass, unless it be represented by the walls of the vesicle, has quite lost its identity. The most conclusive evidence I shall defer, however, until I have entered into a brief historical discussion. Let us consider whether the structure herein termed "prostomal thickening" has been previously observed.

Kowalewski's Account of Kupffer's Vesicle.—M. VON KOWALEWSKI described in *Carassius* a mass of cells, triangular in section, lying at the posterior margin of the embryo and bounded by the pavement layer, the marginal wall and the periblast. Except at this point, the pavement layer spanned a narrow space and ended freely on the yolk as above represented. In his first paper (KOWALEWSKI '85) he considered this cell-mass to be the rudiment of the whole entoderm, considering the cells of the germ-ring to be purely mesodermal in their fate (he asserted this same view in his next paper, '86, a). Later ('86, b) he modified his view to the effect that it represented only that part of the entoderm forming the walls of Kupffer's Vesicle. The latter, according to KOWALEWSKI, was a part of the archenteron, and the cord of cells which he figures (figure 10, B) as extending backward from below the vesicle

FIGURE 9.

Posterior end of sagittal section of brook trout embryo (camera lucida drawing). *Kv*, Kupffer's Vesicle; *N*, neural axis; *Ch*, notochord; *GHy*, gut-hypoblast.

was the solid rudiment of the neurenteric canal. The radial arrangement in this problematic cell-mass at an earlier stage (**figure 10, A**) suggested to him an invagination, and he considers this to be the equivalent of the "prostoma" of KUPFFER, differing from it only in that the latter opened directly to the surface instead of being covered by the superficial layer. Kupffer's Vesicle represents, according to

FIGURE 10.

A. Sagittal section through posterior border of blastoderm of *Carassius* (after Kowalewski) showing supposed invagination occurring at this point.

B. Section showing Kupffer's Vesicle in *Carassius* (after Kowalewski).

KOWALEWSKI, a small but important part of the "Gastruladarm," the rest of which is represented by the uncovered part of the yolk.

It is surprising, in view of this brief and accurate description presented as long ago as 1886, that the prostomal thickening has been all but ignored by subsequent investigators. Both HENNEGUY ('88) and VIRCHOW ('95) have seen cells in this position, but do not regard them as being of constant occurrence nor of any theoretical importance. (Concerning BERENT, see below.)

Conclusive Evidence of the Preceding View.—

The present writer first noticed the prostomal mass of cells in the blastoderm of *Amiurus* (**figure 11**), regarding it at the time as a mere thickening of the superficial layer. Upon following out the subsequent course of development and comparing with the condition in other forms, I was led to a view of its significance quite similar to that expressed in KOWALEWSKI's later paper, even before I had seen the latter. The presence of the nick or indentation often found

FIGURE 11.

Section showing thickening of superficial layer (*Pr*) on posterior margin of early *Amiurus* blastoderm.

in this cell-mass, and the grouping of its cells (**figures 5 and 10, A**) have been noted by KOWALEWSKI and offered by him as an evidence of invagination. Another appearance, which I noticed in an embryo of *Amiurus* at the time of the formation of Kupffer's Vesicle (**figure 12**) pointed to an obvious relation between this vesicle and the supposed invagination. In a paper before the New York Academy of Sciences ('99, b) I advocated a view similar to that of KOWALEWSKI. As then stated, my opinion was somewhat different from that presented in the present paper.

The chain of evidence was not complete, however, until a form had been found

which showed an open invagination in place of the solid ingrowth. The hypothetical primitive condition was shown, with diagrammatic distinctness, in the egg of an unknown eel (*Muraena?*), which it was my good fortune to secure, while at Naples, during the summer of 1899.¹

Here, as in the preceding forms, the process is initiated by a thickening and indentation of the superficial layer on the embryonic side of the blastoderm margin (figure 13). The next ensuing stage is represented in figure 14, which demands little explanation. **Figure 13** represents the condition when the blastopore is still large, while in the next stage only a slender yolk-plug remains. The Vesicle of Kupffer has meanwhile attained a considerable size, so that it is readily visible in the living egg. In the latter there is also to be seen a fine canal connecting the vesicle with the exterior, through the blastopore, and passing just in front of the yolk-plug. This condition is quite enduring, lasting for perhaps an hour after the appearance of the vesicle to view in the living egg. Sagittal sections removed any possible doubt as to the meaning of this appearance. Here, as in the forms

FIGURE 12.

Posterior end of very early *Amiurus* embryo (cam. luc.) showing interesting relation between superficial layer of the epiblast and the vesicle.

FIGURE 13.

Sagittal section through blastopore region of early *Muraena?* embryo (cam. luc.).

previously discussed, there is a direct continuity between the invaginated cell-layer and the lowermost layer of the embryo.

Previous Observations of a Similar Nature.—It is interesting to note that this manner of formation of Kupffer's Vesicle through an invagination from above is identical with that described by KUPFFER himself in 1879. The latter author came to this conclusion from the study of the living eggs of *Osmerus* (see figure 15)

¹In a paper read before the last session of the American Morphological Society (New Haven, December 27, 1899), I described this case, and referred to the egg as being that of *Muraena*. I have later learned from Dr. LoBianco that he is uncertain of the genus.

and the pike (later also of the trout).¹ KUPFFER says that he confirmed these observations by sections, though none are figured. Curiously enough KUPFFER's statements on this subject have been discredited by the majority of later investigators, though they have been confirmed by a few. Professor DOHRN informs me that he years ago noted the open connection in the case of the perch but had never pub-

FIGURE 14.

Contiguous sections of *Muræna?* embryo-cut as above—somewhat later than preceding (camera lucida drawings). *Pr*, "prostoma"; *Yp*, yolk-plug; *Kv*, Kupffer's Vesicle; *GHy*, gut-hypoblast; *NeHy*, "non-embryonic" hypoblast; *Ec*, cells embedded in periblast.

lished this observation. HENNEGUY ('88) noticed this condition in the case of the same fish but later concluded that he had been in error. He did not deny, however, the possible accuracy of Kupffer's observation. RAFFAELLE ('88) observed in the living egg of *Uranoscopus* and in an unknown pelagic egg that Kupffer's Vesicle was for a brief period connected with the exterior through the blastopore. This

FIGURE 15.

Optical section of egg of *Osmerus* (after Kupffer) showing "prostoma."

connecting passage he regarded as equivalent to the neurenteric canal. MCINTOSH and PRINCE ('90) also speak of "what seems to be a tubular connection of the external blastopore and the ventral surface of the embryo" but cannot be sure of an open passage into Kupffer's Vesicle. As none of these authors sectioned the eggs, we do not know whether the canals described had cellular walls or merely lay in the periblast. The "linear canal" mentioned by AGASSIZ and WHITMAN ('84) was evidently merely the disappearing blastopore.

There is then every reason to believe that the open invagination occurring in *Muræna?* and probably certain other fishes is represented by the ingrowing cell-mass found by Kowalewski in *Carassius* and *Gobius* and by myself in *Amiurus*, *Noturus*, *Salvelinus*, *Fundulus* and *Ctenolabrus*. The presumption is that the solid condition is the more frequent among the teleosts. This masked form of invagina-

¹ The vesicle was first described by KUPFFER in 1866 in the embryos of *Gasterosteus* and *Gobius*. Its origin by invagination was not at that time maintained. SOBOTTA ('98) states that COSTE had figured the vesicle in 1847. LEREBOLLETT also ('63, figure 22) represents what appears to be Kupffer's Vesicle.

tion is quite characteristic of the bony fishes, as witness the neural axis and auditory and optic vesicles. The later appearance of the cavity of Kupffer's Vesicle is strictly comparable with the delayed formation of the neural canal or the cavities of the sensory vesicles.

It may be objected that the sort of Kupffer's Vesicle, described for *Ctenolabrus* and other pelagic eggs, in which a cellular floor is wanting, is not reducible to the above type. But as already stated, I find in *Ctenolabrus* a typical prostomal thickening, and I cannot regard the fact that these cells do not completely surround the cavity as of any importance to the theory. I shall later give reasons for believing that this condition is a retrograde one.

Condition in *Amia*.—Now is this condition in the teleosts a unique one or do we find a counterpart in any other group? DEAN has strongly maintained that the key to teleost development is to be found among the ganoids, and from the generally accepted phylogenetic relation of the two groups, this proposition seems self-evident.

It was DEAN's endeavor (DEAN, '95, '96) to show that the ganoids, in their mode of development held a middle position between the elasmobranchs, on the one hand, and the teleosts on the other. My own observations as far as they go, sustain this view. As regards the special subject of the present paper, DEAN described in the eggs of *Acipenser*, *Lepidosteus* and *Amia*, what he considered to be the homologue of Kupffer's Vesicle in the teleosts. This was a cavity beneath and slightly anterior to the dorsal lip of the blastopore, bounded above and behind by the cells of the latter, below by the yolk. DEAN maintained that this cavity simply represented the angle formed by the blastoderm margin as it was mechanically turned in upon itself during its circumrescence of the yolk. This simple mechanical explanation I cannot accept for the teleosts because (among other reasons) the vesicle in some fishes is not formed until the blastoderm has nearly or quite finished its journey over the yolk (*Muraena*? *Perca*) and thus the supposed

FIGURE 16.

Blastopore region of *Amia* cut in sagittal plane (slightly schematized from camera lucida drawing). Yc, yolk cells; Sy, yolk syncytium; Pr, "prostoma"; Sl, superficial layer of epiblast.

mechanical cause no longer exists. But I believe that the homology proposed by DEAN is well founded. Through Professor DEAN's kindness, I have had the privilege of studying some fine *Amia* material, and have found therein the counterpart of the phenomena just described for the teleosts.

Figure 16 shows a longitudinal (nearly or quite sagittal) section through the egg of *Amia* at a time when the yolk-cells are perhaps three-quarters covered. The superficial layer of the epiblast is seen to be much thicker at the blastopore margin than elsewhere and to be directly continuous, around the margin, with the innermost germ-layer. This condition occurs upon the entire periphery, but on the dorsal (embryonic) side of the blastopore the case is somewhat more complicated. Here we find an arrangement strictly comparable to that found in **figure 13**. In both there is an enormous development of the pavement layer at the caudal end of

Blastopore region of *Amia* after closure (drawn as preceding).
Bp, blastopore; *Ne Hy*, "non-embryonic" hypoblast; *GHy*,
 gut-hypoblast; *Kv*, probable homologue of Kupffer's Vesicle;
Sy, rudimentary syncytium.

the embryo and an indentation of its surface around which the cells are arranged in radial manner. In one respect, however, this *Amia* embryo exhibits a more advanced condition than that of the *Muraena?* embryo shown in **figure 13**. The notch in the pavement layer is seen to be continued cephalad into a very noticeable cleft, extending forward for some distance into the hypoblast and separating it into two layers.

Again the similarity between **figures 17 and 14** is evident. The chief difference between the two seems to be that in the former there is wanting the open canal, connecting the cavity of the vesicle with the exterior which occurs in the latter. This open canal is wanting even in embryos slightly younger than that shown in **figure 17**, but the obliteration of its lumen is surely a matter of secondary importance. The ganoidean homologue of Kupffer's Vesicle, like that of many teleosts, has a ventral wall. This is well developed, though not quite complete, consisting of a layer of pavement-like cells, lying directly upon the large yolk cells. This condition has not been mentioned by either DEAN ('96) or SOBOTTA ('96).

III. RELATION OF PROSTOMA TO GERM LAYERS.

Prostomal Thickening and Gut-Hypoblast.—The exact part played by the prostomal cells in the formation of the germ-layers is very difficult to determine and in spite of a painstaking study upon a great many embryos, I cannot regard my observations on this point as quite conclusive. As already shown (**figures 6 and 13**) in sagittal sections at a certain period, a direct continuity is observable between what I have called the "prostomal thickening" and a thin layer of cells which evidently represents the gut-hypoblast. This continuity is strong evidence that the latter has been derived by proliferation from the former. In the trout the prostomal invagination commences before the formation of the germ ring. In *Muræna*? on the other hand, at a time when the blastoderm has covered nearly one-half the circumference of the yolk, neither prostomal invagination nor gut-hypoblast are to be seen, although the germ-ring is well advanced. At the next stage which I have sectioned (**figure 13**) the gut-hypoblast is seen to be in direct continuity with the invaginated cells, which agree with the former in showing a higher staining power than the rest of the blastoderm.

Transverse sections of these same stages unfortunately do not show these conditions with the same degree of clearness.¹ The lowermost cells do not exhibit the distinct epithelial arrangement seen in longitudinal sections. In fact what appears in the latter as a continuous ventral layer, extending beneath the entire embryonic region of the blastoderm, is only to be observed with distinctness in those *cross*-sections which pass through the extreme posterior end. It is clearly marked in several blastoderms which I have cut (**figure 18**). What becomes of this layer in the cephalad portion is not revealed in transverse sections of embryos at such an early stage. At a later period when the gut-hypoblast has finally become distinctly differentiated throughout the entire region of the embryo, it is found that a conspicuous break in its continuity occurs along a considerable extent of the axial line. The gut-hypoblast now occurs in two lateral sheets, each sheet being merged axiad into the undifferentiated cord of cells which form the "primitive streak" (**figure 19**).

The question at once arises—was the lowermost germ-layer, at the time of its first

¹ I have transverse sections of *Noturus*, *Amiurus* and *Salvelinus* embryos during this period, but unfortunately, none of *Muræna*?

FIGURE 18.

Transverse section passing through posterior end of a very early trout embryo (somewhat later than that in figure 6). *PrHy*, hypoblast invaginated at "prostoma."

appearance, a single continuous sheet, which later fused along the axial line with the overlying cells; or was it differentiated from the overlying cell-layer, retaining, however, its original continuity with the latter along the axial line; or finally, has the course of events been complicated by some process which we have left out of consideration? So far, my study of sections has not afforded a final answer to

FIGURE 19.

Transverse section through "primitive streak" region of a *Noturus* embryo of about the stage represented in Figure 22.

this question. In longitudinal sections, such as those shown in figures 6 and 13, I cannot find any evidence of a break in the continuity of this layer across the median line. Since, however, the layer is not perfectly distinct at all points, I cannot feel sure of this fact. As stated above, transverse sections have so far failed to clear up the matter.

The relation of the gut epithelium to the notochord is also an interesting problem. For some time after the chorda has separated from the neural axis above and the mesoblastic plates on each side, there persists a continuity between it and the gut-hypoblast. Does this continuity reveal the actual method of chorda formation in teleost ontogeny, or is the union a secondary one? Also, what is the meaning of the suggestive union occurring at certain points between the mesoblastic plates and the hypoblast? (See figure 20.) Clearer light may be thrown upon some of these points in the ensuing discussion of the meaning of the "prostoma."

Before leaving this topic it is well to emphasize, however, that a part, though at present an uncertain part, of the embryonic hypoblast is derived from that collection of cells which I have called the prostomal thickening. Whether or not this mode of origin is supplemented by differentiation from the overlying "secondary layer" of the germ-ring, I cannot definitely say.

There is certainly one region of the blastoderm where the hypoblast originates quite independently of any connection with the prostomal invagination, namely, the "non-embryonic" part of the germ-ring. Shortly before the blastopore closes,

FIGURE 20.

Section passing through posterior half of embryo of *Noturus*, somewhat in front of Kupffer's Vesicle (cam. inc.). The relations of gut-hypoblast to mesoblast and chorda are suggestive.

that part of the blastoderm forming its now posterior margin (in *Noturus* and *Muraena*?) is seen to be differentiated into all three germ-layers (see **figure 14**). VIRCHOW ('95) describes this condition in the trout and I ('99, a) have already recorded it for *Noturus*.

Historical.—With the exception of the earlier investigators, who derived the entoderm from the periblast, nearly all writers on fish embryology have thought this germ-layer to arise as a differentiation from the inflected layer of the germ-ring. The cells of the latter were held to separate sooner or later into two layers, the lower of which was the entoderm, the upper the mesoderm. The only writers, as far as I know, who have maintained the existence of a distinct entoderm rudiment are KOWALEWSKI (see above), BERENT ('96), and REINHARD ('98).

BERENT maintains that the entoderm arises as a separate rudiment and has figured it with approximate correctness in one stage (**figure 21, B**). But his interpretation of the process is certainly wrong. The accompanying **figure 21, A** from

FIGURE 21.

After Berent—illustrating his view of entoderm formation.

BERENT is probably based upon an observation, though an inaccurate one, of the earlier appearance of this same group of cells. He holds the condition described by H. V. WILSON ('91) for the sea-bass (*i. e.*, a uniform differentiation of the under surface of the whole embryonic germ-ring) to be the more primitive, and considers the condition he finds in the trout to be a derived one. My objections to BERENT's conclusions are two: *first*, that the rapidly developing pelagic egg of the sea-bass would be far more apt to exhibit a *precocious* development of any part than the slowly developing egg of the trout, and *second*, that the account offered by WILSON is undoubtedly incomplete. While I have never studied the egg of the sea-bass, I have carefully sectioned the appropriate stages of the quite similar egg of *Ctenolabrus*, and find that here, as in the eggs of so many widely different fishes, the gut-hypoblast is plainly formed in connection with a prostomal ingrowth. Unlike KOWALEWSKI, BERENT has missed the true meaning of the process. REINHARD regards the entire hypoblast as derived by proliferation from the walls of Kupffer's Vesicle. The cells of the latter arise, he claims, from the periblast.

IV. SIGNIFICANCE OF THE PROSTOMAL INVAGINATION.

I now purpose to offer an interpretation of the foregoing phenomena and to point out their relation to phases in the development of other vertebrates.

My theory in large degree reverts to that proposed by KUPFFER in 1884. (Practically formulated in 1879—see KUPFFER, '79.) KUPFFER's observations, already discussed, led him to a novel view of the development of the fish embryo. It was his endeavor to make a complete comparison between the conditions found in the *Teleostei* and that found in the *Amniota*. KUPFFER was the first to point out the dorsal invagination in the reptilian blastoderm, and he considered that in this he had found the homologue of the gastrula mouth or blastopore of

FIGURE 22.

Early blastoderm of *Noturus*. Gr, germ-ring; Pr, "prostoma."

FIGURE 23.

Early embryo of *Noturus*, before appearance of caudal knob. The bifid caudal end of the embryo is seen to embrace a thin cellular sheet as in case of *Muraena*? (see figure 27).

Amphioxus. The yolk-filled aperture of the blastoderm margin had nothing to do with blastopore proper. For this aperture he invented the name of "BLASTOTREMA." He considered the process by which the blastoderm covered the yolk as in no sense a process of gastrulation but as a process of blastosphere formation, thinking that the completed blastosphere would surround the yolk on all sides. KUPFFER sought for and found a homologue of the reptilian prostoma in teleosts. This invagination, he says, at first + shaped in surface view, greatly elongates in a forward direction, resulting in the formation of a longitudinal groove which he calls the "primitive groove," extending along the axis of the embryo. He differs in his interpretation of the primitive groove and streak from BALFOUR, who, as well known, considered these structures to be homologous with the seam along with the blastoderm edges united behind the embryo in the elasmobranch (figure 29, A, Bl). Thus, according to BALFOUR ('78 and '81) the primitive streak is posterior

to the neurenteric canal, while KUPFFER held it to be anterior to the latter. The real representative of the "Raphe" or line of union of the blastoderm edges of the shark, KUPFFER found in the so-called "Caudal Knob" (Randhugel) appearing at an early period at the posterior end of the trout embryo. This comparison was based upon the supposition that the "prostoma," or blastopore of the meroblastic vertebrates, was originally situated upon the blastoderm margin as in the shark, and that its more anterior position in *Teleosts* and *Amniota* had been due to a removal from its primitive situation. It is not plain, however, whether or not KUPFFER regarded this change of position as occurring in the *ontogeny* of the teleosts.

This original invagination, occurring immediately in front of the caudal knob, resulted in the formation of the much discussed vesicle, which represented that part of the archenteron forming the allantois of the amniota.

There seems to be no doubt now that the "primitive groove" seen by KUPFFER in the trout was nothing more than the medullary furrow. KUPFFER apparently made little use of sections and he does not give a single figure of one illustrating this point. But his main ideas, namely, that there occurs in the teleosts a dorsal invagination comparable to that in the reptile, and that this invagination has been removed from a primitive marginal position, are fully borne out by the present investigations. I differ from KUPFFER, however, in regarding the "prostoma" as representing only a limited part of the blastopore, the remainder being constituted by the entire blastoderm margin (KUPFFER's "Blastotrema"). Again I find strong evidence that this detachment of the "prostoma" from the margin occurs in *ontogeny* in the teleost as well as in the elasmobranch, a point left uncertain by KUPFFER.

If such a process occurs, it must on theory take place some time before the appearance of Kupffer's Vesicle, since this represents the expanded inner end of the "prostoma." **Figure 22** shows the condition of a *Noturus* blastoderm at this period. The indentation of the blastoderm margin and its relation to the future axis of the embryo recall at once the figures, given us by Duval ('84) of the early chick blastoderm. These will be referred to later. **Figure 23** represents a somewhat more advanced stage and demands no further explanation. **Figure 24** shows the appearance of an embryo in which Kupffer's Vesicle has formed. The indented posterior margin has now given place to a rounded projection, a condition which we should expect to exist after the final union of the sides of the indentation, in other words, after the detachment of the prostoma from the yolk blastopore.

FIGURE 24.

Noturus embryo after appearance of caudal knob (Ck).

The emarginate condition is also well shown in the transparent living egg of *Scorpena*. It is true that **figure 25** shows a shallow bay rather than sharp nick, but the conspicuous shingle-like overlapping of the cells along the whole posterior margin speaks strongly for a process of concrescence. This appearance of the blastoderm margin recalls the descriptions of RYDER ('86) for *Elacate* and of LOCY ('95) for *Squalus*, and it seems quite possible that the structures which were by those writers given a segmental value find their true explanation here. In **figure 26** the emarginate condition has for some time ceased to exist and Kupffer's Vesicle has come into view at a considerable distance from the margin. Behind this the caudal knob projects into the yolk blastopore.

In the egg of *Muraena*? which, as above described, exhibits the prostoma in its least modified form, there is also the strongest evidence of this view of its formation.

FIGURE 25.

Living egg of *Scorpena*. *Pr*, emarginate region at posterior end (not often as deeply concave as in figure). Arrangement of cells on either side strongly suggests concrescence.

FIGURE 26.

Scorpena embryo after caudal knob and Kupffer's Vesicle have appeared to view.

Figure 27 shows a deep bay extending forward from the blastopore into the posterior end of the embryo. It is evident from transparent view that the yolk is not, however, exposed in this bay as in the rest of the blastopore (compare with condition in *Noturus*, shown in **figure 23**). The section (**figure 28**) verifies this opinion. It is at once seen that at this point the invagination to form the hypoblast (as above discussed) is occurring.

In this connection it is significant to note that there seems to be a certain relation between the time of appearance of the caudal knob and the age at which Kupffer's Vesicle is formed. In the embryo of the trout, the caudal knob appears at a period when the blastoderm occupies but a small part of the upper hemisphere of the egg. The definitive Kupffer's Vesicle is formed some time before the equator has been passed. In *Noturus*, the caudal knob is not developed until about one-half

of the yolk has been covered, and Kupffer's Vesicle appears somewhat later; in *Scorpena*, the relative time of formation of both of the structures is still later, at least four-fifths of the yolk being covered; while in *Muraena?* the vesicle does not appear to view until the blastopore is nearly closed, and the caudal end of the embryo is conspicuously bifid up to the time when the blastopore is very small. Of course this line of argument is open to the reply that the time of appearance of both these structures is conditioned by the general rate of development of the embryo in any particular case and that the coincidence stated does not prove any necessary relationship between the two.

As might be expected, an indentation of the posterior end of the embryo has been already noted by several investigators. AGASSIZ and WHITMAN ('84) once or twice observed this condition in living pelagic eggs and regarded it as strong evidence for the formation of the embryo by concrescence. HENNEGUY ('88) noted its

FIGURE 27.

The posterior end of embryo of *Muraena?* just prior to closure of blastopore. Yp, yolk-plug; Pr, "prostoma"; X-Y, plane of section shown in FIGURE 28.

FIGURE 28.

Oblique section of preceding embryo.

existence in a single egg and from this concluded that concrescence occurred in the fish embryo in a very limited region, *i. e.*, enough to form the caudal knob, although not giving to this process any such interpretation as we have had under discussion. M'INTOSH and PRINCE ('90) speak of a "terminal bay" in certain pelagic eggs. EYCLESHYMER ('95) notes that this indented condition has been already observed in *Amiurus* by Miss O'GRADY, of Vassar. JABLONOWSKI ('98) describes the artificial production of a similar condition in the *Salmonidæ*, by the use of salt solution.

Reference might also be made to the *Mesodidymi* and *Hemididymi* of various authors, although I do not consider it necessary to discuss them here.

Thus the prostoma of the teleost, like the neurenteric canal of the shark, represents a specialized portion of the blastopore which has become detached from the remainder by a process of concrescence or union of the blastopore lips. **Figure 29, A and B,** illustrate this comparison. It will be seen that the homology suggested

by KUPFFER between the caudal knob (**Ck**) of the telcost and line of fusion of the blastoderm edges behind the embryo of the shark is in principle true, though not quite exact. The caudal knob of the teleost represents, rather, the embryonic tail-end of the shark, enclosing the neurenteric canal. (This is the view maintained by SCHWARTZ '89, though I did not know it when the foregoing words were written. Concerning KOPSCH, see below.) The posterior line of fusion (**Bl**) has, strictly speaking, no counterpart in the telcost, inasmuch as the embryo retains its connection with the blastoderm margin.

H. VIRCHOW ('95) proposes a view of the teleost embryo similar in some respects to those of KUPFFER and myself. He considers the caudal knob to result from a process of folding similar to that in the elasmobranch. In the latter, he says, all three of the germ layers are folded off and the tail projects freely, while in the trout the folding process affects merely the ento- and mesoderm, the ectoderm taking no

FIGURE 29.

Schematic figures of **A**, early elasmobranch (after Balfour), and **B**, teleost embryo illustrating the author's view of the formation of the teleost embryo. *Mg*, medullary groove; *Ne*, neurenteric canal; *Bl*, line of fusion of edges of blastoderm behind the latter; *Yk*, yolk.

part in the process. This ventral folding off of the entoderm results in the formation of Kupffer's Vesicle which is thus, in its origin, like any other part of the gut. But as VIRCHOW does not recognize the presence of the "prostoma," nor the part played by concrescence, his account is incomplete. He attributes a similar view to OELLACHER, though I do not recall the latter's statement. The views of KOPSCH and JABLONOWSKI will be considered below.

If the theory I have advocated is correct, it is evident that the development of the teleost egg differs far less from that of the other meroblastic vertebrate eggs than has usually been held. In all of these, it seems probable that the originally simple blastopore, consisting of the whole exposed surface of the yolk, has been separated by a process of concrescence into an anterior, embryonic portion and a posterior, non-embryonic portion. For the bird's egg, the case has been convincingly presented by DUVAL ('84). The primitive streak, although appearing to originate at some distance

from the blastoderm margin, has been shown by him to arise in connection with the latter, in fact, to arise from a portion of the latter.¹ In the egg of the reptile, the connection of the plate of cells in which invagination occurs with the blastoderm has not yet been established, but we cannot doubt that even here the invaginate part has, in phylogeny at least, been detached from a primitive position on the margin. An interesting parallel occurs between the fate of the prostomal cavity (Kupffer's Vesicle) in the teleost and that of the invaginate cavity in the reptile. It has been shown that the latter breaks through at its inner extremity, thus becoming connected with a large sub-germinal cavity. Two regions of the archenteron, at first separate, are thus brought into union. Now SOBOTTA ('98)—at least I so interpret Sobotta's statement—and GREGORY ('99) have described a union, at a certain period, between the cavity of Kupffer's Vesicle and the lumen of the gut in front of it. This parallel was called to my attention by Professor MINOT.

FIGURE 30.

A, B and C. Showing three stages in the formation of the embryo of the toad-fish. (After Miss Clapp.)

This dichotomizing of the blastopore is, in the elasmobranchs and the sauropsida, very evidently due to the great relative amount of the yolk, which renders the epibolic growth of the blastoderm a slow process, thus making it necessary for the form of the embryo to be established long before the close of gastrulation. In the case of the teleost, the yolk as a rule is far smaller, so that the yolk blastopore is able to close at a period when the embryo is much less advanced. In this group, accordingly, we find the caudad growth of the embryo to take place at a rate about equal to that of the blastoderm margin. Consequently, although the folding-off of the tail end occurs here, as in the shark, resulting in the detachment of a portion of the blastopore, the embryo retains throughout its connection with the border of the blastoderm. That this is the true explanation of the difference in the position in the two groups is shown by certain exceptional teleosts where this marginal position of the embryo

¹ It is true that the experiments of ASSHETON ('96) have not supported Duval's theory. [Since writing the foregoing, I have fully confirmed such of Assheton's results as go to prove that the primitive streak of the bird does not arise from the blastoderm border in ontogeny. This in no way disproves, however, that it so arose in *phylogeny*, and I believe that there still remain strong reasons for such a view.—September 21, 1900.]

is lost. In the toad-fish (*Batrachus*) according to Miss CLAPP ('91) the caudal end of the embryo parts company with the margin of the blastoderm at a relatively early period (**figure 30, A, B, C**). EYCLESYMER reports a similar condition for *Lophius*, though his description and figures do not bear out such a contention. The latter author infers from certain appearances that the same process occurs in *Amiurus* as well. In this, EYCLESYMER is certainly wrong. The embryo of this fish invariably retains its connection with the germ-ring until the complete closure of the yolk blastopore. JHERING, also, ('88) has described what seems to be a similar condition for the South American cat-fish *Arius*. The eggs of *Batrachus*, *Lophius* and *Arius* are all extraordinarily large, the latter being as much as 18 mm. in diameter. Again, CORNING ('96) reports this separation of the caudal end of the embryo from the germ-ring to exceptionally occur at a relatively late period in the salmon.

It will be recalled that *Amia* was included among the forms exhibiting the "pro-stoma," although it is now certain that the egg of *Amia* is a holoblastic one. However this may be, the egg of this ganoid is probably *secondarily* holoblastic, for it shows other evidences than the one considered of having been derived from a meroblastic egg. For instance, my discovery in *Amia* (SUMNER, '00, a) of a rudimentary syncytium or "periblast" with typical giant nuclei confirms the above view (**figures 16 and 17**) although this fact is also open to the interpretation, as Professor Minot has suggested to me, of being anticipative of the condition found in the teleost rather than derived from that in the shark. I do not, however, offer the case of *Amia* as being nearly as well established as that of the teleosts mentioned.

V. CONCRESCENCE AND CONFLUENCE.

Problem Stated.—In the foregoing pages concrescence has been discussed only in relation to that process by which the hinder end of the embryo becomes folded off from the margin of the blastoderm. It will be remembered that BALFOUR ('81), while admitting the occurrence of concrescence to this extent, denied to it any part in the formation of the embryonic body itself. So far, I have affirmed the process only to the degree admitted by BALFOUR.

I cannot go further, however, without facing the general problem of concrescence. I must defer to a subsequent paper a review of the endless mass of literature bearing upon this interesting subject. In this article, I shall merely point out that my conclusions are quite in harmony with the results reached by the two investigators who have given most labor to the experimental study of concrescence in the teleost embryo.

"CONCRESCENCE" as applied to the growth of the embryo, may be taken in two entirely different senses and a failure to recognize this difference may lead to much confusion. As originally conceived by HIS,¹ concrescence was a process by which the lateral portions of the germ-ring were actually *apposed* to one other behind the embryo, which thus grew backward *pari passu* with their union. In this case, the embryo was looked upon as formed by the coming together of the two halves of the germ-ring as such, the successive levels in the body of the former being at the outset represented by successively distant portions of the circumference of the latter. The term "concrescence" may be extended in its application, however, to a process quite different from this. The growth of the embryo may still be regarded as taking place at the expense of the germ-ring, but merely in the sense that the latter furnishes undifferentiated building-material, which is first organized after reaching the embryonic region. In this case, the cells composing the opposite halves of the germ-ring are conceived to undergo a gradual concentration toward the axis of the embryo as the latter grows backward. This axial concentration is part of the same general process by which the broad "embryonic shield" gives rise to the narrow, but greatly thickened body of the definitive embryo. It is clear that such a centripetal movement of the germ-ring cells would not necessarily result in a growth of the embryo by accretion at its posterior end. On the contrary, its growth might, in this case, occur by intussusception, the newly added cells reaching the embryo at some point anterior to the caudal end. This second possible mode of concrescence I prefer to call "CONFLUENCE."

Turning to my own studies, it is evident that I have explained the detachment of the prostoma from the yolk blastopore by a process of concrescence in the former sense (apposition). The duration and extent of this process I have not so far determined, but for many reasons I feel convinced that the folding-off of the hinder end of the body is merely the last step in a process of concrescence by which a whole or part of the length of the then-existing embryo has been formed. I am equally convinced that after the detachment of the prostoma and the appearance of caudal knob, concrescence in this sense ceases entirely. For unless we believe this to have come to a close, it would be difficult to account for the continued presence of the projecting caudal knob at the posterior end. Again, if there occurred a union of the halves of the germ-ring behind the embryo, after the formation of Kupffer's

¹First stated in print in *Verh. d. Leipziger naturforsch. Ges.*, June 5, 1874. In the same year appeared "Unsere Körperform." After this, HIS reiterated the theory many times, his last utterance on the subject being in 1891 (see HIS '91). HIS was in large measure anticipated by LEREBoullet ('63) who distinctly affirmed that the two sides of the embryonic body arose from the two halves of the "*bourellet blastodermique*," or "*bourellet embryogene*," as he calls it in view of its supposed fate. Lereboullet did not, however, describe the *process* by which the body arose from the "*bourellet*."

Vesicle, there would be a rapid increase in the distance between the latter and the caudal end. But this, as HENNEGUY was first to point out is untrue. The writer has verified HENNEGUY's observations, which were made upon trout embryos, by camera lucida measurements made upon the living egg of *Scorpaena*. Whether, however, there occurs, after the appearance of the caudal knob, a process of concrescence in the second sense ("confluence") is a question upon which my own observations throw little light. [See Supplement.] The experiments of other investigators, as we shall see, speak strongly for this view.

Experiments of Morgan and Kopsch.—MORGAN ('95),¹ working upon the egg of *Fundulus*, performed the crucial experiment of cutting the germ-ring to one side of the embryo. In a number of cases, nearly normal embryos developed in spite of their severance from the hypothetical sources of building material. He rejects, on what seems to me to be insufficient grounds, the supposition that a process of regeneration has taken place on the injured side. MORGAN's accounts, it is important to note, mentions two cases in which injury to the germ-ring resulted in a deficiency of mesoblast on the corresponding side of the embryo. For this and other reasons he concludes that the germ-ring normally does, in part at least, pass into the embryo during growth, but he also holds that it furnishes a relatively small part of the substance of the latter.

The experiments conducted by KOPSCI ('96)² upon the egg of the trout differ from those of his predecessor mainly in being more systematic and thorough. He finds that the effects of injuring the germ-ring vary with the position of the injury and the stage at which it is inflicted. After the caudal knob has appeared, any injury to the germ-ring laterad to this does not prevent the corresponding side of the embryo from developing with a normal number of somites. This side is, however, less strongly developed, in point of quantity of mesoblast, than the opposite (thus agreeing with MORGAN). Injury to the embryo during an early "embryonic shield" stage, before a tail-bud has appeared, gives results varying according to the location of the injury. If the latter is in the center, no growth takes place here, but the "non-embryonic" borders of the blastoderm continue to surround the yolk, leaving the punctured spot, at the time of closure, at the (anterior) end of a long slit. If the injury is slightly laterad, an embryo develops, having a perfect head, but the trunk fails to develop on the injured side. If the injury is still further laterad, an embryo results but not so strongly developed on the injured side (see above).

¹ A preliminary account of these experiments is to be found in the *Anat. Anz.*, 1893. KASTSCHENKO, in 1888, had performed similar experiments upon the *Selachian* embryo, his results leading him to oppose the concrescence theory.

² KOPSCI's later experiments ('99) in producing artificial Hemididymii I shall not discuss, inasmuch as his results are equally well explained upon the orthodox view of concrescence.

KORSCH concludes from these results that, at the time of the first appearance of the embryonic Anlage, the head rudiment (**K**, figure 31, **A**) is median, the cell-masses (**R**) destined for the halves of the trunk and the tail are laterad to this. The formation of the caudal knob ("Knopf," **R**, figure 31, **B**) results from the union of the two lateral masses behind the head rudiment. The "Knopf" is the repository for the trunk and tail Anlagen, which thus, even at this early stage, come to lie in the middle line. The caudal knob does not, however, depend for its growth entirely upon a multiplication of its own cells but, during the circumescence of the germ-ring, receives successive portions of the latter, which serves merely as so much undifferentiated building material. A conerescence in the sense employed by HIS, he says, does not occur in the *Salmonidae*. The process by which the two rudiments (**R**) came together KORSCH does not consider as conerescence, although he does not give sufficient reason for his position. He makes the very significant

FIGURE 31.

Diagrams illustrating Korsch's view of the formation of the embryo from the germ-ring. **K**, head anlage.
R, material destined to form the trunk.

remark that the *Knopf* "contains the neurenteric canal," although not stating just what he means by this. In a later paper ('99), he says that the *Knopf* is "composed of two symmetrical halves which are separated from one another by the ideal neurenteric canal."

The above conclusions of MORGAN and KORSCH are in no way inconsistent with the results of HENNEGUY's measurements. HENNEGUY located the growing region of the embryo between Kupffer's Vesicle and the most recently formed somite. This HENNEGUY offered as convincing evidence against conerescence by apposition, and in doing this he seems to have struck the first decisive blow against HIS's theory. If conerescence (confluence) is occurring at all, he says, it occurs in front of Kupffer's Vesicle. This supposition, however, he also rejects for reasons which I shall not here discuss. It will be remembered that HENNEGUY believed the caudal knob (and this only) to be formed through an actual process of conerescence proper.

There is no evidence, however, that HENNEGUY attributed any such significance to this last phenomena as has been done by KOPSCH and myself.

JABLONOWSKI ('98) offers a view based largely upon that of KOPSCH, though not identical with it. He regards the first formed section of the embryo as resulting from an "excentrischer Zusammenziehung" of the blastopore lips, comparable to that described for *Amphioxus* by HATSCHEK. After the definite establishment of the embryonic body (he seems to have in mind the appearance of the caudal knob) growth occurs "through multiplication of material situated in the Endwulst." The latter represents only the anterior (dorsal) wall of the neurenteric canal, which is thus, properly speaking, an "*incisura neurenterica*" (His) like that of the early elasmobranch. In favor of this view he cites the artificially produced bifid condition above described. JABLONOWSKI, accordingly, recognizes no detachment of the neurenteric canal from the rest of blastopore. Neither JABLONOWSKI nor KOPSCH hint at any relation between neurenteric canal and Kupffer's Vesicle.

Conclusions.—From the previous discussion, it is evident that I have been led, on purely morphological grounds, to a view of the formation of the fish embryo in full accord with the results of the latest work in the experimental field. The neurenteric canal I have shown, moreover, to be much more than an "ideal" structure, it having, in some cases at least, an open lumen, and being in others represented by a solid ingrowth of cells. Of course, such a neurenteric canal as occurs in *Amphioxus* is impossible in any teleost, owing to the solid condition of the neural axis.

That the process of conrescence, which leads to the formation of the neurenteric canal, was previously instrumental in the construction of the embryonic body anterior to it seems to be proven by KOPSCH's experiments, although he rejects the word "conrescence" in this connection. He admits, however, an apposition of the two laterally situated halves, which is all that is necessary for the present discussion.

The long-continued emarginate condition at the caudal end of certain embryos (see above) leads me to the belief that the process of conrescence is one of considerable duration, and the fusion of the germ-layers along the embryonic axis ("primitive streak") in front of this point, gives further evidence of such a process. (JABLONOWSKI also regards this region as due to "Nahtbildung.") That conrescence (apposition) ceases, however, with the formation of the neurenteric canal is certain. That it is thereafter replaced by a process of *confluence* seems proven by the experiments of both Morgan and Kopsch. [Also by my own.—See Supplement.]

VI. FURTHER MORPHOLOGICAL CONSIDERATIONS.

The generally accepted view that Kupffer's Vesicle represents a certain part of the archenteron seems to me to be true beyond doubt. How much of the gastrula cavity is represented by this structure is less obvious. It has been variously interpreted as the allantois, as part or whole of the post-anal-gut, or as the latter plus the neurenteric canal. The case of *Muraena*? seems to show that the neurenteric canal is not included in the vesicle proper, but that the latter forms the dilated inner end of the invagination which gives rise to both. The neurenteric canal, in most teleosts, is represented by a solid ingrowth as maintained by KOWALEWSKI. At the inner end of this, the cavity of the vesicle forms secondarily.

In many fishes, the post-anal portion of the gut (Kupffer's Vesicle) possesses from the first, as we have seen, a ventral as well as a dorsal wall. In the more anterior portion, or gut proper, the lumen is formed by a real or virtual upfolding of a horizontal sheet of cells, the axial portion of which represents the dorsal wall of the gut, the lateral portions representing the ventral. We should thus expect the gut-hypoblast to be continued into the dorsal, rather than the ventral wall of Kupffer's Vesicle. This relation is impossible to determine at an early period, but **figure 32** representing a late stage of the vesicle in the trout embryo, exhibits the expected condition.

The structure of the fully formed Kupffer's Vesicle and adjacent parts have been so often carefully described that they need not be discussed here except in connection, with certain differences, already mentioned, in the form which it assumes in various types of fishes. Two different types of Kupffer's Vesicle have been described above, one with no cellular floor, the other from the first bounded on all sides by cells. That these two types exist there can no longer be any doubt. According to the view maintained in this paper as to the significance of this structure, it is obvious that I must regard the second type as the more primitive. The forms in which the cellular lower wall has been described as wanting are, as far as I know, all rapidly developing pelagic eggs, in which many developmental processes are modified by abridgement. On the other hand the more slowly developing cat-fish, trout and salmon, in which there is reason to believe that the type of development is less modified, display a vesicle with a complete cellular boundary. An exception

FIGURE 32.

Sagittal section showing late Kupffer's Vesicle in *Salvelinus*.

to such a rule, however, is found in the case of *Muraena*? a form having a rapidly developing pelagic egg, for this fish exhibits, in the formation of its vesicle, the hypothetical primitive condition more clearly than any other teleost that has been carefully described.

I have observed both in *Muraena*? and in the trout an incompleteness of the lower wall of the vesicle at one period. The cells forming the floor of the vesicle are embedded in the underlying periblast (**figures 13 and 14, A**) and are not at all points in contact with one another. This fact, and the presence of free cells in the periblast in this neighborhood, might lead to the suspicion that the lower wall of the vesicle is formed by cells derived from the syncytium. In fact, this is a conclusion adopted by REINHARDT ('98). I do not find any evidence that these free cells are formed out of the periblast proper. They are present from a very early period in the development of *Salvelinus*, and occur beneath the whole blastoderm, but especially at the posterior end. It seems probable that their differentiation as cells dates back to the segmentation period. If so, they are to be regarded merely as detached segmentation spheres which have lost all connection with the blastoderm. They are surrounded by distinct cell-walls and generally contain normal nuclei. Whether such free cells play any part in the building of Kupffer's Vesicle in *Salvelinus* or *Muraena*? I cannot say definitely. In *Amiurus* and *Noturus* such free cells are of very rare occurrence. I have observed them only once or twice in all the *Amiurus* eggs examined, and do not recall having seen any in the eggs of *Noturus*. OELLACHER ('72) was probably the first to describe this conditional though he undoubtedly made no distinction between these embedded cells and the periblast nuclei.

Such an incompleteness in the ventral wall may be looked upon as a condition transitional between the two types of vesicle described. SOBORTA ('98) has already offered as a transitional form the case of the rainbow trout, which exhibits a complete, though extremely thin, lower wall. That such cases as I have described may have, however, some other morphological significance is suggested by the occurrence of a like condition in *Amia*. Here I find, in both transverse and longitudinal sections that a gap in the cellular floor of the vesicle occurs along the median line, the floor being completed by the syncytium (**figure 17**).

VII. YOLK VESICLES.

In certain fishes, there exists, in addition to Kupffer's Vesicle, a second vesicle lying in the yolk beneath the floor of the former. This structure is well shown in

the egg of the "Stone Cat" (*Noturus*) although entirely lacking in the closely related *Amiurus*. It appears a little earlier than Kupffer's Vesicle and attains a somewhat greater size (see **figure 34**). This "yolk vesicle" is surrounded by a complete wall of periblast, except on the upper side, where it is bounded directly by the cells of the embryo. There is at no time any connection between the two.

EIGENMANN ('92) describes in the case of *Cymatogaster* a single large vesicle lying partly in the yolk and partly in the embryo. This latter subdivides into three portions: a lower one, which he calls the "yolk vesicle"; an intermediate portion, which he homologizes with the post-anal-gut, and an upper cavity, which he considers to be the equivalent of the neurenteric canal. KINGSLEY and CONN ('83) originally described Kupffer's Vesicle in *Ctenolabrus* as arising by the fusion of a mass

FIGURE 33.

Section through posterior end of same embryo as that in figure 20 (camera lucida). Kupffer's Vesicle (*Kv*) is beginning to make its appearance as a group of small vesicles.

FIGURE 34.

Transverse section through embryo of *Noturus*, showing fully developed Kupffer's Vesicle and yolk vesicle. A layer of pavement cells, continuous with the gut-hypoblast, is here seen to underly the columnar epithelium forming the lower wall of Kupffer's Vesicle. This pavement layer has probably reached its present position by growing in from the sides, as it is not present in an earlier stage (figure 33).

of vacuoles in the yolk, and this account has been confirmed by several subsequent workers on pelagic eggs (*e. g.*, AGASSIZ and WHITMAN, see p. 52, ante). The dorsal cellular wall of the vesicle becomes differentiated above this resulting single vacuole. HENNEGUY ('88) states that there frequently occurs beneath the posterior end of the trout embryo a vesicle in the yolk. In the case of *Salvelinus* the whole periblast is much vacuolated, but in some specimens I have found a particularly large vacuole lying in the appropriate position. AGASSIZ and WHITMAN have described certain "secondary caudal vesicles" probably of a similar nature. M'INTOSH and PRINCE ('90) speak of a "multiplicity of vesicles" in the gurnard and some other fishes. EYCLESHYMER ('95) describes two such accessory vesicles in *Lophius*, one of which communicates at one period with Kupffer's Vesicle.

I am convinced, from the mode of formation of Kupffer's Vesicle, that it has no morphological relation to these lower cavities in the periblast. In such cases as those of *Otenolabrus* the connection is doubtless a secondary one. But there probably exists, as I maintain below, a physiological connection between the two.

VIII. THE FUNCTION OF KUPFFER'S VESICLE.

The preceding pages have been largely concerned with a morphological interpretation of the embryonic structure known as Kupffer's Vesicle. It is obvious that this morphological interpretation in no way accounts for the dimensions attained by the vesicle during development. Why should the lumen of this quite transitory post-anal-gut reach such a relatively enormous size at a time when, in the remainder of the gut, no lumen has appeared? Kupffer's Vesicle must be regarded as an embryonic organ having some definite part to play in the economy of the growing embryo. The measurements made by Henneguy located the region of growth in the unsegmentated part of the embryo lying between Kupffer's Vesicle and the last formed somite. Here, then, metabolism is most active, and the material needed for growth ought to be the most abundant. It is now a generally accepted fact that the periblast with its giant nuclei play a leading role in the assimilation of the yolk. Passing upward from the deeper layers of the yolk, we find that the yolk spheres become successively smaller, while in the periblast syncytium they are reduced to minute granules. Finally, there seems to occur a thin fluid layer between the periblast and the cells of the embryo proper. I have already noted the occurrence of a large vesicle in the yolk beneath the growing region of the embryo in certain fishes. Around this vesicle the periblast and its contained nuclei are especially abundant, and within its interior there is to be seen in sections a fine coagulum, reticulate in appearance, which seems to be of an albuminous nature. It has for some time been my view (SUMNER, '99, a), that this vesicle contains a more fluid yolk, partially assimilated through the activity of the periblast, and intended for the nourishment of the growing embryo. I have, also expressed the view ('99, b) that Kupffer's Vesicle itself represents an embryonic digestive organ (more properly an organ of *absorption*). Its relation to the yolk vesicle has already been dwelt upon. Within it, moreover, occurs a coagulum which is similar to that found in the other. SOBOTTA ('98) has commented upon the fact that Kupffer's Vesicle contains a fluid having a refractive index higher than that of water. I find in the case of *Scorpena*, that this is noticeably true at an advanced stage in the development of the vesicle,

when the outlines of the chorda, seen through the contents of the former, are considerably distorted, this fluid mass serving as a bi-convex lens.

Microchemical tests as to the contents of the vesicle gave only negative results. I subjected the eggs of *Muraena*? and *Scorpæna* to the reagents employed by LE DANTEC ('90) and Miss GREENWOOD ('94) for the detection of acid in the vacuoles of protozoa. None of these were found applicable to fish eggs, as they either killed the embryo or failed to stain it; but I also used Bismarck Brown. This stain, when neutral, shows a very characteristic reaction in the presence of acids. But no appreciable reaction was exhibited by the contents of Kupffer's Vesicle.

IX. RECAPITULATION.

1.—The hypoblast arises in connection with an invagination of the superficial layer ("Deckschicht") occurring on the posterior border of the blastoderm. This invagination may be an open one ("*Muraena*" possibly some others) or it may be a solid ingrowth of cells (*Amiurus*, *Noturus*, *Salvelinus*, *Fundulus*, *Ctenolabrus*. A similar condition was found in *Amia*.

2.—This invagination is the "prostoma" of KUPFFER, whose descriptions and theoretical conclusions upon this subject are in the main correct.

3.—Kupffer's Vesicle arises from the expanded inner end of this invagination, when it is an open one; it is secondarily formed in the invaginated mass of cells, when solid.

4.—Kupffer's Vesicle, as is usually stated, represents the post-anal-gut, the neurenteric canal being represented by the open duct leading from the vesicle to the exterior in "*Muraena*" by the solid ingrowth in the other forms named.

5.—A process occurs in the teleosts exactly similar to that folding off of the tail end of the embryo which results in the formation of the neurenteric canal of the elasmobranchs. The main difference between the two cases is that the teleost embryo continues to grow backward at an equal pace with the blastoderm margin, while in the elasmobranch, the embryo, owing to its relatively slower growth, is left behind, thus losing its continuity with the border of the blastoderm.

6.—I have adduced evidence of a purely morphological character for a view of concrescence which is supported by the most recent experimental work. This is, briefly, that true concrescence (apposition) occurs at an early period in embryo formation. It ceases with the appearance of the caudal knob, which arises as a result of the above-mentioned folding-off of the neurenteric canal. No true concrescence

can occur after this event, though it is probable from the experiments of MORGAN and KOPSCH, [See also Supplement] that a modification of the process, which I have termed "CONFLUENCE," continues till the closure of the blastopore.

7.—A second vesicle, lying in the yolk below the embryo, is present in *Noturus* and some other forms.

8.—Kupffer's Vesicle has an important function in embryonic life. Its position and some other facts suggest that it plays the part of a transitory digestive (absorbent) organ.

DEPARTMENT OF NATURAL HISTORY, COLLEGE OF THE CITY OF NEW YORK, April 14, 1900.

X. NOTE ON METHODS.

As the result of considerable experimenting upon the fixing of teleost eggs, I have settled upon two methods which I now use almost exclusively. One of these is treatment with ZENKER's Fluid. This reagent gives very good results with the eggs of *Noturus* and *Amiurus*, but I have found it to be far less satisfactory for fixing the eggs of the trout or those of pelagic fishes. The second method, which I have found applicable to all the eggs I have studied, consists in a brief fixation in sublimate acetic (10% acetic) followed by preservation in formalin. The eggs are allowed to remain in the fixing fluid till the blastoderm or embryo becomes whitened, one minute being usually sufficient. After hasty rinsing in water, they are transferred to 10% formalin. This method, in addition to securing good histological fixation, has the advantage of not hardening the yolk and of preserving the natural appearance of the egg far better than any other treatment which I know of. This method, Doctor STRONG tells me, originated with Doctor C. M. CHILD.

The eggs of *Amia* (kindly furnished me by Professor DEAN) were preserved by the late Doctor ARNOLD GRAF. Those which I sectioned had been fixed in ZENKER's Fluid or in GRAF's Chrom-oxalic mixture. (See *New York State Hospitals Bulletin*, Vol. II, 1897.) Both gave satisfactory results.

The teleost material was stained according to HAIDENHAIN's "Iron hæmatoxylin" method. Occasionally I used an anilin counterstain, though this was of no real advantage. For the sections of *Amia* I employed both the "Iron hæmatoxylin" and DELAFIELD's hæmatoxylin. The latter was far preferable for these eggs.

XI. SUPPLEMENT.

Since writing the foregoing pages, I have had an opportunity of putting to experimental test certain of the views therein maintained. At Naples, during the past July, I carried on experiments with a view to determining the manner of formation of the embryonic body in *Exocoetus* sp. My results will shortly be published. I will, however, briefly record the confirmation of two of the conclusions maintained above.

First, that the germ-ring passes, in large part at least, into the embryo. *Proof*:—Glass needles were inserted into the eggs, piercing the germ-ring far laterad to the caudal end of the embryo during an early stage. At a late period, the point of insertion of the needle was, in certain instances, found to be close beside the caudal end of the embryo. In these cases the latter was *conspicuously bent* as if the posterior part had been drawn towards the needle. One can only conclude that the segment of the germ-ring which lay between the needle and the embryo had passed into the latter, but the germ-ring being held fast on one side, the embryonic body itself was drawn in that direction.

Second, that, at least after a certain period, there occurs a process of confluence, rather than one of concrescence. *Proof*:—In cases where a needle was inserted at the mid-caudal point of the early embryo, the embryo none the less continued to grow, but necessarily *in a forward instead of a backward direction*, while the blastopore continued to close in a seemingly normal manner. Indeed there was nothing to show that the usual concentric growth of the blastoderm had been disturbed. The only possible inference is that the germ-ring material entered at a point anterior to the needle causing the embryo to elongate in the only direction in which it was free to move.

FRANCIS B. SUMNER.

C. C. N. Y.—Sept. 21, 1900.

BIBLIOGRAPHY.

This table includes only those papers referred to in the present article and lays no claim to completeness. The papers of Henneguy ('88), McIntosh and Prince ('90) and Berent ('96) contain lists of papers bearing on early teleost development, and Morgan ('95) and Kopsch ('99) give the literature of concrescence. Kopsch ('00) gives a very complete bibliography of Kupffer's Vesicle. (This last appeared after the present memoir had gone to press.)

Agassiz, A., and Whitman, C. O.

- '84. On the Development of some Pelagic Fish Eggs. Preliminary notice.
Proc. Amer. Acad. Arts and Sciences, Vol. XX, pp. 23-75, pl. I.

Assheton, R.

- '96. An Experimental Examination into the Growth of the Blastoderm of the Chick.
Proc. Roy. Soc. London, Vol. LX, pp. 349-356.

Balfour, F. M.

- '78. A Monograph on the Development of Elasmobranch Fishes. London, 1878.

Balfour, F. M.

- '81. Comparative Embryology. London, 1881.

Berent, W.

- '96. Zur Kenntniss der Parablastes und der Keimblätterdifferenzierung im Ei der Knochenfische.
Jenaische Zeitschrift, Bd. 30, pp. 291-349, Taf. XVI-XVIII.

Clapp, Cornelia M.

- '91. Some Points in the Development of the Toad Fish (*Batrachus tau*).
Journ. Morph., Vol. V, pp. 494-501.

Corning, H. K.

- '96. Merocyten und Umwachsungsrand bei Teleostiern.
Festschrift f. Carl Gegenbaur, Bd. 2, pp. 103-132.

Dean, B.

- '95. The Early Development of Gar, Pike and Sturgeon.
Journ. Morph., Vol. XI, pp. 1-63, pl. I-IV.

Dean, B.

- '95. Fishes, Living and Fossil. New York, Macmillan Co.

Dean, B.

- '96. The Early Development of *Amia*.
Q. J. Micr. Sci., Vol. 38, pp. 413-445, pl. XXX-XXXII.

Duval, M.

- '84. De la Formation du Blastoderme dans l'Oeuf d'Oiseau.
Ann. des Sci. nat., Sér. 6, Tome XVIII, pp. 1-208, pl. I-V.

Eigenmann, C. H.

- '94. On the Viviparous Fishes of the Pacific Coast of North America.
Bull. of U. S. Fish Com. for 1892, pp. 381-478, pl. XCII-CXVIII.

Eycleshymer, A. C.

- '95. The early Development of *Amblystoma*, with Observations on some other Vertebrates.
Journ. Morph., Vol. X, pp. 343-419, pl. XVIII-XXII.

Götte, A.

- '73. Beiträge zur Entwicklungsschichte der Wirbelthiere. I, Der Keim des Forelleneies.
Arch. f. mikr. Anat., Bd. IX, pp. 679-709, Taf. XXVII.

Greenwood, (Miss) M. and Saunders, E. R.

- '94. On the Rôle of Acid in Protozoan Digestion.
Journ. of Phys., Vol. XVI, pp. 441-467, 1 pl.

Gregory, E.

- '99. Die Kupffer'sche Blase bei der Forelle (*Trutta fario*).
Festschrift zum siebenzigsten Geburtstag von Karl von Kupffer, pp. 711-715, Taf. LX, LXI.

Henneguy, F.

- '88. Recherches sur le Développement des Poissons osseux. Embryogénie de la Truite.
Journ. de l'Anat. et de Physiol., 1888, pp. 413-502 and 525-617, pl. XVIII-XXI.

His, W.

- '74. Unsere Körperform.
 Leipzig, 1874, pp. 186-191.

His, W.

- '91. Zur Frage der Längsverwachsung von Wirbelthierembryonen.
Verh. d. anat. Ges., 1891, pp. 70-83.

Jablonowski.

- '98. Ueber einige Vorgänge in der Entwicklung des Salmonidenembryos, nebst Bemerkungen über ihre Bedeutung für die Beurteilung der Bildung des Wirbeltierkörpers.
Anat. Anz., Jahrg. 14, pp. 532-551.

Jhering, H. von.

- '88. Ueber Brutpflege und Entwicklung des Bagre.
Biolog. Centr., Bd. VIII, pp. 268-271.

Kingsley, J. S., and Conn, H. W.

- '83. Some Observations on the Embryology of the Teleosts.
Memoirs Bost. Soc. Nat. Hist., Vol. III, pp. 183-212, pl. XIV-XVI.

Kopsch, F.

- '96. Experimentelle Untersuchungen über den Keimhautrand der Salmoniden.
Verh. d. anat. Ges., 1896, pp. 113-127.

Kopsch, F.

- '99. Die Organisation der Hemididymi und Anadidymi der Knochenfische.
Internat. Monatschr. f. Anat. u. Phys., 1899.

Kopsch, F.

- '00. Homologie und Phylogenetische Bedeutung der Knipffer'schen Blase.
Anat. Anz., Bd. XVII, pp. 497-509.

Kowalewski, M. von.

- '85. Ueber Furchung und Keimblätteranlage der Teleostier.
Sitzungsber. d. phys.-med. Soc. zu Erlangen, Heft 18, pp. 1-6.

Kowalewski, M. von.

- '86a. Ueber die ersten Entwicklungsprocesse der Knochenfische.
Zeitschr. f. wiss. Zool., Bd. 43, pp. 434-480, pl. XVII.

Kowalewski, M. von.

- '86b. Die Gastrulation und die sogen. Allantois bei den Teleostiern.
Sitzungsber. d. phys.-med. Soc. u. Erlangen, Heft 18, pp. 31-36, Taf. I.

Kupffer, C. von.

- '66. Untersuchungen über die Entwicklungen des Harn- und Geschlechtssystems. (Second part.)
Arch. f. mikr. Anat., bd. II., pp. 473-489, Taf. XXIV.

Kupffer, C. von.

- '79. Die Entstehung der Allantois und die Gastrula der Wirbelthiere.
Zool. Anz., 1879, pp. 520-522, 593-597, 612-617.

Kupffer, C. von.

- '84. Die Gastrulation an den meroblastischen Eiern der Wirbelthiere und die Bedeutung des Primitivstreifs, III.
Arch. f. Anat. u. Phys. (Anat. Abth.), 1884, pp. 1-40, Taf. I, II.

Le Dantec, F.

- '90. Recherches sur la Digestion intracellulaires chez les Protozoaires.
Ann. de l'Institut Pasteur, 1890, p. 776.

Lereboullet, M.

- '63. Recherches sur les Monstruosités du Brochet.
Ann. des Sci. nat., 4 Série, Tome XX, pp. 177-271, pl. 2, 3.

Locy, W. A.

- '95. Contribution to the Structure and Development of the Vertebrate Head.
Journ. Morph., Vol. XI, pp. 497-594, pl. XXVI-XXX.

M'Intosh, W. C., and Prince, E. E.

- '90. On the Development and Life History of the Teleostean Food- and other Fishes.
Trans. Roy. Soc. Edinb., Vol. XXXV, pp. 665-946, pl. I-XXVIII.

Morgan, T. H.

- '93. Experimental Studies on the Teleost Eggs.
Anat. Anz., Jahrg. 8, pp. 803-814.

Morgan, T. H.

- '95. The Formation of the Fish Embryo.
Journ. Morph., Vol. X, pp. 419-472, pl. XXIII-XXV.

- Oellacher, J.**
 '72. Beiträge zur Entwicklungsgeschichte der Knochenfische nach Beobachtungen am Bachforelleneie.
Zeitschr. f. wiss. Zool., Bd. 22, pp. 373-421, Taf. XXXII, XXXIII.
- Raffaëlle, F.**
 '88. Le uova galleggianti e le larve dei Teleostei nel golfo di Napoli.
Mitth. aus der Zool. Station zu Neapel, Bd. 8, pp. 1-84, pl. 1-5.
- Reinhard, W.**
 '98. Die Bedeutung des Periblastes und der Kupffer'schen Blase in der Entwicklung der Knochenfische.
Arch. f. mikr. Anat., 1898, pp. 793-819, Taf. XXXV-XXXVI.
- Ryder, J. A.**
 '86. On the Development of Osseous Fishes.
Rep. of U. S. Fish Com. for 1885, pp. 116, pl. XXX.
- Schwarz, D.**
 '89. Untersuchungen des Schwanzes bei den Embryonen der Wirbelthiere.
Zeitschr. f. wiss. Zool., Bd. 48, pp. 191-223, Taf. XII-XIV.
- Sobotta, J.**
 '96. Die Gastrulation von *Amia calva*.
Verh. d. anat. Ges., 1896, pp. 108-111.
- Sobotta, J.**
 '98. Die morphologische Bedeutung der Kupffer'schen Blase.
Verh. d. phys. med. Ges. zu Würzburg, Bd. XXXII, pp. 16, 1 pl.
- Sumner, F. B.**
 (See below.)
- Virchow, H.**
 '95. Ueber das Keimhautrand der Salmoniden.
Verh. d. anat. Ges., 1895, pp. 201-219.
- Wilson, H. V.**
 '91. The Embryology of the Sea Bass (*Serranus atrarius*).
Bull. U. S. Fish Com. for 1889, pp. 209-277, pl. LXXXVIII-CVII.

The following is a list of brief abstracts of papers read by the author of the present memoir and referred to in the text :

1. 1899 a. On the Early Development of the Catfish (*Noturus*). *Read before Amer. Morph. Soc.* Dec. 28, 1898.
 Abstracted in *Science*, Mar. 3, 1899, pp. 313, 314. (No mention is made in the abstract of the "prostomal thickening" of the blastoderm, although this appearance was described in the paper.)

2. 1899 *b*. Observations on the Germ-layers of Teleost Fishes. *Read before Section of Biology, N. Y. Acad. Sci.*, Mar. 14, 1899
Abstracted in *Science*, May 19, 1899, p. 718.
3. 1900 *a*. The Teleost Gastrula and its Modifications. *Read before Amer. Morph. Soc.* Dec. 27, 1899.
Abstracted in *Science*, Feb. 2, 1900, p. 169.
4. 1900 *b*. Kupffer's Vesicle and its relation to Gastrulation and Concrecence. *Read before Section of Biology, N. Y. Acad. Sci.*, Feb. 12, 1900.
Abstracted in *Science*, Mar. 16, 1900.

14 DAY USE
RETURN TO DESK FROM WHICH BORROWED

BIOLOGY LIBRARY

This book is due on the last date stamped below, or
on the date to which renewed.

Renewed books are subject to immediate recall.

OCT 27 1961	
OCT 31 '61 ST	
NOV 6 1961 T&E	
NOV 20 1961	
NOV 17 1961	
NOV 25 1961 Mc Kenzie	
DEC 2 1961 Gin	
DEC 13 1961	
DEC 13 1961	
JAN 31 1962	
JAN 25 1962	

LD 21-50m-6,'60
(B1321s10)476

General Library
University of California
Berkeley

299288

Summer

QL959

58

1900

BIOLOGY
LIBRARY

UNIVERSITY OF CALIFORNIA LIBRARY