STUDIES ON GANOID FISHES.¹

II. THE DEVELOPMENT OF THE HYPOPHYSIS OF AMIA.

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In describing the origin and early development of the hypophysis it is convenient to refer to definite stages in the development of *Amia*, as designated in the preceding article on "The Development of the Adhesive Organ and Head Mesoblast of *Amia*."

By referring to Fig. 1 accompanying the article just mentioned it will be noticed that there is a slight depression between the anterior end of the neural tube and the adhesive organ. This depression marks the position of the fundament of the hypophysis. A median longitudinal section (Fig. 1) through an embryo of this stage, shows the ectoblast to consist of two layers. The outer layer is thin and quite uniform in thickness. It is composed of a single tier of rounded opaque cells which are slightly flattened. The inner layer, also composed of a single tier of cells, approximately cubical in form, is about as thin as the outer except over a considerable area in the region dorsal to the anterior end of the neural tube, where it gradually becomes thicker from all sides, extends inward and becomes continuous with the walls of the neural tube which at this point extend outward forming a small funnel-shaped evagination in the dorso-anterior region of the neural tube, or forebrain. A similar evagination is designated lobus olfactorius impar by von Kupffer (1893) in *Acipenser*. In the following description we shall, following Haller (1897), speak of the tissues (Fig. 1, np.) between the

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above-mentioned evagination of the forebrain cavity and the exterior as neuropore (mediane Riechplatte and lobus olfactorius impar of von Kupffer).

Immediately in front of the neuropore the thickened portion of the inner layer of the ectoblast becomes still thicker, extending postero-ventrally in the form of the segment of a sphere. (Fig. 1, hy.)

This is the fundament of the hypophysis. The ectoblast of the neuropore and that of the hypophysial fundament are thus seen to form parts of the same thickening of ectoblast. The nervous or inner layer of the ectoblast anterior to the fundament of the hypophysis as well as that on both sides of it is composed of a single tier of columnar cells. In the ectoblast which forms the fundament of the hypophysis, the columnar cells, judging from the arrangement of the nuclei, appear to have divided transversely, thus forming approximately cubical cells. The cell walls could not be seen distinctly in the hypophysis in this stage, consequently it was impossible to determine the exact form of the cells and their arrangement. Some little distance anterior to the fundament of the hypophysis, there is a broad, low, thick-walled evagination (Fig. 1, ad.) of the dorsal wall of the alimentary canal near the anterior end. This is the crescent as described in the preceding paper. Immediately in front of the crescent there is a smaller but deeper evagination (Fig. 1, m.) marking the position of the mouth opening. In the space between the ventral surface of the hypophysis the dorsal wall of the alimentary canal and the anterior wall of the forebrain there is a mass of rather closely packed mesenchyme cells (Fig. 1, me).

A median longitudinal section (Fig. 2) of an embryo corresponding in age to stage three (Fig. 6) of the preceding article, shows that the fundament of the hypophysis which formed a part of the inner layer of ectoblast in the preceding stage, is now connected with other ectoblast tissue only at its posterior or dorsal end where it is continuous with the neuropore. It has become a solid rod of cells slightly flat-

tened. The original anterior end of the hypophysis is now directed postero-ventrally and projects into the slightly acute angle formed by the floor of the forebrain and the dorsal wall of the alimentary canal. The posterior surface (ventral surface of the preceding stage) comes in contact near its dorsal end with the wall of the forebrain in the region of the lobus olfactorius impar and near its ventral end with the wall of the forebrain in the infundibular region (Fig. 2, in.). The central portion of the posterior surface is separated from the forebrain by a mass of mesenchyme cells which in the preceding stage was situated between the anterior end of the forebrain, the dorsal wall of the alimentary canal, and the ventral surface of the hypophysis. The anterior surface of the hypophysis, which in the preceding stage was the dorsal surface and which was then in contact with the outer layer of ectoblast, is now in contact with the adhesive organ and button, which, developing from the crescent of the preceding stage, have grown up and separated the hypophysis from the ectoblast and forced it against the anterior wall of the forebrain. The cells composing the hypophysis are more or less columnar in form, arranged so that their longitudinal axes are approximately parallel with the longitudinal axis of the hypophysis, especially at its dorsal end, as if the entire organ had been forcibly elongated.

Mechanically, the change in the position and form of the hypophysis between stages 1 and 2, may be referred to the rapid enlargement of the cavity of the forebrain and to the development of the adhesive organ. The enlargement of the cavity of the forebrain causes the ectoblast in front of the neuropore, which in the preceding stage was nearly parallel with the dorsal wall of the alimentary canal to assume a position nearly at right angles to it. The upgrowth of the adhesive organ at the same time separates the hypophysis, anteriorly and laterally, from the ectoblast, and as already stated forces it against the anterior wall of the forebrain.

Sections of several embryos both older and younger than those of the stage just described were studied, but, contrary

to the observation of von Kupffer (1893) in *Acipenser*, no connection was found between the hypophysis and the entoblast, the cells of which may readily be distinguished from those of ectoblastic origin found in the hypophysis by the large round yolk granules they contain in contrast with the fine granules characteristic of ectoblast cells. Fig. 2, which is from a photograph, shows how distinct these two sorts of cells are and how sharply the ventral end of the hypophysis is separated from the entoblast.

Fig. 1a represents a median longitudinal section of an embryo somewhat younger than that represented in Fig. 6 of the preceding article, from which Fig. 2 is taken. The section is slightly oblique. It shows the hypophysis more broadly connected with the neuropore at its dorsal end than in Fig. 2, and lying against the posterior face of the button. Otherwise the condition of the hypophysis is not greatly different from that shown in Fig. 2.

In the fourth stage of the preceding article, the hypophysis (Fig. 3, hy.) has lost its connection with the ectoblast entirely and lies in a horizontal position in the somewhat enlarged space between the forebrain and the adhesive organ close to the dorsal wall of the alimentary canal with its posterior end projecting into the angle between the forebrain and the dorsal wall of the alimentary canal. The hypophysis considered as a solid has become somewhat tongue-shaped, and is now composed of a mass of more or less cubical cells. No definite arrangement of these cells could be determined. It contains three small inter-cellular cavities, none of which were found in younger stages. These cavities, some little distance apart, are situated on a median longitudinal line a little nearer the posterior than the anterior end of the mass of cells.

A stage between Figs. 2 and 3 is represented in Fig. 2a. The hypophysis is here more recently separated from the neuropore than in the stage of Fig. 3. The embryo from which this section was taken is externally scarcely distinguishable from that shown in Fig. 6 of the preceding article, except by the single character that its tail is free from the yolk for a distance equal to thirty degrees of the yolk's circumference.

Sections of embryos corresponding in age to the fifth or hatching stage in the preceding article (Fig. 8), show the hypophysis (Fig. 4, hy.)² to have changed considerably in position. It now lies between the ventral wall of the infundibulum and the dorsal wall of the alimentary canal with its posterior end extending to a point some little distance ahead of the anterior end of the notochord (Fig. 4, n.), which reaches the postero-ventral region of the infundibulum. At this point the infundibulum presents a slight evagination (Fig. 4, sv.), the fundament of the saccus vasculosus. The cavities first observed in the preceding stage are somewhat larger in this stage, and the cells, more columnar in form, are arranged apparently in a single layer around them, forming small vesicles (Fig. 4, c.). The ends of the cells next the cavities are clear, while their opposite ends contain numerous small granules among which the nuclei are found.

By comparing the position of the hypophysis in Figs. 3 and 4, it seems to have migrated backward between the dorsal wall of the alimentary canal and infundibulum independently of surrounding parts. Mechanically, this change in position may be explained by supposing that the entire forebrain moves forward in its courses of development, its ventral wall thus passing over the hypophysis, and that later its infundibular region moves backward, due to the formation of the cerebral flexure, carrying the hypophysis with it. The first supposition is supported by the fact that the large space between the anterior end of the forebrain and the ectoblast, seen in Fig. 3, disappears; the second by the fact that the saddle cleft is much narrower in Fig. 4 than in Fig. 3.

The hypophysis in embryos about 22 mm. long is found to be located in the same relative position as in the preceding stage. It has, however, increased rapidly in size and has become broader and flatter than it was in the earlier stages. The vesicles (Fig. 5, c.) have increased in number and be-

²The relative difference in size between Figs. 2, 3 and 4 is not due to a difference in magnification, as might be supposed, but to a difference in the actual size of the embryos, all being magnified 190 diameters.

come elongated to form canals, all of which end blindly at both ends, as far as could be determined. One exceptionally large vesicle (Fig. 6, c.) was found situated but a short distance from the anterior end of the hypophysis in the median plane, near its ventral surface. This vesicle is approximately cylindrical, its length being about ten times as great as its diameter, which was found to be about five times as great as the diameter of any of the other vesicles. It is formed by a layer of columnar cells whose inner ends are hyaline while their outer ends are granular. No opening could be found leading either into or from this cavity.

At this stage the hypophysis was found to be well supplied with blood by vessels (Figs. 5 and 6, b. v.) which enter both along the median dorsal and the median ventral surfaces from the posterior end.

On either side of the median line on the dorsal surface near the posterior end, there are depressions into which project anteriorly directed ingrowths (Fig. 5, hy.) from the ventral wall of the infundibulum. These ingrowths distinguish the nervous portion of the hypophysis, which is designated *ramus hyoideus* by Goronowitsch (1883), in *Acipenser*. They vary in number in different individuals, there being sometimes two on either side of the median line and sometimes one on one side and two on the other. They are composed of neuroglia (Haller) similar to that found in the walls of the brain tube at this stage. In the middle of each protuberance there is a rod-shaped core of nuclei which is surrounded by a layer of fibrous neuroglia tissue which is separated from the hypophysis by a limiting membrane.

We thus have here indicated a division of the hypophysis into two portions homologous with the two portions described by Haller (1897) and others in other vertebrates.

SUMMARY OF OBSERVATIONS.

1. The hypophysis of *Amia* originates at a much earlier stage than described by Dean (1896) as a solid mass of cells from the inner layer of the ectoblast between the funda-

ment of the adhesive organ and the neuropore. Its tissue is continuous with that of the neuropore and remote from the stomodæum.

2. It loses its connection with the ectoblast and the neuropore tissue and comes to lie between the infundibulum and the dorsal wall of the alimentary canal.

3. In changing its position it never unites with the entoblast.

4. Its cells contain smaller yolk granules than those found in entoblast cells and may consequently be readily distinguished from cells of entoblastic origin. This is true of the earliest stages.

5. In 22 mm. larvæ it shows a division into a number of elongated vesicles with well marked cavities.

6. By the penetration into it of nervous tissue (neuroglia, Haller, 1896) from the infundibulum, it becomes divided into an anterior and a posterior portion.

7. It is richly supplied with blood-vessels.

DISCUSSION OF RESULTS.

From the foregoing account it appears that the hypophysis of *Amia* shows in its development a striking likeness in some features to the hypophysis of *Acipenser* as described by v. Kupffer (1893).

In both cases the hypophysis does not originate from the ectoblast of the stomodæum or near it, but from the ectoblast at the anterior end of the neural tube in connection with the anterior neuropore (mediane Riechplatte and lobus olfactorius impar of v. Kupffer). In both cases there is an adhesive organ intervening between the fundament of the hypophysis and the stomodæum. In both cases the hypophysis detaches itself from the ectoblast and neuropore and takes up a position posterior and dorsal to the stomodæum. In both cases its further history is not essentially different from that of other vertebrates (Haller, 1896).

Acipenser differs from *Amia* in that its hypophysis is, according to v. Kupffer, tubular in an early stage. This hypophy-

sis differs further from that of *Amia* in being continuous at its ventral end (in early stages) with the entoblast of the dorsal wall of the archenteron. The lumen of the tube opens at one end into the archenteron, while at the other end it is closed by the outer layer of ectoblast. In *Amia* we have not found the hypophysis at any time tubular, nor have we found any lumen until some time after the separation of the organ from the ectoblast. We have not at any time found a connection of the hypophysis with the entoblast.

The hypophysis of *Amia* is in close contact with the entoblastic button, which in an earlier stage is the middle of that part of the archenteric entoblast designated in the preceding article as the crescent (Fig. 1). The plane of demarcation between the ectoblast of the hypophysis and the entoblast of the crescent or button is not always easy to find. Its visibility depends on the plane of section. In suitable sections we have always found it as shown in Fig. 1, and we believe that it always exists. In respect to these two points, the existence in it from the beginning of a lumen and the continuity of its walls with the entoblast, the hypophysis of *Amia* may be in a less primitive condition than that of *Acipenser*.

It is not our purpose to discuss at length the theory advanced by v. Kupffer, on the basis of his work on *Acipenser* and *Petromyzon*, to the effect that the hypophysis is a palæostome. The development of the organ as we have described it in *Amia* certainly adds force to the array of facts adduced by v. Kupffer in support of his theory.

In this connection it should be noted that we know but three vertebrates in which the hypophysis originates in connection with or near the neuropore, *Petromyzon*, *Acipenser* and *Amia*. In *Acipenser* and *Amia* the adhesive organ intervenes between the hypophysis and the stomodæum. In *Ammocetes* the upper lip occupies the same position as the adhesive organ of the Ganoids. This suggests the possible homology of the *Ammocetes* upper lip with this adhesive organ.

Von Kupffer believes the position of the hypophysis as found in these forms to be primitive, the hypophysis to be a

palæostome and its connection with the stomodæum in other vertebrates to be a secondary condition correlated with the increased size of the forebrain and the loss of the adhesive organ. That such a condition as the connection of hypophysis and stomodæum could come about through the secondary displacement of the hypophysis he considers "schwer zu verstehen." Whether the position of the hypophysis in these three forms is primary or secondary, depends, it seems to us, on whether the adhesive organ is a primitive organ formerly possessed by all vertebrates, or an organ which has made its appearance in only a few groups and has in these groups caused the displacement of the hypophysis from a position in the stomodæum to a more dorsal position. This question can scarcely be answered until we know more of the adhesive organ itself and its homologues. Until our knowledge on this subject is increased one of these alternatives seems to us nearly as probable as the other; although, as v. Kupffer points out, the early appearance and large size of the *Acipenser* hypophysis is probable evidence of its primitive character.

Since the foregoing was written there has appeared a paper by Prather (1900) on the development of the hypophysis in *Amia*. The earliest embryo in which Prather has found the hypophysis is one a few hours before hatching, corresponding, therefore, very closely to Fig. 8 of the preceding article. In this stage he finds it in process of being differentiated out of the entoblast of the dorsal wall, of the foregut, ventral to the infundibulum, that is essentially in its adult position. His account thus differs from ours chiefly in two particulars: (1) He has not seen the early stages in the development of the hypophysis as described by us. (2) He derives the hypophysis from the entoblast; whereas, according to our account, it is ectoblastic.

Through the kindness of Dr. Eycleshymer one of us has been able to examine the sections on which Prather's studies were made. They show what Prather has described. Having had an experience of many years in the preservation of *Amia* material and in the preparation of sections from it, we be-

lieve that we are justified in saying that the differences between Prather's results and ours are due to the insufficiency of the methods used in the preparation of his material. His Fig. 1 shows that in the material used by him, the egg membranes had not been removed from the eggs before preserving them. The figure shows also that in this case the membranes had shrunk down close against the embryo. The result of this is that the tissues of the embryo are pressed together and the limits between adjacent tissues are frequently obliterated. The hypophysis is not then visible in its early stages, and in the stages some time before hatching it is so pressed into the entoblast of the dorsal foregut wall as to be scarcely distinguishable. Such a condition is shown in Prather's Fig. 3. At the time of hatching or upon removal of the egg membranes the pressure from them is, of course, removed, and the hypophysis, being no longer pressed into the dorsal foregut wall, becomes visible and appears to be differentiated in situ out of the entoblast.

We have tried every feasible method of preserving these eggs without shrinkage of the membranes and have not found any method hitherto in use that accomplishes this. One of us has, however, devised a fluid, consisting of a five per cent formalin solution (i. e., containing two per cent of formaldehyde) to which is added two or three per cent of potassium bichromate and ten per cent of glacial acetic acid. This fluid used for eight hours or longer causes not more than a very insignificant shrinkage of the membranes or none at all. It is not stable and should be used fresh. Eggs are transferred from it to four per cent formalin which is changed until no longer discolored by the bichromate, and are then preserved permanently in the formalin. The histological results are similar to those obtained from Flemming's or Hermann's fluids and are excellent, as may be judged from the figures of cell divisions visible in Fig. 2. In spite of the results obtained from this fluid we have made it a rule, before preserving the eggs, to remove the membranes from them in the earliest stages practicable. This may be done in the stage represented by Fig.

6 of the preceding article, and (by using very great care) in somewhat earlier stages.

A comparison of our Fig. 4 with Prather's Fig. 3 of approximately the same stage will show the differences between eggs preserved with and without removal of the membranes.

From the foregoing we believe that Prather is in error in regarding the hypophysis as of entoblastic origin. Prather gives a brief summary of opinions that have been hitherto held as to the origin of the hypophysis. From this it appears that the view that the hypophysis is of entoblastic origin exclusively has not been maintained since 1882. Nearly all recent writers have maintained its ectoblastic origin (though a few have regarded it as of mixed origin). We believe that we have shown that *Amia*, also, must now be placed among those forms concerning the ectoblastic origin of whose hypophysis there is no question.

Prather believes that the structure which v. Kupffer (1893) has described and figured in early stages of *Acipenser* (Figs. 13, 14) as the hypophysis is in reality the adhesive organ of that form. This belief he bases on the two facts; that the hypophysis is described by v. Kupffer as laden with food yolk, and that it has the appearance of being a dorsal diverticulum of the entoblast. Both of these characteristics distinguish the adhesive organ of *Amia*, and may be expected to be characteristic of that of *Acipenser* also. An investigation in progress in this laboratory on the adhesive organ of *Lepidosteus* has given figures of median sections strikingly like Fig. 13 of v. Kupffer (1893). In these sections there is in front of the brain an entoblastic tube with its lumen and walls continuous below with those of the foregut. The tube is flattened and may be traced in series of longitudinal sections from one side of the brain to the other. It is therefore as wide as the brain. Its position and relation to surrounding structures are the same as that figured for the hypophysis of *Acipenser* by v. Kupffer, but its subsequent history shows it to be the adhesive organ. These observations on *Lepidosteus* certainly lend weight to Prather's suspicion. On the

other hand, the very circumstantial account which v. Kupffer gives of the transformation of the tubular fundament of the hypophysis of the embryo of *Acipenser* into what is undoubtedly the hypophysis of the larva, and his clear though very diagrammatic figures make it necessary to wait for a re-investigation of *Acipenser* before pronouncing judgment.

With our present knowledge it seems to us most likely that what v. Kupffer describes as the fundament of the hypophysis in *Acipenser* is really such. This view is strengthened by the likeness of its development to that described for *Amia*. The dorsal diverticulum of the archenteron shown in v. Kupffer's Fig. 13, between en. and vd., is the probable homologue of the *Amia* adhesive organ. V. Kupffer speaks of this structure as a "quer gelagerten Wulste des Entoderms, . . . der jederseits von der Mittellinie sich taschenartig gestaltet." Its form is thus not unlike that of the *Amia* adhesive organ in an early stage, and its position is the same. V. Kupffer himself suggests its possible homology with the anterior head cavities of *Acanthias*, structures which in the preceding paper are considered to be the homologues of the adhesive organ of *Amia*.

If it be true that this diverticulum of the foregut of *Acipenser* seen in v. Kupffer's Fig. 13 corresponds to the adhesive organ of *Amia* we should expect that the structure marked hf. (Haftscheibe) in the same figure is in reality a part of this entoblastic diverticulum and not, as stated by v. Kupffer, a thickening of the ectoblast. With this slight alteration v. Kupffer's account of *Acipenser* would be in substantial agreement with that given for *Amia* in this and the preceding papers.

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EXPLANATION OF PLATE.

All figures are photographs of sections and are magnified about one hundred fifty diameters. They have been slightly retouched with the pencil, for the purpose of adding to the distinctness of certain features. Nothing has been *added* with the pencil to that which was visible in the original photographs.

REFERENCE LETTERS FOR ALL FIGURES.

ad—adhesive organ (crescent).
al—alimentary canal.
b—button.
bv—blood-vessel.
c—cavity in hypophysis.
h—heart.
hy—hypophysis.
hy'—posterior lobe of hypophysis.
in—infundibular region of brain.
m—position of future mouth opening.
me, me'—mesenchyme.
n—notochord.
np—neuropore.
st—stomodæum.
s. v.—saccus vasculosus.

FIG. 1.—A median longitudinal section (sagittal) of an *Amia* embryo of the stage of the one shown in Fig. 1 of the preceding article.

FIG. 1a.—An approximately longitudinal section of an embryo somewhat younger than the one represented in Fig. 6 of the preceding article.

FIG. 2a.—A median longitudinal section of an embryo a little older than of the preceding article.

FIG. 2a.—A median longitudinal section of an embryo a little older than the one represented in Fig. 6 of the preceding article.

FIG. 3.—A median longitudinal section of an embryo still a little older than that shown in Fig. 6 of the preceding article, and older than the last.

FIG. 4.—A median longitudinal section of the hypophysis and surrounding structures of an embryo of the hatching stage (Fig. 8 of the preceding article).

FIG. 5.—A longitudinal section of the hypophysis and surrounding tissue of a larva of *Amia*, 22 mm. long, taken a little to one side of the median plane.

FIG. 6.—A median longitudinal section of the hypophysis and surrounding tissue of a larva of *Amia*, 22 mm. long.