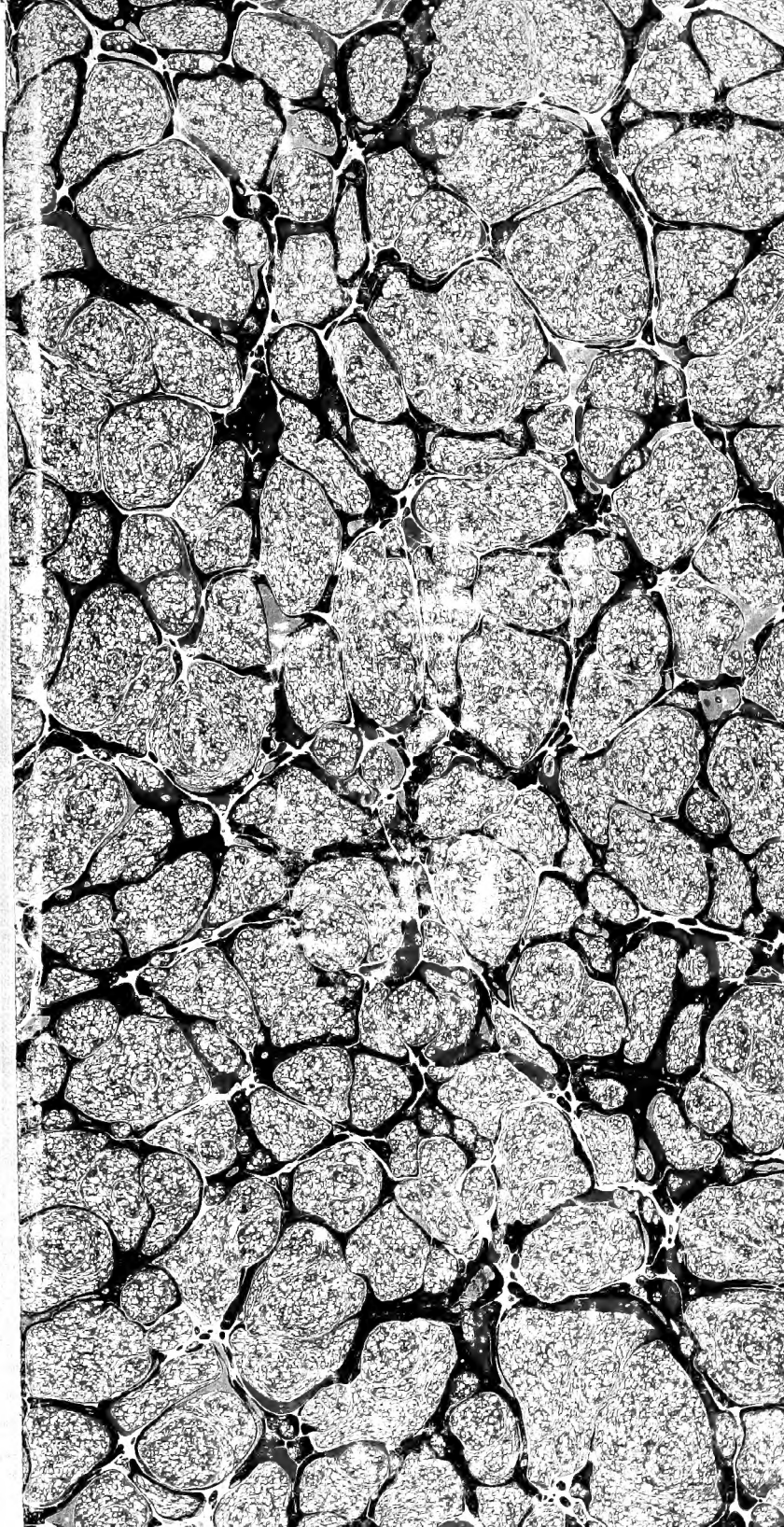


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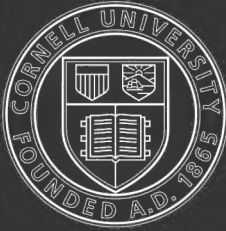
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Radcliffe College Monographs

No. 8

THE PRIMITIVE STREAK

AND

NOTOCHORDAL CANAL IN CHELONIA

BY

GERTRUDE CROTTY DAVENPORT, B.S.

PREPARED UNDER THE DIRECTION OF
EDWARD LAURENS MARK, PH.D., LL.D.
HERSEY PROFESSOR OF ANATOMY IN
HARVARD UNIVERSITY

WITH ELEVEN PLATES

BOSTON, U.S.A.
PUBLISHED BY GINN & COMPANY

1896

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Radcliffe College Monographs

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By GERTRUDE CROTTY DAVENPORT.

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I. COLLECTION, PREPARATION, AND PRESERVATION OF THE
EMBRYOS.

ALL the embryos figured and described in this paper were collected in the neighborhood of Boston. The eggs were taken from the nests at different periods after deposition. In some instances the surface of the freshly disturbed soil had not had time

* Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the Direction of E. L. Mark. No. LXVII.

to dry. Indeed, in one instance the female—*Chelopus insculptus*—was found in the act of laying, and the eggs were taken from the uncovered nest. The nest was of the same form as that described by Professor Agassiz ('57, p. 500) for *Chelydra serpentina*. The perpendicular excavation was about five inches deep. From the bottom of this excavation a chamber extended horizontally for some three or four inches. The female stood over the external opening in such a manner that the axis of her body was parallel to and directly above the horizontal chamber. The blind end of the horizontal chamber pointed anteriorly in relation to the axis of her body. During each act of deposition she pressed the cloacal end of the body as far as possible into the perpendicular hole and let the egg fall to the bottom. Then she extended one hind foot into the hole and pushed the egg anteriorly into the horizontal chamber. What seemed remarkable to me was the fact that she used the left foot to arrange one egg, then the right foot for the next, and so on alternately for the eight eggs she had to deposit. Did a deposition of the egg from the right or left oviduct induce this alternation of foot movement? Do the eggs come alternately from the right and the left oviduct?

An effort was made to obtain eggs from females kept in confinement; but, although males and females were kept in a pen out of doors with sufficient food,—earthworms,—and with water for swimming, no eggs were laid by the end of June. The females were then chloroformed, and unfertilized eggs were found still retained in the oviducts.

Since all attempts to gain fertilized eggs from captive turtles failed, all the embryos described in this paper were taken from eggs laid by free turtles. Upon removal from the nest, the eggs were packed in moist sand and thus transported to the laboratory. As soon as possible after arrival in Cambridge the embryos were removed from the eggs, not longer than two or three hours after their first discovery.

During the early stages, as Mitsukuri ('93, p. 230) has already said, the embryo is not yet attached to the shell; therefore, if precaution against desiccation is taken, the eggs may be transported with perfect safety to the embryos.

In order to remove the embryo, the egg shell was broken and a portion carefully torn away by means of forceps. When a sufficiently large opening had been made, the whole contents were

poured from the remaining portion of the shell into normal salt solution. By means of a pair of small forceps and a pipette, as much as possible of the surrounding albumen was removed. An incision was then made into the yolk sac near the edge of the blastoderm. The yolk contents flowed out instantly, but the embryo was preserved from submergence in yolk granules by washing gently with a pipette in a direction contrary to that in which the yolk was streaming. When freed from the mass of yolk, the embryo was floated into a watch crystal, in which the blastoderm was fixed in Kleinenberg's weaker picrosulphuric solution. After remaining from one to two and a half hours in this solution, the blastoderm was transferred to seventy per cent alcohol and eventually into ninety per cent.

The only staining reagent used was seventy per cent alcoholic hæmatoxylin. This stain only was used, since it gave entirely satisfactory histological and differential results. The different germ layers are often made out satisfactorily by the aid of this stain when a separation into definite layers could not otherwise be traced. As a clearing reagent cedar oil was used. In this the embryos could remain without fear of brittleness, and thus an excellent opportunity was afforded for restudying surface views. The blastoderms were embedded in paraffin and cut into sections $10\ \mu$ in thickness. Since all embryos were treated in the same manner, histological differences between the embryos described cannot be due to the effect of different reagents. After a slight staining in hæmatoxylin in order to render the outlines of the shield more distinct, dorsal and ventral surface views of the embryos were outlined by means of the camera lucida. It seems very probable that the dorsally vaulted appearance of my shields is partially due to my method of hardening, i. e. after removal from the yolk. Mitsukuri ('93, p. 269) has thus accounted for the bulged appearance of his own earlier embryos. Unfortunately, I have no embryos hardened upon the yolk to compare with the isolated shields.* This vaulted condition of the shield tends to throw into shadow the notochordal cavity; hence, when viewed from the ventral side, it is often difficult to make out, on views of the depressed ventral surface, the honeycomb structure described by Mehnert and Mitsukuri. In

* Since this paper was written, I have had an opportunity to harden shields upon the yolk before removal. The embryos thus killed show little or no tendency to bulge.

sections, however, this honeycomb condition is seen to be present to a greater or less degree.

In drawing sections all outlines were made with the camera lucida. The nuclei were filled in freehand, except in a few instances in which it was deemed expedient to use high magnification. In these instances the outline of every cell, nucleus, and yolk globule was faithfully drawn by means of the camera. Those therefore who disagree with my interpretation of facts may at least have conditions faithfully reproduced upon which to base their own explanations.

II. SCOPE OF THE PAPER.

The scope of this paper is narrow. Not all the questions upon which my sections throw light are discussed. The question concerning the homologies of the notochordal invagination is purposely avoided for the present. Some observations, however, have been made upon the manner in which the invagination of this canal takes place.

My sections also throw some light upon the question controverted between Will and Mitsukuri concerning the length and width of the notochordal canal. The facts which these sections afford lead me to agree in this matter with Will's theoretical conclusions.

The much contested question concerning the origin of the mesoderm will not be considered in the present paper. However much Will and Mitsukuri may disagree as to the manner of it, they both testify to the fact that the gastral mesoderm (Rabl, '88, p. 660) arises from the entoderm lateral to the chorda, therefore they both furnish evidence for Hertwig's cœlom theory. Their point of disagreement seems scarcely to warrant the words already spent upon it. Some of my series give me that condition which Mitsukuri ('91, p. 199) considers as positive evidence of cœlomic outpocketings, while in other series the "Zwischenplatte" appears solid and flat.

Concerning the homology between the Rusconian plug of Amphibians and a clump of cells behind the blastopore of Reptilia, I find myself in agreement with Robinson and Assheton ('91, p. 477), Keibel ('93, p. 105), and Bonnet ('91, pp. 78, 79). This is the structure which Kupffer ('82) has observed in

Emys europæa, *Lacerta*, and *Coluber asclepii*, and denoted as "Zapfen"; which Strahl ('82 and '84) has figured for *Lacerta agilis* and named "Caudalknoten"; and which has been repeatedly figured by Mehnert ('92) for *Emys lutaria taurica*, by Will ('92^b and '93) for *Platydictylus facetanus* and *Cistudo lutaria*, by Mitsukuri ('93) for *Chelonia caouana*, *Trionyx Japonicus*, and *Clemmys Japonica*, and by van Beneden ('88, p. 711) for *Mammalia*. The last four writers have made this structure homologous with the yolk plug of Amphibia. This structure I noted constantly in older shields, but the homology, as made out by the above mentioned observers, I cannot accept.

My sections moreover compel me, so far as concerns the species I have studied, to disagree with Will in regard to his theory of an uncovered entodermic streak. Furthermore, I agree with Mitsukuri in so far as he is unable to discern that sharp boundary between ectoderm and entoderm which Will figures for the lateral and posterior margins of the streak.

Sections of embryos which show conditions later than the early formation of the notochord, gastral mesoderm, and backward bending of the lateral portions of the blastopore, will not be described.

This paper will be confined to the conditions found in a small collection of twenty-six embryonic shields, including (1) stages in which the blastoporic opening is a crescentic slit concave in front, (2) those in which the slit becomes a transverse opening, and still later (3) those in which the crescent is concave behind. These stages furnish proper material for the study of the questions concerning the yolk plug and the entodermic streak.

III. DESCRIPTION OF THE NOTOCHORDAL INVAGINATION.

1. *Chelydra serpentina*. — Only two series of sections were obtained of embryos with a developing notochordal canal *intact*. Nor can these two series be considered to show absolutely normal conditions, for the embryos from which they were taken were a pair of twins, or doublets, upon the same embryonic disk. Plate II. Fig. 8, and Diagram I., represent the dorsal surface view of these twins. The blastoporic opening of the embryo *a*,* which

* The letters *a* and *β* refer to Diagram I. (page 6). In Figure 8 these letters have been accidentally *interchanged*.

possesses an extraordinarily elongated streak, is seen to be a perfectly normal transverse slit, whose lateral horns are directed anteriad and whose dorsal lip presents the median notch which we shall later consider. The other embryo (β) lies anterior to the first, and the axes of the two are almost perpendicular to each other. At the hinder end of the second embryo (β) occurs a crescentic depression.

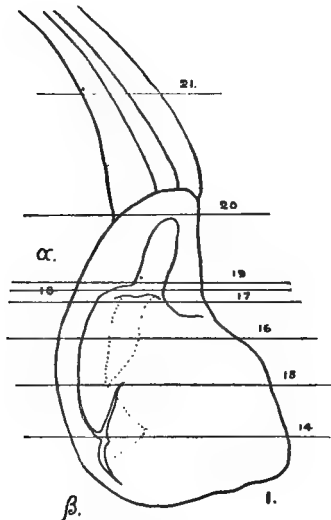


Diagram I.

The axes of these twins were so nearly at right angles, that it was possible, without changing the plane of sectioning, to get an approximately sagittal section of one embryo and a transverse section of the other. For the sake of ease in description, the embryo which possesses the elongated streak will be designated α , and the one which lies anterior and at right angles to α will be named β . The embryos were so oriented that α was cut into transverse and β into sagittal sections.

The forty-seventh section (Plate IV. Fig. 14), counting from the right edge of β , is median, but makes a small angle with the sagittal plane of β . This section discloses the fact that the invaginated cavity possesses "two limbs, one vertical and one horizontal," which makes it correspond with one of Mitsukuri's ('93,

p. 238) earliest stages. This condition, in which the invagination has begun to grow anteriad, would fall, according to Will's classification ('93, pp. 541-544), under his Stage III.

The invagination of β extends anteriad about *one third the length of the shield*, and there ends blindly. At the anterior end of the invagination, cells along its floor are seen to be assuming a columnar arrangement. Lining the pocket there is also a layer of cornified degenerating substance, without nuclei and for the most part without appearance of cytoplasm. The apex or blind end of the cavity seems to terminate against a triangular mass of this degenerating substance. It looks as though the cavity is extending itself forward at the expense, i. e. by the destruction, of cells which formerly composed a solid infolding. Ventral and anterior to the layer of columnar cells which lines the invaginated cavity there exists another cell layer, which seems in every section to be sharply separated from the former. The cells of this second layer also have assumed a columnar form for a certain distance back of the apex of the cavity.

Mitsukuri ('93, p. 238) has not detected below the primitive knob that independent layer of cells which, according to Wenckebach ('91) and Mehnert ('92), is continuous with the lower layer of the shield,—a layer which the latter author, in agreement with Kupffer, designates as paraderm, but which Wenckebach calls cœnogenetic entoderm. At first glance it seems as though we have here that layer which, according to Will ('92^b), develops later than the invaginated entoderm, and is therefore called by him secondary entoderm. In the present instance, however, I find myself unable to decide whether to consider this lower layer as at all belonging to embryo β . Embryo β is crowding its invagination into the wall and lumen of the already developed invagination of embryo a . What I interpret as a cross section of the lumen of the notochordal cavity of a (Fig. 14) is situated above and to the right of the notochordal cavity of β , which seems to be growing into the entoderm of a ; consequently, it is impossible to determine what portion of the surrounding entoderm belongs to a and what to β . Also underneath the primitive knob and streak of a (Plate IV. Figs. 17-19) an entodermic layer lies upon the yolk. In the posterior region of the primitive knob and the anterior portion of the streak this layer is distinctly separated from the mesoderm. If we pass anteriad along the notochordal area

of embryo *a*, we find that this distinct entodermic layer thins out and eventually disappears, so that the floor of the canal is composed of one layer only (Figs. 15 and 16).

2. *Chrysemys picta* and *Ozotheca odorata*.—A series of sections through the shield of an embryo of the former genus shows the same peculiarity in regard to the entoderm. Figures 1 and 1' represent dorsal and ventral surface views respectively of this embryo. From the ventral view we see that the lower wall of the notochordal canal is breaking away. The *breaking through* is not yet completed, however, for anterior to the existing opening a portion of the floor still remains. Posterior to the opening a considerable portion of the primitive knob also remains intact. From the region of the knob I have drawn two sections. Figure 45 (Plate IX.) is taken from the anterior region of the unbroken knob; Figure 46 falls just anterior to the dorsal lip of the open blastopore. In Figure 45 the ventral floor of the notochordal canal is composed of a layer of cells about three rows thick. For a short space at the extreme right, however, a few cells mingled with yolk globules, and indicated by an asterisk, are separated off by a distinct line as an independent layer. According to Will this is the region of the developing secondary entoderm. Mitsukuri ('93, pp. 256, 257), on the other hand, if I correctly understand his idea and his application of Hubrecht's ('90, p. 522) theory of precocious segregation, would be compelled to say that in this instance palingenetic and cœnogenetic hypoblast have failed to fuse. As we proceed backward we find that with each succeeding section this lateral area increases in its extent toward the axial line, and finally (Fig. 46,*) is continuous with a similar area, developed a little farther back on the opposite side, forming a complete and distinct layer next the yolk.

A lack of sections of earlier stages compels me to leave untouched the question concerning primary and secondary hypoblast. I may, however, state that in two instances I have found that the free edges of the secondary entoderm stood out along the lateral margins of the streak behind the blastopore in such a way as to give one the impression that this secondary entoderm is growing in from the sides underneath the streak in the manner described by Will for the ectoderm on the dorsal surface of the streak. I believe, however, that the secondary entoderm is not developing in this region, but is in process of dissolution. My

evidence for this conclusion will appear later. All other series of sections in my possession disclose only one layer of entoderm below the notochordal canal. On the posterior floor of this canal (Plate VII. Fig. 32) is situated that solid mass of cells which is described as the commencing mesoderm. Anteriad this compact mass gradually passes into a vacuolated condition, as is seen in sagittal sections (Plate VII. Figs. 32-34). I have repeatedly observed in cross sections this same vacuolated condition, and have figured it in Plate VIII. Figs. 36 and 37, and in Plate X. Figs. 49 and 50.

Another point of contention between Will and Mitsukuri concerns the length and width of the blastoporic canal at the time of its opening toward the yolk. Views of the ventral surface of two embryos—*Chelydra serpentina* (Plate II. Fig. 10') and *Chrysemys picta* (Plate I. Fig. 1')—illustrate the variability in the *width of the canal* in different genera. Transverse sections of the canal of the embryo of *Chelydra serpentina* (Figs. 10 and 10') show that its width nowhere exceeds one half that of the shield, whereas in a transverse section of the *Chrysemys picta* embryo (Plate IX. Fig. 45) it is seen to be almost coextensive with the width of the shield. If now we compare surface views of two shields of *Chrysemys picta* (Plate I. Figs. 1 and 1', and Figs. 3 and 3'), we shall at once see that great variability in regard to the width of the notochordal canal exists even in the same species. *Chelydra serpentina* also seems to show some variability in this respect, as a comparison of Figures 7' and 10' (Plate II.) will show.

Concerning the *length or anterior extent of the canal*, I am able to give evidence for three genera of American turtles; *Chelydra serpentina*, *Chrysemys picta*, and *Ozotheca odorata*. I have already had occasion, in considering vacuolated entoderm, to call attention to a series of sagittal sections of an embryo of *Chelydra serpentina* (Plate VII. Figs. 32-34). Figure 32, which passes through the axis of the embryo, gives little information in regard to the length of the canal, for the whole anterior part of the floor of the canal has already disappeared. A view of the ventral surface of this embryo (Plate II. Fig. 7'), however, gives one the impression that all of the lateral portion of the canal has not opened below. If we examine a parasagittal section made along the inner margin of this lateral unbroken area (Plate VII. Fig. 33), we find that the posterior ventral thickened area extends much farther anteriad than

in Figure 32, and that its anterior end approaches the roof of the canal at the point marked by an asterisk. The cells forming the roof of the canal somewhat in front of this point have lost in great part their columnar arrangement, and have taken on that vacuolated condition seen in the region of Figure 32 marked by a dagger. The central area of the entoderm beneath the shield is not only vacuolated, but a ventral layer seems to be splitting off from it. If the cavity of this central area is continuous with the notochordal invagination, and I believe that it is, then the lumen of the latter extends forward a distance equal to *two thirds* the length of the shield. In the next section, Figure 34, the posterior solid ventral region has become completely fused with the anterior ventral strand. At their point of fusion, however, they are united for the space of two cells with the dorsal wall of the canal. From this series of sections one is led to believe that the lumen of the blastoporic canal had not attained its definite lateral extent at the time when it first opened towards the yolk. In the lateral regions the lumen of the notochordal canal cannot be considered to extend forward for more than two thirds the length of the shield. Returning now to the median section (Fig. 32), we find that the columnar arrangement of the cells forming the roof of the canal has extended forward over about two thirds of the length of the shield; it seems probable, therefore, that the anterior end of the columnar region was the anterior limit of the canal at the time its floor broke through. It is possible that cells from the primitive knob have gone on proliferating to form the vacuolated entoderm of the remainder of the anterior part of the shield. The extent of the canal at the time it breaks through seems to indicate that the embryos correspond with the stages described by Mehnert ('92, p. 411) and Mitsukuri ('93, p. 241) rather than with those of Will's tortoise ('92^a, pp. 191, 192). Will says: "Aus diesen Stadien geht nun die wichtige Thatsache hervor, dass auch der Urdarm der Schildkröte noch in seiner ganzen Ausdehnung hohl ist und dass seine Ausdehnung absolut und relativ diejenige des Gecko noch übertrifft. Während derselbe beim Gecko die vorderen und seitlichen Ränder des Schildes nie vollständig erreicht, nimmt derselbe bei der Schildkröte stets die ganze Fläche des Schildes ein."

Sections through the anterior unbroken area of the shields of embryos of *Chrysemys picta* (Plate I. Fig. 1') and *Ozotheca*

odorata show that the lumen of the notochordal canal continues forward for three or four sections in front of the anterior margin of the break, and then ends blindly. A cross section of the lumen in this anterior region is represented for *Ozotheca* in Figure 57 (Plate XI.). The *Chrysemys* embryo shows in cross sections of this region the same condition. From the blind end of the lumen, in both species, a solid layer of tissue extends forward under the remainder of the shield. In these three genera, namely, *Chelydra*, *Chrysemys*, and *Ozotheca*, the invagination cavity at the time of its union with the subgerminal cavity is not always coextensive with the area of the shield. The original figures of Agassiz ('57), and Will's reproduction of them, show the lateral extension of the invagination cavity to be less than that of the shield.

The statement quoted above from Will, to the effect that the invagination is hollow throughout its whole extent, leads us now to the question concerning *the method in which the lumen of the invagination is formed*. Referring again to Plate IV. Fig. 14, the walls of the notochordal cavity of individual β , as has previously been noted, are lined with cornified, degenerating cells. The apex or anterior end of this lumen is likewise filled with these same disintegrating cells. Figure 52 (Plate X.) shows the floor of the notochordal cavity of another embryo, — *Chrysemys picta*, — which is lined by the same cell products. Figure 30 (Plate VI.) represents the notochordal canal of an individual (α) which is one of a pair of twins of *Chelydra serpentina* shown in surface view in Plate II. Fig. 5. The notochordal canal of this embryo has been much retarded in its development, for reasons which will be stated later. Even into this small lumen, which appears as though formed in the main by a normal separation of cells, a few unstainable shreds or fragments of cells (unfortunately not brought out by the lithographer) project. Such an appearance along the walls of the canal I have repeatedly observed. In a few instances, however, there was no evidence of cells degenerating within the canal.

We now come to the description of an invaginated cavity which shows, I believe, an anomalous condition, at least one which has not been described, to my knowledge, by writers on reptilian embryology. The egg (*Chrysemys picta*) from which this shield was obtained was found on June 16, 1893. The embryo was removed three hours after the discovery of the egg. It was possible to make use of the camera lucida to outline both dorsal and ventral

surface views of the hardened embryo (Plate I. Figs. 3 and 3'). The dorsal view exhibits the three-pronged or trident-like marking which Mitsukuri and Ishikawa ('86) have already observed and described. The central prong ends abruptly. Cross sections show this to be the point at which the columnar arrangement of the thick dorsal wall of the notochordal cavity ends. From this point a thin layer of loosely arranged cells continues anteriorly underneath the remaining portion of the shield.

The reconstruction (Plate VIII. Fig. 44) from transverse sections is intended to illustrate the condition which would have been found along the axis of the shield if the shield had been cut in the sagittal plane. The notochordal entoderm, which is continuous with the axial ectoderm at the dorsal lip of the blastopore, stretches anteriorly for some distance as a thickened layer of columnar cells several cells deep. In this instance the entoderm for a considerable distance in front of the blastopore is thicker than the ectoderm which lies above it. About two thirds of the distance towards the anterior end of the shield the entoderm suddenly diminishes in thickness, and continues forward as the thin layer already mentioned.

The anterior portion only of the notochordal cavity has become continuous with the subgerminal space. Half of the remaining portion of the floor, however, shows indications of being about to disintegrate. Anterior to the region in which only a few scattered cells exist, the floor of the notochordal canal continues for some distance as a jelly-like granulated sheet. Here and there in the anterior portion of the canal small, free clumps of granulated substance also exist. Along the walls of the canal are seen fragments of degenerating cells, such as have been previously described. Between these shaggy margins the lumen of the canal extends *posteriorly* for some distance, to end blindly in a plug of cornified or degenerating cells, which completely fill the lumen of the canal as far as the blastopore itself. The notochordal entoderm above this plug has assumed a perfect columnar arrangement. Below the plug exists a mass of cells which has been identified by many embryologists as the beginning of Rabl's gastral mesoderm. The plug itself is composed mainly of what appear to be empty cell walls, which either refuse to stain at all, or stain but slightly. Figure 35 (Plate VIII.) represents, under higher magnification than Figure 44, a cross section through this region. The lumen

of the canal appears definitely outlined, except at the extreme dextral angle, where the process of disintegration seems to be still widening and deepening the lumen at the expense of cells in that region. Here nuclei have disappeared entirely from several cells, being replaced by a finely granular substance. Other cells, scattered here and there along the wall, or even within the degenerating area, possess the same granular contents. The greater portion of the plug, however, is composed of completely empty cornified cell walls.

This process of degeneration so closely resembles the cornification described by Unna ('76), Kusnetzoff ('67), and Gardiner ('84), and that seen in my own preparations of the developing hoof and Müllerian tegumentary gland of the embryo pig, that I have no hesitancy in considering that the lumen of this portion of the notochordal canal, in the present instance, is being formed by a process of either horny or fatty degeneration. It is difficult to distinguish one of these forms of degeneration from the other. In the present case there seems to be no evidence that resorption aids in the formation of the posterior portion of the notochordal canal. In the anterior region, however, it is not impossible that the floor at least disappears in this manner, since nuclei and cell walls disappear at one and the same time, and are replaced by a sheet or reticulum of granulated substance.

It is interesting to note in the present instance that the anterior portion of the lumen of the canal has been completed, and has become confluent with the subgerminal cavity before the posterior portion of the lumen is formed.

Lieberkühn ('82, p. 412) finds that in the case of the guinea-pig the notochordal canal is formed with a ventral entodermal, but no dorsal ectodermal opening. In one instance he finds in the mole ('81, pp. 449 and 551) an indication of a dorsal opening. The lumen therefore cannot, he says ('82, p. 412), be formed by invagination. The head-process of *Cavia* he finds in early stages to be solid, or, when a lumen exists, it opens neither to the ectodermal surface above nor through the entoderm below ('84, p. 436). In an embryo with one protovertebra, the notochordal canal opened below for the space of eight sections ('84, p. 440). The notochordal cavity of the guinea-pig (Lieberkühn, '82, p. 412) opened below by means of a linear split. This split increased in size after its first formation, as a study of older embryos showed. Kölliker ('82) found

the ventral entodermal opening in the rabbit also. The dorsal opening is so small, and the time of its existence so brief, that Kölliker failed altogether to find it in the case of the rabbit, and Lieberkühn saw it but once in the mole. Hubrecht ('90, p. 509) observed in the mole a thickening, which he has designated the protochordal wedge. A slight canal enclosed within the ectoderm appeared within this wedge. All efforts to find so distinct a canal as Heape has recorded were in vain. Strahl ('86, p. 160, Taf. IV. Fig. 7) figures and describes a distinct dorsal opening of the notochordal canal in the cat. Heape ('83, p. 429) finds an ectodermal involution at the anterior end of the streak of the mole in the region in which the neurenteric canal had existed in younger embryos, and considers the presence of this unobliterated remnant of a canal evidence that the streak does not grow forward, but must lengthen in a posterior direction. He ('86, p. 437) also discusses the presence of the neurenteric canal in younger embryos.

Bonnet ('84, '88, '89) has found the neurenteric canal in the sheep. He states that the notochordal canal opens above and below ('84, p. 217). The opening of the canal on the ventral side is accomplished by a process of dehiscence ('88, p. 121). Dorsally the canal opens into the primitive groove of the streak ('88, p. 122). Kölliker ('82) saw the ventral opening of the notochordal canal, but later ('83, pp. 7 and 9) he stated that he found the ectoderm and entoderm united at the posterior end of the embryo rabbit and an indication of a neurenteric canal. Van Beneden ('86, p. 289) describes not only a dorsal and a ventral opening of the notochordal canal in the bat, but also a mass of cells at the posterior end of the canal which he homologizes with the Amphibian yolk plug. He ('86, p. 289) finds an opening of the canal at the anterior end of the streak in both the rabbit and the bat. Graf Spee ('88, p. 315) observed in the guinea-pig only a neurenteric strand, but in the rabbit (p. 322) he found the neurenteric canal to have a distinct lumen. Keibel ('88, p. 410) described in 1888 a rudimentary neurenteric canal in the guinea-pig which did not open dorsally, and in the following year he described a dorsal opening which he observed in one instance ('89, p. 344). Robinson ('92, Plate XXII. Fig. 13^a, Plate XXIV. Figs. 14^a and 15^d) figures a neurenteric canal for the rat, and also (Plate XXVI. Fig. 17) for the mouse. According to the observations of Fleischmann ('87, p. 12), the notochordal canal of the cat opens toward the

yolk below. Only in one case did he observe a small ectodermal sinking at the anterior end of the streak, and in this instance the cavity was plugged with cells. Selenka ('82, p. 556) considers a small ectodermal pocket in his so called gastrular region of the mouse, to be a rudiment of the neurenteric canal.

The foregoing examples merely serve to illustrate the fact that the posterior dorsal opening of the notochordal canal has become in Mammalia a rudimentary structure. Indeed, a few examples do exist in Mammalia in which the neurenteric canal has a distinct lumen, but none show a dorsal opening comparable in extent to that seen in reptiles.

In the *Chrysemys* embryo under consideration (Plate VIII.) we see no diminution in the size of the potential canal as compared with other embryos. The limits of the canal are not determined even at this stage, for we have seen in Figure 35 that the lumen is still expanding on the right at the expense of the cells in that region. A retardation in the development of the ectodermal opening of the notochordal canal in the present instance may be a forerunner of the condition found in higher vertebrates, — birds and mammals, — a condition in which the ectodermal opening develops late or not at all. The neurenteric canal of birds not only develops as a very restricted lumen when compared with that of reptiles, but it develops at a stage in which many protovetebrae are found. Gasser describes a neurenteric canal in the goose at a stage when fourteen protovertebrae are present. According to Balfour ('85, p. 163) the second neurenteric canal opens in the duck at the stage with twenty-six protovertebrae.

In this *Chrysemys* embryo it seems evident that the lumen of the notochordal canal could not have been invaginated from the dorsal surface. The notochord must therefore have been laid down as a solid process, which is secondarily becoming hollowed out. It seems interesting that even in one instance the development of the notochordal canal of a reptile should proceed in a manner so comparable to that which exists as the rule in mammals.

All students of reptilian embryology, so far as I know, have stated that the lumen of the notochordal canal develops from the posterior toward the anterior region of the shield. Will figures one instance of the gecko ('92^b, Taf. 7, Fig. 47) in which there can be little doubt of an ectodermal invagination, since entoderm

fails altogether underneath the invagination. In other cases for the gecko and the turtle Will is not certain in regard to the method by which the lumen is developed. Yet Will in all his papers, Kupffer ('82), Mehnert ('92), Mitsukuri ('86 and '93), Wenckebach ('91), Balfour ('79), Weldon ('84), and Strahl ('82) give unanimous testimony to the effect that the lumen proceeds in its development from the posterior towards the anterior end of the shield.

In regard to the method by which the lumen is developed Strahl ('82, p. 257) writes as follows:—

“Entweder ist der Canal eine Einstülpung der obersten Zellenlage nach unten, oder er entsteht durch ein Auseinanderweichen der Zellen des Primitivstreifen und läge dann also oben im Ectoderm weiter unten im Mesoderm. Dass auch die letztere Entstehungsweise möglich ist, geht daraus hervor, dass ohnedies eine Zellverschiebung bei der weiteren Entwicklung des Canals in Betracht gezogen werden muss, nämlich bei dem Durchbruch durch das Entoderm.”

The evidence which the sections of my embryo mentioned above afford leads me to the same conclusion as that to which Strahl came by inference; namely, that, in some instances at least, the canal is formed by an actual yielding or degeneration of cells *in loco*. This may be accomplished by a process of cornification aided perhaps to a limited extent by resorption.

IV. PRIMITIVE STREAK AND DORSAL NOTOCHORDAL OPENING.

1. *The Concavity of the Crescentic Opening is directed anteriorad.*
— Up to this time we have concerned ourselves chiefly with the extent of the notochordal canal, that is to say, with its length and width, and have paid little attention to the character of its dorsal opening. The history of the dorsal opening is so intimately bound up with that of the primitive streak, that both will be considered together.

Mitsukuri ('92, p. 36) has shown that almost as soon as the dorsal opening of the invagination cavity has assumed a transverse position, it becomes a crescentic slit, whose concavity is directed forward and ('93, p. 248) that later this opening bends so that its concavity is directed backward. It is during this last stage that, according to Will ('93), the ectodermic margins of the blastopore

overgrow the entodermic streak, or primitive plate, and when the ectoderm has approached the axial line of the streak it fuses with the "Randfeld" of the primitive plate, and the "Mittelfeld" is soon overgrown; thus the entodermic primitive plate is covered by ectoderm, and the primitive groove is formed.

In discussing the question whether or no my material affords support to Will's observations and theories in regard to the entodermic streak and the primitive groove, I will take up the consideration of my embryos in two groups. In the first group I will consider embryos whose open blastopore is either a transverse slit or one whose lateral arms bend forward. Under the second group I will consider only those shields whose blastoporic crescentic opening has its arms directed backward.

a. *Chelydra serpentina*. — I will describe first a specimen in which two embryos are formed on the same germ disk, the second case of this sort that has occurred in my small collection of twenty-six embryos. As in the case next to be described, so also in the present one, it will be observed that the two individuals (Plate II. Fig. 5) are not in the same stage of development. The younger individual, which we will designate as α , is distinguished from the older individual (β) primarily by the lack of the opening of the blastoporic or notochordal canal on the dorsal surface. The blastoporic opening in individual β , which is a narrow elongated slit whose concavity is directed forward, separates its medullary groove from its primitive streak. Individual α however shows, at least on surface view, no such separation into primitive streak and medullary groove. If both exist, they are continuous. Embryo α , which lies exposed throughout its whole length, is, as has been previously stated, somewhat younger or more retarded in development than the one (β) which it partly conceals.

Rauber ('77, p. 79) has found unequal rate of development to exist in the case of double embryos of bony fishes. Burckhardt ('88, p. 431), however, has observed the existence of two chick embryos on the same disk which are in the same stage of development. These chick embryos possess a primitive groove in both head and tail regions.

At the point of fusion or contact between the two turtle embryos (Fig. 5) an irregularity — a kind of folding — is noticeable on the ectodermal surface.

In order to determine the cause of this surface irregularity and

to investigate the continuity of the primitive and medullary grooves of individual *a*, we will direct our attention to sections of these two individuals. Individual *a* was laid into transverse sections as far as the right horn of the blastoporic slit in β . The remaining portion of β was then cut into sagittal sections.

Beginning at the hind end of individual *a*, the primitive streak and groove appear in two sections before the condition seen in section three (Plate VI. Fig. 30) is reached. Free thickened entoderm, however, occurs in two sections posterior to the streak or region of fused layers. In section three (Fig. 30) there is a sudden and decided deepening of the primitive groove, — a deepening which seems to pass through the ectoderm and end blindly in the midst of the entoderm below. It is impossible to know how much of this groove lies within ectoderm and how much within entoderm, for it is as difficult for me to determine how much of this thickened area in which the groove lies owes its origin to ectoderm and how much to entoderm, as it was for Selenka ('87, p. 116) to determine the limits of ectoderm and entoderm in the region of the primitive blastopore described by him for the opossum. This fusion of layers with accompanying thickening in the fused area extends through six sections, all of which show a primitive groove, which displays this decided deepening in the third (the one figured) and fourth sections counting from the posterior end of the fused area. This deepening of the groove seems to be effected by a separation and a disintegration of cells. The opening has the form of a wedge which terminates below in a mere fissure. The cells which border this opening show no indication of columnar arrangement, but one or two of them show a tendency to degenerate or become cornified. The fusion of ectoderm and entoderm continues for the space of two sections anterior to the region in which a lumen has appeared. The accumulation of cells accompanying this fusion is, however, somewhat less, while the groove suddenly becomes shallow again. The third and fourth of the sections which possess the deep groove (Fig. 30), I consider to include either the beginning of a developing neurenteric canal or else a dwarfed canal.

This restricted extent of the blastoporic opening is comparable to the condition figured by Weldon ('83, Figs. 6 and 7) for *Lacerta muralis*. Upon comparing Figure 30 with transverse sections of the blastoporic opening of other Testudinidæ, it will be seen that

the blastoporic canal has not yet attained its full normal transverse extent, and certainly its antero-posterior length falls short of the normal condition. Mitsukuri ('93, p. 248) found that the invagination cavity at an early stage is a "squarish pit rather elongated in the antero-posterior diameter." Kupffer ('82, Taf. VIII. Figs. 7, 8, 9) illustrates stages found in blastoderms of the chick incubated between nine and twelve hours, in which the prostoma possesses an anterior linear extension. A posterior prolongation also is shown in his Figures 8 and 9. This condition, which sometimes appears in the chick, is comparable to that found in the Chelydra embryo in the present instance. The transverse extent of the Chelydra prostoma is, however, much more limited than that of Kupffer's chick, while the extension of the groove anterior to the prostoma is greater in the present instance than in the chick. In both cases the groove continues anterior to the region of fusion between ectoderm and entoderm. The seventh to ninth sections of this Chelydra embryo show a condition in which ectoderm and entoderm are separated from each other, and in which both are grooved in the axial line. A drawing of section nine (Fig. 31) illustrates this condition. It is anomalous that the ectoderm in this embryo seems to contain as many yolk globules as the entoderm, or even more. From section ten onward, we find the ectodermal halves of embryo *a* to be separated in the mid line. The drawing of section thirteen (Plate V. Fig. 24) has been less highly magnified than the two sections already described, in order that it might include that portion of individual β which appears in this region. Figure 24 illustrates a condition which occurs with but little variation for twenty-two sections. The ectoderm of the two halves of embryo *a* no longer meets in the mid dorsal line. The entoderm, however, is continuous across the median plane, sometimes, as in Figure 24, with little or no indication of an axial thickening. That portion of Figure 24 included between the letters β' and β'' will, upon reference to the surface view (Plate II. Fig. 5), be seen to belong to the antero-dextral portion of individual β . Individual β is growing in an anterior direction, and, being the more advanced of the two and the more normally developed, it crowds *a* by pushing itself antierad, either over *a*, as at the point β'' (Fig. 24), or underneath *a* (Plate V. Figs. 25, 26, and Plate VI. Fig. 27). As soon as the line of continuity between the ectoderm of the two individuals is broken, β slips underneath *a*

and comes almost to reach the axis of a (Plate VI, Fig. 27). This upward pressure exerted by β upon one side of a lifts one half of a above the level of the other half, so that the ectoderm fails to fuse in the line of the medullary groove (Plate V. Figs. 24-26).

This division of the ectoderm in the axial line reminds one so strongly of the ectodermal spina bifida produced by Kollmann ('93, pp. 135, 136) in the chick and duck by means of overheating, and of the many grades of spina bifida induced by Hertwig ('92) in the frog, — a spina bifida which also oftentimes healed later, — that one has little hesitancy in considering this to be another case of partial spina bifida. The ectoderm remains separated in the axial line for a distance of sixty-one sections. At the anterior end of the embryo, however, the ectodermal halves again fuse. Hence we find that at the far anterior and posterior ends of a , — the points where the pressure exerted by β is least, — the ectoderm is not severed. In the region of non-fusion I cannot detect the slightest evidence of mechanical rupture. Each ectodermal half is smoothly rounded off at its axial edge (Plate V. Fig. 24) without the slightest evidence of a former union. In section forty-one (Plate V. Fig. 25) there is decided evidence of fusion, not however between the ectoderm of the two sides, but between the ectoderm and entoderm of the right side. Upon comparing Figure 26 (Plate V.) with Figure 27 (Plate VI.) it appears that the entoderm (*en'drm. d. β*) lying immediately beneath the ectoderm on the left hand side of a does not belong to a at all, but is that portion of the entoderm of β which has pushed forward underneath a . What then has become of one half of the entoderm of a ? If we compare Figures 24, 25, 26 (Plate V.), and 27 (Plate VI.), it will be seen that this portion fused with the notochordal entoderm of β (*en'drm. d. β*), and that it probably broke away together with the ventral entoderm of β (*en'drm. v. β*) when the notochordal canal of that individual became continuous with the subgerminal space. Perhaps the entoderm of a , like the ectoderm, was bifid in the axial line in those seven sections in which it has disappeared on the left side only; for we find that, when the entoderm was continuous in the axial line, as in Plate V. Fig. 26, the rupture occurred lateral to the axis. The section which is shown in Figure 27 (Plate VI.) falls near the dorsal notochordal opening of β and towards the anterior end of a . The ectodermal and likewise the entodermal halves of a are now continuous. The

entoderm, which has an axial thickening, is fused with the entoderm of β at the point marked by an asterisk. The notochordal canal of β has opened below (Plate V. Fig. 26) between the points *en'drm. v. β* and *en'drm. d. β* . In Figure 27 (Plate VI.) the notochordal canal of β is still losing its anterior floor at the points *en'drm. v. β* and *en'drm. d. β* , but is cut off from the yolk by the presence of the lower-lying entoderm of *a* (*en'drm. a*).

From the preceding description and the figures cited it is evident that it will be most difficult to determine the anterior limit of the primitive groove of individual *a*. If we adopt the interpretation that the primitive groove is limited to those sections in which ectoderm and entoderm are fused, then it is continuous with the medullary groove. We find, moreover, a groove on the streak in front of the small invagination, as well as on that behind it; in other words, the invagination falls within the streak, as Strahl has observed for *Lacerta agilis* ('82, pp. 242 and 246) and *Lacerta viridis* ('83, p. 9).

Miss Johnson ('84, p. 663) states that in the newt — *Triton cristatus* — the primitive groove extends throughout the whole length of the medullary groove, and that in one instance it even extended anterior to the medullary groove and there ended in a triangular pit. Keibel ('93, p. 117) has recently maintained that the primitive streak of the embryo pig at one stage reaches the anterior end of the chorda, — hence the anterior end of the future embryo, — and then recedes. Still more recently he ('94, p. 158) expresses the opinion that the primitive groove of the embryo pig in one stage extends into the region in which the medullary groove will later develop. According to the experiments of Hertwig ('92) on the frog, the blastopore extended primarily along the whole axis of the embryo. Roux ('88, p. 143) states that in the frog the chorda is formed by the fusion of the free margins of the blastopore. Kölliker ('82) for the rabbit, Heape ('83) for the mole, Bonnet ('84) for the sheep, Hubrecht ('90) for the mole, and van Beneden et Julin ('85) for the cat, have figured a forward extension of the primitive streak, but not of such a length as Keibel ('93, p. 120) has stated it to be in the case of the pig. According to Gasser the streak in the case of the chick takes part in the formation of the embryo. He ('79, p. 26) also observes a shortening of the streak, which, in agreement with the observations on *Mammalia*, takes place at its anterior end. In his stages two

and four Koller ('82, pp. 194, 195) finds no sharp boundary between the cells of the germ wall and the crescent. A decrease in the size of the crescent is accompanied by an increase in the length of the streak. But to Duval ('78, '84, '89) is due the credit of first applying from observation the theory of concrescence to birds.

When we come to reptiles the evidence that the streak takes part in the formation of the axis of the embryo is meagre. Strahl ('82) states that the notochordal invagination arises within the streak, and that portions of the streak anterior to the invagination are rapidly converted into the axis of the embryo. Moreover, he asserts that the neurenteric canal travels backward along the streak. According to Will the primary neurenteric canal in the gecko, and possibly in the turtle, closes to open later in a posterior region of the streak. Kupffer ('82, Taf. IV. Fig. 40, *e, f*) has figured for *Coluber* a short posterior axial continuation of the blastoporic opening. "In reptiles, then," writes Minot ('92, p. 123), "concrecence can only be inferred from the presence of the 'Sichel' and the growth backward of the primitive axis."

Despite a lack of corroborative evidence of concrecence in reptiles, I consider the present case one of true spina bifida. The decided evidence of continuity between ectoderm and entoderm in one half of one anterior section (Plate V. Fig. 25) justifies me, I believe, in concluding that the primitive groove extended forward at least to this point. Perhaps the failure of the ectodermal halves of the blastoporic rim to fuse may account also for the failure in the development of the normal notochordal canal (compare Roux, '88, p. 143).

In regard to embryo β of this set of twins, little need be said. The remaining portion of embryo β was cut, as stated, into sagittal sections. A section which passes through the blastopore of this embryo in the sagittal plane is represented in Plate VI. Fig. 28. Posterior to the ventral lip of the open blastopore a primitive streak is well developed. The ventral opening of the notochordal canal does not extend so far posteriad as to be included in this section. The section next figured (Fig. 29) passes through the left side of β lateral to the blastopore, and includes the region of fusion between embryos α and β . The point where this fusion takes place will be recognized by the ectodermal fold. The cavity of the notochordal canal of β lies between the layers marked

en'drm. d. β and *en'drm. v. β*. The layer of cells marked *en'drm. a* comprises a portion of the entoderm of α , which is fused with that of β at the posterior end of the streak at a point in the section marked by an asterisk.

A second example of a double embryo was found two weeks later than the one just described. As in the preceding case, so in this one, we will designate the two individuals as α and β . The embryo with an elongated streak posterior to the blastopore (Plate II. Fig. 8, left half) will be distinguished as α . (By an error α and β are interchanged in Fig. 8. See Diagram I., p. 6, for correct lettering.) This streak, which is so much elongated as to resemble the handle of a dipper, the bowl of which is represented by the second embryo, β , furnishes a striking parallel to the condition of the streak in the emu figured by Haswell ('87) in his Plate VIII., and again in his Diagrams 5 and 6 (p. 584), which "are intended to illustrate the manner in which, as pointed out by Duval, the anterior end of the primitive streak comes in its later stages to be situated so far forwards simply by the considerable extension of the area pellucida on all sides."

In the turtle (individual α) the streak is seen to be divided at its anterior third by a sort of ridge. The portion anterior to the ridge is comparable in extent to the streak in the emu described by Haswell, and possesses a true primitive groove, shown in Figure 8 as a fine sharp line, whereas the posterior two thirds show along the axis a rather broad depression instead of a true groove. Sections show, however, that these two grooves are structurally continuous with each other, and that therefore the posterior portion is not due simply to an accidental folding of the blastoderm induced during the process of hardening. Unfortunately, the whole blastoderm was not preserved; consequently the condition of its edge behind the elongated streak of α cannot be stated; it is uncertain whether this streak extended to the edge of the blastoderm and to a marginal notch, or even whether a marginal notch existed. The margin of the shield of embryo α extends around to, and is continuous with, the blastopore of embryo β . One horn of the blastopore of embryo β (Plate II. Fig. 8) was unobserved under the low magnification at which this figure was drawn. Diagram I. has been reconstructed from the sections in order to show the position of this horn of the blastopore, and the extent of the notochordal

invaginations; the positions of the sections figured in Plates IV. and V. are indicated by parallel lines, which are numbered to correspond with the numbers of the figures.

Since these embryos, a and β , were situated almost at right angles to each other, a series of sections could be made to furnish nearly sagittal sections of β and cross sections of a . The sections were cut in succession from the right toward the left portions of β . A description of a sagittal section through embryo β (Plate IV. Fig. 14.) has already been given. Figure 15 (Plate IV.) represents a section which passes through the horn of the blastopore of embryo β (the depression near the letter a in Figure 15), and the margin of the shield of embryo a (at the letter β of Figure 15). Compare Diagram I.

A little below and to the *right* of the cut horn of the blastopore of β is seen a cavity which seems to be continuous with the notochordal canal of a ; it has been correspondingly lettered in Figure 14. The section shown in Figure 16 (Plate IV.) passes beyond the left horn of the blastopore of embryo β , and across the axis of a . The region of the notochordal canal of β is indicated in Figure 8 (Plate II.) by the line along the axis anterior to the blastopore. This line is much too sharp, for it is impossible from the sections to make out any corresponding depression of the surface. The ventral wall of the notochordal canal of a is unbroken (Fig. 16), and there is no division into primary and secondary entoderm discernible. The letter β indicates in this section, too, the margin of the shield of embryo a . Figure 17 (Plate IV.) represents the seventh section anterior to the dorsal lip of the notochordal canal of a . We have already reached a point in this embryo, in passing from in front backwards, where the ectoderm and entoderm of the dorsal lip are fusing. This fusion becomes more and more pronounced as we pass backward through each of the six sections which lie in front of the dorsal opening of the notochordal canal. The mesoderm shows a differentiation into splanchnic and somatic portions, and is separated from the entoderm. In the mid-line on the floor of the canal a slight groove (*sul. pr. p.*) is evident, even under low magnification. A cross section of this groove is shown under higher magnification in Figure 22 (Plate V.). The cell walls, nuclei, and yolk granules were all carefully outlined in this drawing by means of the camera lucida. In both Figure 17 and Figure 22 this groove appears to

terminate ventrally in a seam or closed fold, which dips down into the mesoderm. The presence of this seam is indicated in section by the fact that the boundaries of the cells here stain more deeply than mere cell walls, and that the cells which adjoin it are more or less flattened perpendicular to the plane of the fold. A single cell on the left bounding the seam and reaching to the surface is particularly remarkable in this respect. Its dorso-ventral length corresponds with that of two and a half somewhat elongated cells on the opposite side of the seam. Even the nucleus of this greatly flattened cell is correspondingly altered in form. This cell contains, besides the nucleus, two yolk globules. This condition of the groove occurs, as already stated, on the floor of the canal in the seventh section anterior to the dorsal lip of the blastoporic opening. The groove, however, continues anterior for four or five sections more before it fades away. If we follow the groove in sections posterior to the one described, we find that it is distinguishable in all the sections in the median line of the floor of the invagination; as we pass backwards, it increases in distinctness, and becomes continuous with the primitive groove occupying the axis of the streak behind the blastopore.

The next figure (Plate IV. Fig. 18) represents the fourth section behind the blastopore, and passes through the region of the so called plug of Will, Mehnert, and Mitsukuri (compare also Plate V. Fig. 23). Mesoderm and entoderm are still distinguishable as separate layers, but ectoderm and mesoderm are fused. Along the margins or lateral boundaries of the streak I can distinguish in sections no such separation into ectoderm and entoderm as Will has described for both the gecko and the turtle. Will criticises Mitsukuri's drawings for their failure to bring out this separation. I have repeatedly and carefully gone over all my sections in order to discover such a line of demarcation. Among twenty-six embryos of about the same stages as those described by Will, I have found but once and in only one section — then on only one side of the streak — evidence of such a separation. I consider this separation to be mechanical, for I cannot find a trace of it in the preceding or succeeding sections.

Figure 23 (Plate V.) represents under greater magnification the fused area or streak shown in Figure 18 (Plate IV.). Here also the outline of each cell and nucleus was carefully made by aid of the camera lucida. At the extreme right of the figure are

represented the first two ectoderm cells, which show a distinctly columnar form. Adjoining these on the side toward the primitive groove are four ectoderm cells which are distinctly separated from the lower lying cells. The last of the four is the one indicated by the dotted line running from the letters *ec'drm.* These four cells, however, are not of columnar form. The next cell which adjoins these four, again on the side toward the groove, is exactly like them in character, but is not separated from the cell layer below. The two succeeding cells, continuing in the same direction, are dividing along a plane parallel to the ectodermal surface, and are undoubtedly contributing to the formation of the deeper cell layer. In the centre of the streak a V-shaped groove exists, from the bottom of which a sharp line extends still deeper, as though marking the direction of a fold. This groove continues along the axis of the streak for the space of seven sections behind the open blastopore. In Figure 19 the groove is no longer evident. The ectoderm remains fused with the mesoderm in this and four succeeding sections, yet it is discernible as a well marked layer continuously across the region of the streak, as Mitsukuri and Ishikawa ('86, p. 30) have described it in *Tryonix*. There is not the slightest indication in the grooved region, or behind it, of that differentiation of the streak into "Mittelfeld" and "Randfeld" described by Will for the gecko ('92^b, Plate II.) and for the turtle ('93, pp. 569-571). In Figure 19 it is shown that the mesoderm and entoderm are fused. From this section posteriad the entodermic layer begins to grow thinner, until, after a long stretch, a condition is reached in which there is below the ectoderm, and entirely free from it, a series of clumps and strands of cells scattered on the yolk or partially embedded in it. This condition extends for some distance laterad to the axis of the streak on either side (Plate V. Fig. 20). This stretch of cells is really narrower in extent than the mesoderm of the streak in Figure 19, for Figure 20 is outlined under a higher magnification than the preceding figure.

We now pass along the streak until we reach the condition seen in Figure 21 (Plate V.). In this section the ectoderm seems to be fused in the median line with a lower lying continuous row of cells, which stretches laterad for a short space on either side of the region of fusion. This lower layer shows a tendency to fuse here and there all along its extent with the ectoderm. Unfortunately

this is the last section of the series which has been preserved, for during the process of embedding the remaining portion of the blastoderm broke away. An explanation of this thickened line of entoderm, continuous with the primitive streak, and extending backward through the greater portion at least of the area opaca, will be suggested during the description of the next older shield.

The notochordal canal of this next older embryo (Plate I. Figs. 3, 3', and Plate VIII. Fig. 35) has already been described; therefore we will immediately direct our attention to the study of a series of cross sections of its primitive streak. Figure 36 (Plate VIII.) represents the condition found in the fifth section behind the blastoporic opening. The histological conditions of this section and the four anterior to it are almost identical. The positions of this and the following sections are shown on Figure 44 (Plate VIII.). Mesoderm and entoderm are continuous along the whole breadth of the streak. The ectoderm is continuous with the lower layer at two points, one on either side of a central region, in which the ectoderm is completely separated from the underlying cells. In the ninth section (Plate VIII. Fig. 37) posterior to the open blastopore, this central region is somewhat arched. The ectoderm is still proliferating cells into the lower layer at the right of this central arch. In Figure 38 the central area has become still more elevated, and the two lateral areas, which were centres of proliferation in Figure 36, have now become two compact clumps of cells, which are indissolubly fused with the entoderm below. Upon reference to surface views (Plate I. Figs. 3 and 3') a central elevation is seen to continue backward along the region of the streak. The portions at the two sides of the streak seem to be composed each of two terraces. Figure 38 passes through the posterior end of the anterior (or inner) terrace. Figure 39 passes through the posterior terrace twenty-three sections behind that of Figure 38. In this section (Fig. 39) the streak has become narrower and much flatter. On account of the presence of a layer of hardened yolk, which lies upon the dorsal surface of the streak at this point, it is impossible to make out the exact condition of the streak from surface views. The next posterior section, of which Figure 40 is a representation, passes very near the posterior margin of the shield. In this section the ectoderm has parted in the median plane, so that the yolk has streamed out through the gap and spread over the surface of the streak. In the thickened central

area in this figure, as well as in Figure 39, it is impossible to distinguish ectoderm from entoderm, excepting at the margins through which the yolk has streamed out. Wherever the ectoderm of opposite sides of the median plane has succeeded in uniting in this central area, a differentiation into ectoderm and entoderm is discernible (Figs. 38 and 39). Seven sections behind that shown in Figure 40 (Fig. 41) the edges of the ectoderm fail by a considerable distance to meet in the mid-line. At the margins of the uncovered area the ectoderm bends downward and lateralward to become continuous with the cells of the entoderm and yolk. This central area may be considered to be one of uncovered yolk. Figure 42 is a drawing under a higher magnification of this uncovered area and the adjacent covered area on one side of it, twelve sections behind that of Figure 41. This figure was outlined with the camera lucida even to the smallest details. In this figure the layer which is folded in under the ectoderm shows no tendency to fuse with the ectoderm above it. In other sections, however, this whole lateral region is composed of a mass of cells upon whose dorsal surface the ectoderm is not well differentiated. The margins of the ectoderm continue to be separated in the axial line for the space of twenty-five sections. Wherever the yolk has streamed out over the dorsal surface of the ectoderm, the dorsal ectodermal surface seems to be absorbing this yolk, for in many sections the external surface of the ectodermal layer has no distinct boundary, and a few nuclei are present in the yolk above it.

As we pass posteriad the separated ectodermal margins gradually approach each other, and finally fuse (Fig. 43). In the immediate vicinity of the point of fusion the ectoderm is not separated from the entoderm below. From this point of union posteriad the entoderm begins to thin out, and finally, when we arrive at the sixty-fourth section behind the posterior end of the uncovered area, we meet such a condition of ectoderm and scattered entoderm as exists over the whole area opaca. The cellular condition of this streak within the area opaca behind the uncovered area is similar to the condition found in the streak within the area pellucida of embryo *a* (Plate II. Fig. 8, and Plate IV. Figs. 18 and 19, and area opaca, Plate V. Fig. 20) of the preceding pair of twins. In embryo *a* (Fig. 8) the ectoderm must have fused in the axial line as rapidly, or nearly as rapidly, as the

embryonic area progressed backward. In the present instance, however, the lips have failed to fuse in the anterior region of the area opaca. Whether the conerescing margins failed to fuse on account of a mechanical obstacle, the presence of yolk, or whether the yolk subsequently protruded through the area of nonfusion, can only be conjectured. This region in this single instance is the only one observed by me that is in any way comparable to Will's uncovered entodermic streak, and in this case the region lies almost entirely outside the area of the shield. It is possible that the streak within the shield may have been formed in this retarded manner. Such a method of development might account for the persisting areas of lateral proliferation and the early differentiation of ectoderm in the axis of the streak. The crescentic dorsal opening of the notochordal canal (Fig. 3) still directs its concavity anteriorly; therefore the development of the ectoderm over the streak and the backward turning of the horns of the open blastopore are two entirely independent processes. Moreover, in the present instance there can be no division of the streak into the "Mittelfeld" and "Randfeld" in the sense in which Will employs these terms, since the middle area has become covered with ectoderm before the separation between ectoderm and entoderm is accomplished laterally. According to Will's theory, this "Mittelfeld" is entodermic, and should be the last region to be covered.

b. *Chelopus insculptus*.—Views of the under surface of the embryo show (Plate I. Fig. 2') that the notochordal canal has opened ventrally. The concavity of the dorsal crescent-shaped opening (Fig. 2) is still directed anteriorly. Only two drawings of sections through the streak region of this embryo will be reproduced, for they will illustrate the histological condition of the whole streak. Figure 47 (Plate IX.) exhibits the third section behind the blastopore. The ectoderm is much thickened, and composes the greater portion of the streak. A shallow groove is present along the axis of the streak, and lateral to it there are two depressions, one on either side. These lateral depressions are the first stages in the development of the posteriorly directed horns of the open blastopore. These depressions later sink deeper into the streak and divide it into a central elevation—the so called plug of several reptilian embryologists—and two lateral portions. In Figure 47 there is no sharp boundary between the columnar ectoderm of the

lateral areas and the ectoderm of the central area. Six sections behind the open blastopore (Plate IX. Fig. 48) the ectoderm over the surface of the streak becomes completely differentiated from the underlying cells. Here and there, however, it is fused for the space of a few cells with the entoderm. In the lateral regions it is possible to distinguish the splanchnic and somatic layers of the mesoderm; but in the centre of the streak entoderm and mesoderm are indistinguishable.

This and the preceding examples are sufficient to show that the primitive groove exists along the streak before the turning backward of the margins of the open blastopore. Therefore, if the backward growth of the blastopore should produce a groove on the streak, this groove must be a structure quite different from the primitive groove.

Although it is impossible for me in the two instances of an elongated streak previously described, to connect these streaks with the edge of the blastoderm, as Whitman ('83) has done in the case of an abnormal chick, yet it seems impossible to account for the presence of a streak over so great a portion of the area opaca, unless we grant that the edges of the germ ring have continued to unite for a much longer distance than usually occurs in birds and reptiles. Therefore my two embryos seem to me to form an intermediate stage between the normal streak of reptiles and that described by Balfour for Elasmobranchs, and by Whitman for his abnormal chick.

I have little to add to the question whether the whole germ ring represents the blastopore (Rauber, '77, '80; His, '78), and the embryonic axis only a portion of the blastopore, or whether only a portion of the germ ring represents the gastrula mouth (Hertwig, '92, '93; Duval, '78, '84; Minot, '92; and Cunningham, '86). If the blastopore has experienced a hernia, as Cunningham suggests, concrescence of the non-blastoporic germ ring could continue nevertheless. The condition of the separated ectodermal borders along the streak in the region of the area opaca, as seen in Figures 41 and 42, would seem to yield evidence for the His-Rauber theory, since in this region the ectoderm is decidedly infolded. At the prostoma marginale the ectoderm goes over into the entoderm (Rauber, '83, p. 165). But this infolding and proliferation, as seen in Figures 41 and 42, may have a mechanical cause only. It is difficult, however, in the case of Figure 42, to conceive of

a mechanical cause which could have prevented the coalescing of the germ ring, and yet produced an increased accumulation of entoderm along the median line. The yolk in this instance appears to lie in a perfectly normal position. In the specimen from which Figures 3 and 3' were drawn, there is a decided difference between the streak of the embryonic region and that of the area opaca, — a difference which was evident on surface view. This difference would seem to argue for the interpretation of Duval and Hertwig.

2. *The Concavity of the Crescentic Opening is directed posteriad.* — Hitherto we have considered sections through the region of the primitive streak of embryos whose dorsal notochordal crescentic opening has not yet bent posteriad. We proceed now to those stages in which the crescentic opening is bent posteriad, so as to include in its concavity that portion of the streak which has been considered by several reptilian embryologists to be homologous with the amphibian yolk plug. Embryos of this stage fall distinctly into two groups, namely, those embryos along whose streaks a groove extends up to the open blastopore, — a groove which can be recognized on surface view, or in section, or both, — and those whose posterior blastoporic horns have sunk so low into the streak that the central plug-like area has lost its primitive groove.

a. *Chelydra serpentina.* — Figures 4 and 4' (Plate I.) are surface views, dorsal and ventral respectively, of an embryo *Chelydra serpentina*. From a ventral surface view it is seen that the notochordal canal has opened ventrally. Upon dorsal view the horns of the open blastopore are seen to be bent somewhat posteriad, and to embrace the so called yolk plug region. The posterior bending of the horns is however as yet very slight. In the mid-dorsal line a distinct groove leads posteriad from the blastopore across the so called yolk plug region, and continues backward along the whole surface of the streak.

Mitsukuri ('93, Plate VII. Fig. 8) figures a similar condition, which he considers to be teratological. In his example, however, the groove does not reach the posterior end of the embryonic shield. Will ('93, Taf. 33, Fig. 9^b, p. 561) has figured and described a condition of the blastopore of *Cistudo lutaria*, which he considers to be a condition intermediate between that of a blastopore of the gecko figured by him ('92^b, Taf. 1, Fig. 7), and Kupffer's *Coluber*. This *Cistudo* blastopore possesses a short posterior extension.

According to Mitsukuri ('86, p. 28) the so called plug is composed of undifferentiated cells. Behind the plug the ectoderm extends over the whole surface of the streak and proliferates cells along the axis into the cell mass below. Will, on the other hand, considers the plug and streak to be composed of exposed entoderm. In the gecko a primitive groove does not exist on the streak until the open blastopore becomes elongated in an antero-posterior direction. He says ('92^b, p. 132), "Die beiden Schenkel der winklig geknickten vordern Urmundlippe nähern sich einander immer mehr und mehr, so dass sie einander bald parallel verlaufen und dadurch eine Primitivrinne entsteht, deren Ränder von der Urmundlippe selbst, deren Boden von dem Entodermpfropf, resp. dem Primitivstriefen gebildet wird"; and on page 141 he says that this union of ectoderm begins to take place first at the anterior lip of the dorsal notochordal opening, and spreads gradually posteriad along the streak. Will ('93, Taf. 34, Figs. 17^b-17^d and Taf. 35, Figs. 18^a-18^c) represents transverse sections through the streak region of a *Cistudo*, in which the horns of the notochordal opening have begun to turn posteriad. In these sections a distinct separation is shown between the entodermic streak and the ectoderm, and the ectodermal borders still lie at some distance from the axial line of the streak.

In the present instance (Plate I. Figs. 4 and 4') the horns of the blastopore have only just begun to bend backward, and yet a deep groove extends along the whole surface of the so called plug and streak region. Cross sections show that a deep groove exists in this region. After these drawings were made the embryo suffered somewhat from overheating in preparation for sectioning, so that I will not describe its histological condition. There is the less need of it, since I possess two other embryos, whose histological condition is faultless, which show the same groove.

Figure 7 (Plate II.) represents an embryo possessing a much shorter streak region, but a groove such as existed in the embryo just described extends along its axis. This embryo (Fig. 7) was cut parallel to the sagittal plane, and therefore does not furnish such suitable sections for studying the groove question as are afforded by series cut crosswise to the axis. Another embryo, which in surface view so resembled Figure 7 that it is unnecessary to figure or describe its surface appearance, was cut crosswise, thus furnishing the necessary sections. Figure 50 (Plate X.) represents the

third section behind the dorsal notochordal opening of this embryo. The dorsal lip of the crescentic blastopore is cut nearer the median plane on the left side than on the right, so that it overhangs the primitive streak region more on that side than on the right. On both sides the lip of ectoderm is much thickened. Between these lateral ectodermal elevations is situated a third, the region called by Will and Mitsukuri the plug. I can distinguish no boundary between the cells which constitute the surface of the so called plug and the cells of the ectoderm lateral to the plug. The surface of the plug is divided in the median line by a distinct groove, which begins at the posterior lip of the blastoporic opening, and extends over the plug and along the streak for twenty-two sections. Figure 49 represents the tenth section behind the open blastopore; it passes through the posterior end of the plug. The cells at the surface of the plug show a tendency to become columnar, but this layer is indissolubly fused with the entoderm below, while the groove is still very distinct. The groove continues on backward over the streak, but becomes fainter and fainter, until finally at the twenty-third section it disappears.

b. *Ozotheca odorata*.—The conditions which I have described for *Chelydra* are not peculiar to that genus. As another example of a grooved plug, I will describe the condition in a single case of *Ozotheca odorata*. A dorsal surface view (Plate II. Fig. 9) presents an anomalous condition of the blastoporic opening. The notochordal canal is interrupted at the anterior end of the posterior third of the shield by a thickening of cells through which the neurenteric canal runs perpendicularly to open below. This lumen appears in two transverse sections only, while the thickening itself is fused with the ectoderm for the distance of five sections anterior to the neurenteric canal. As seen in dorsal view, two pairs of horns extend anteriad from this canal, and one pair posteriad. The plug region, which is included between the two posterior horns, is grooved. Figure 54 (Plate XI.) represents an oblique section through the neurenteric canal. Figure 55 represents the third, and Figure 56 the sixth, section behind the canal. On surface view and in sections one horn of the open blastopore, the right, is seen to extend farther posteriad than the other. In cross section the surface of the plug shows a median groove, which continues backward along the streak. Figure 58 is a more highly magnified representation of the plug region of the section seen in

Figure 55. The depression in the centre of this plug is not prolonged ventralward into a closed fold, as was the groove on the plug of Figure 23 (Plate V.).

It seems unnecessary to add to this list descriptions of the streak region of other embryos for the purpose of emphasizing the fact that a groove may exist along the axis of the streak up to the very lip of the notochordal opening, both before and after this opening has begun to bend posteriad. This condition exists too frequently in my collection of twenty-six embryos taken from five genera for me to accept Mitsukuri's explanation ('93, Fig. 8, pp. 248, 249) that, in this respect at least, they are teratological. In an earlier paper, Mitsukuri ('86, pp. 29, 30) refers to the fissure in the "Zapfen" of *Coluber* described by Kupffer. He writes: "We have also observed a similar appearance in some of the earlier embryos of *Trionyx*, but we are satisfied that there is no true median fissure. What appears to be such is the optical expression of the primitive streak, along which the ectoblast is proliferating, and giving cells to the mesoblast below. Even in the earliest embryos with this appearance, it is doubtful if it ever extends to the extreme tip of the plug. . . . The reason why the yolk plug in *Trionyx* is more conspicuous at this stage than earlier stands, we think, in close connection with the fact that the blastopore has become a much better defined horseshoe-shaped slit."

3. *Concerning the so called Plug.*—A yolk plug, to be in agreement with that of the frog, ought to be more clearly defined in early stages. Moreover, Will ('90, p. 599) writes that, after the horns of the blastopore bend backward, "Die Schenkel nehmen mit dem Auswachsen des Primitivstriefs an Länge zu, rücken einander immer näher und näher und bilden so eine Primitivrinne, welche auf der Oberfläche des Primitivstriefens verläuft und an ihrem vordersten Ende in den Kupfferischen Gang sich hinabsenkt." Again ('92^b, pp. 125, 126) he writes: "Der entodermpfropf geht so allmählich in den hintern verdickten Theil der untern Urdarmwand über, dass es den Bildern Zwang anthun hiesse, wollte man irgendwie eine Grenze statuiren. . . . Der Entodermpfropf stellt somit auch beim Gecko nur einen Theil der untern Urdarmwand dar, wenn mann nicht vorzieht, sich dahin auszudrücken, dass in Folge des raschen Wachsthums der Urdarmeinstülpung ein Theil des Entodermpfropfes mit in die Einstülpung hinabgezogen wird." If now we return to Will's ('90, p. 599) earlier statement, it seems

that, after the entodermic streak has been overgrown by ectoderm, and the primitive groove is formed, this primitive groove at its front end sinks down into Kupffer's duct. According to Will, not only a portion of the entodermic plug comes to lie on the ventral floor of the primitive gut, but still later a portion of the streak likewise occupies the same position.

Robinson and Assheton ('91) had already criticised the comparison of the streak with the yolk plug as follows: "According to Mitsukuri and Ishikawa, the streak consists in the first instance mainly of a mass of hypoblast or yolk, which they compare to the yolk plug of Amphibians. To this, however, it cannot correspond, for we have already shown that the yolk plug of *Rana* is a portion of the ventral wall of the archenteron, whilst the primitive streak is formed by the fusion of the lateral lips of a deficiency in the posterior wall of the same cavity." If Will had recognized the fact that the lengthening of the notochordal canal is produced, in part at least, by a progression backward of its dorsal opening, I believe he would not have been drawn into these statements. He would then have appreciated the fact that, during those stages in which the neurenteric canal descends so obliquely anteriad, the anterior or dorsal lips are fusing more rapidly than the posterior or ventral lips are reopening. The apparent occupation of the ventral floor by the primitive streak is only transitory. What now lies ventral will divide, pass laterad, upward, and then mesiad to again fuse in the dorsal lips in a position posterior to that which it at one time occupied on the ventral floor. In later stages the opening of the posterior lip more nearly keeps pace with the closure of the anterior lip, so that the neurenteric canal comes to occupy a perpendicular position in relation to the embryo and yolk. I shall recur to this point again, when my meaning will be made clearer by the aid of diagrams.

Will maintains that the primitive groove is formed upon the entodermic streak by the growing over and union of the ectodermal lips of the blastopore. It is formed, therefore, after the horns of the blastopore bend posteriad. Mitsukuri ('93, pp. 259, 260) is inclined to the same belief, although in an earlier paper he refers to a primitive streak which exists posterior to and contemporaneous with the yolk plug. My sections, on the other hand, compel me to conclude that during these later stages the primitive groove disappears. It is at the time when the horns of the open blasto-

pore bend backward, extend along the streak, and sink down into it, that the streak becomes so modified that it loses its groove. It is only in rare instances that the groove persists so long as in the case shown in Figure 9.

Dorsal surface views of the embryos shown in Figures 9, 11, and 13 indicate that these embryos, with the possible exception of Figure 9, belong to a stage later than any embryos previously described in this paper. Figures 51 and 53 (Plate X.) are representations of the third sections behind the open blastopores of Figures 13 and 11, respectively. Neither Figure 53, which is drawn under a high power, nor Figure 51, drawn under a lower magnification, shows any line of demarcation between lateral ectoderm and streak. I am unable to find such a line of separation in any of my sections. Mitsukuri ('93), after carefully re-examining his sections, fails to find such a separation. The ectoderm seems to pass insensibly into the cellular condition of the plug. The plug has become a rounded, dull-pointed structure, along whose surface a groove is no longer discernible. The cracks or blastoporic horns which bound this central area laterally contain a few fragments of degenerating cells. Moreover, Figure 51 shows a most interesting condition, which I have also observed in two other series of sections. On the outer side of the right horn of the blastopore the ectoderm and entoderm are fused. It is impossible in this region to distinguish a histological condition different from that which exists in the so called plug region. At the angle between the plug and the elevation at the right of it in Figure 51 there is a tendency of the superficial layer of cells to become columnar. The mere loss of surface indication of ectoderm is not sufficient therefore to establish the existence of an entodermic plug or yolk plug; for otherwise in the present case we should have a second plug at one side of the blastopore.

The opening of the notochordal canal on the ventral floor, as seen in Figure 11', is so far posterior that it persists for only four sections in front of the dorsal lip. The dorsal lip or roof shows evidence of a fusion of ectoderm and entoderm for two sections in front of the anterior termination of the ventral lip. Figure 52 (Plate X.) is a representation of the third section in front of the posterior margin of the dorsal lip. A fusion of the halves of the anterior lip can be traced distinctly through six sections.

Will accounts for the presence of his so called plug and primi-

tive groove on the floor of the notochordal canal by the supposed fact that the streak grows forward, presses into the blastopore, and causes it to assume a horseshoe shape. In the course of rapid invagination a portion of the plug is drawn down into the canal. In agreement with Mitsukuri ('93), I am unable to accept this explanation. Strahl ('86, p. 160) produces evidence which leads him to believe that the streak grows backward.

What is the meaning of this fusion of the halves of the anterior lip of the open blastopore which I have so repeatedly observed in my sections? What, moreover, is the significance of the median notch which exists in the anterior lip of the blastopore of so many reptilian embryos? Such a notch has been figured by Will for the gecko; by Strahl, Wenckebach, Weldon, and Balfour for the lizard; by Agassiz and Clark, Kupffer, Mehnert, Mitsukuri, Hoffman, and myself for the turtle.

The primitive groove does not come to lie upon the floor of the notochordal canal by a forward growth and invagination of the streak. Such a forward proliferation would, it seems to me, obliterate all traces of the groove. The primitive groove comes to lie temporarily on the floor of this canal, because in the passage posteriorly of the neurenteric canal the halves of the anterior lip fuse more rapidly than the ventral lip opens. The rapid differentiation of the medullary and notochordal areas would tend quickly to obliterate all trace of fusion of the halves of the dorsal lip, while a groove may persist on the ventral lip for a long time.

According to Will ('92^b, p. 132), there are two neurenteric canals formed in the gecko. The first of these he names Kupffer's canal. This canal closes and a second neurenteric canal opens later. Concerning the neurenteric canal in *Cistudo* he writes ('93, p. 577): "Demnach ist es wenn auch nicht vollkommen sicher, so doch höchst wahrscheinlich, dass der Kupffersche Gang, wie beim Gecko, etwa um die Zeit der Bildung der Medullarinne schwindet, und dass der bei ältern Embryonen aufgefundenene weite Canal einen neuen Durchbruch, einen *canalis neurentericus* s. str. darstellt." Van Wijhe ('88, p. 76) has found a stage in the development of selachians in which the neurenteric canal is present, while the blastopore still persists, and according to Goette ('88, p. 161) the prostoma of *Petromyzon* remains to form the anus. Shipley ('86, p. 331) also testifies to its permanency in *Petromyzon*. We are accustomed, Will ('92^b, p. 132) writes, to find the neurenteric

canal of most mammals and lizards open until its definite disappearance. Mehnert's statement, that in *Emys* "verödet das Anfangsstück des Urdarmcanals gänzlich," is considered by Will as not conclusively demonstrated.

My collection contains a sufficient number of older stages in which to trace a disappearance and reopening of the canal, if such a condition exists in turtles.

Figure 6 (Plate II.) illustrates a stage in which the medullary folds are formed, and Figures 12 and 12' (Plate III.) one in which these folds have begun to close dorsally. The lumen of the horseshoe-shaped canal still persists in both cases, but is much smaller than in the stages previously described. This diminished lumen still encloses between its horns a reduced portion of the streak. I believe that at no time does the neurenteric canal of *Chelydra serpentina*, at least, close to be later reopened.

Strahl was the first to maintain that the open portion of the blastopore migrates backward along the streak in *Lacerta agilis*. Sections show, he says, that in spite of the lengthening of the embryo the streak becomes shorter; and since the streak is not absorbed, the neurenteric canal must close in front and open behind. This backward progress of the anterior lip of the neurenteric canal resulting from a process of fusion may take place, and yet all traces of such fusion may be so quickly obliterated that any evidence of it is difficult to find in section.

Agassiz and Whitman ('84, p. 74) have stated that in the case of Teleostei, the whole germ ring is converted into the axis of the embryo. "The concrescence appears under the disguised form of a migratory movement of cells, which accompanies the epibolic growth of the blastoderm." A marginal notch existed in two of their embryos. A notch is almost always distinctly seen on the anterior lip of the notochordal canal of the turtle embryo. Kingsley and Conn ('83) describe only an inflection of the epidermal layer, while Ryder ('84, p. 565, p. [71] of separate) and Locy ('94, pp. 398-400) have seen metameric segments lying outside the axis in the germ ring of *Elacate* and *Squalus acanthias* respectively. Therefore, in the case of those fishes in which concrescence seems to be a certainty, this process may be so evanescent as entirely or almost entirely to escape observation.

Of all the theories to account for gastrulation in reptilian development formulated by embryologists, that set forth by Wenckebach

('91, pp. 74, 75), seems to me more nearly to fulfil all the conditions than any other. If we conceive that invagination takes place normally, not as in Amphibia, at the edge of the blastoderm, but a little anterior to this growing margin, then we can at once comprehend the meaning of the entodermic layer which forms in early stages the floor of the gastrula cavity. In abnormal cases, however, gastrulation might take place at the edge of the blastoderm. Such a course of development would account for the appearance seen in Whitman's abnormal chick, and possibly that in my own two stages in which the groove extends so far posteriad beyond the embryonic shield.

Concerning the question of a primitive streak, Wenckebach ('91, p. 73) writes as follows: "Denkt man sich dazu das Lumen der Gastrulaeinstülpung mehr oder weniger reduziert, so wird, wie schon vielfach aus theoretischen Gründen verteidigt wurde, die hintere Blastoporuslippe zum Primitivstreifen (Gastrulaleiste), die Stelle, wo die Einstülpung stattfindet, wird zum Hensen'schen Knoten." Hensen ('76, p. 266) describes on the primitive streak of the guinea-pig a groove which later disappears. I have already quoted the objection of Robinson and Assheton ('91) to Mitsukuri's yolk plug theory. The yolk plug represents a portion of the ventral floor of the archenteron, while the streak "is formed by the fusion of the lateral lips of a deficiency in the posterior wall of the same cavity." Bonnet, describing in mammals the plug structure in question, says: "Der Endwulst selbst ist geschildertermassen der verdickte Rest des Primitivstriefens"; and Heape refers to it in the mole as "the primitive streak forced upwards as a rounded knob at the posterior end of the latter." Kiebel ('93, pp. 104, 105) writes as follows: "So lässt sich van Beneden, wenn er den unregelmässigen Zellpfropf, welcher im Grunde der Primitivrinne steckt, als Dotterpfropf auffasst und dem Dotterpfropf der Amphibien homologisirt, meiner Meinung nach, nur von einer äusserlichen Aehnlichkeit leiten, die sich, wenn man den Dingen näher nachforscht, als trügerisch erweist. Zunächst muss es auffallen, wie bei Eiern von so extremen Dotterarmuth, wie es die Säugethiereier sind, ein Dotterpfropf sich bilden und den Blastoporus auseinander drängen kann oder, besser gesagt, am Verschlusse hindern soll. Er ist ein Erbtheil von dem Amphibien, könnte man antworten. Wie will man sich das aber vorstellen? Der Dotterpfropf hat bei den Amphibien keine grosse morpho-

logische Bedeutung, er ist ein Entwicklungshinderniss, er hat keine Funktion. Warum sollte gerade diese Bildung so zäh festgehalten werden, während doch so vieles andere, das von ungleich grösserer Bedeutung ist, undeutlich wird und verschwindet. Bei den Sauropsiden aber hat sich der Theil des Urmundes, der dem Primitivstriefen der Säuger entspricht, auch schon in eine Primitivstreifenbildung umgewandelt. . . . Der Dotterpfropf der Amphibien ist Darmentoderm, das, wenn es erhalten bleibt, zum Darmentoderm sich umwandelt und sogar zum Entoderm der ventralen Darmwand. Der Zellkomplex, den van Beneden als Dotterpfropf bei Säugern auffasst, jene Zellmasse, welche sich bei verschiedenen Säugern im Grunde der Primativrinne nachweisen lässt, wird aber bei Säugern Mesoderm und liefert auf keinen Fall Material für die ventrale Darmwand."

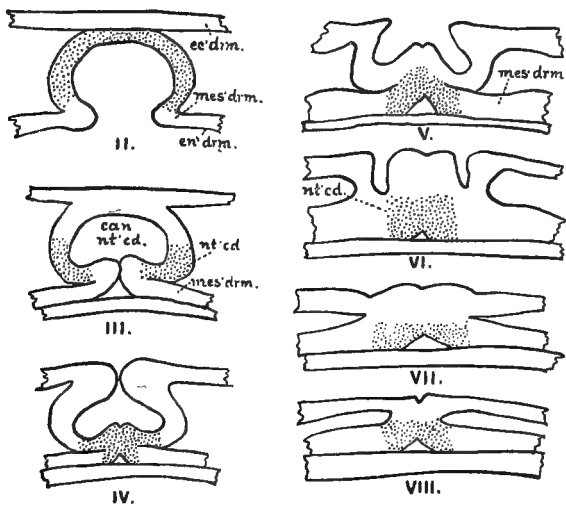
V. REGRESSION OF THE NEURENTERIC CANAL ALONG THE STREAK.

While I believe that the so called plug is only a modified portion of the primitive streak, I cannot entirely agree with Heape, for in the turtle at least this portion of the streak is not in reality "forced up." The plug-like eminence never rises higher than the level of the lateral ectodermal surface. Indeed, it either just equals the lateral ectoderm in elevation, or else falls below the level of it. That such a condition exists is evident not only in many of my own figures, but also in those of Mitsukuri and Ishikawa ('86, Fig. 9, Plate III., and Fig. 18, Plate IV.) and Will ('93, Taf. 36, Fig. 24"). Mitsukuri and Ishikawa ('86, p. 28) write as follows: "The considerable space between the two lateral lips of the blastopore is filled almost entirely by a plug (*yk. p.*) of considerable size, which projects upwards from the axial mass of cells as far as the level of the general surface of the embryo."

This so called plug is formed, not by a forcing upward, but by a sinking downward, of the streak. Mitsukuri and Ishikawa ('86, p. 80) realized this fact, I believe, when they wrote: "The reason why the yolk plug in *Trionyx* is more conspicuous at this stage than earlier stands, we think, in close connection with the fact that the blastopore has become a much better defined horse-shoe-shaped slit." This sinking begins in two areas parallel as well as lateral to the axis of the streak, and together with the

space they enclose equals in width the lateral extent of the open portion of the blastopore. Indeed, these two depressions are the posterior extensions or horns of the crescentic portion of the open blastopore. As this process of sinking continues, the central area by degrees falls lower and lower, until it reaches the level of the floor of the notochordal canal, at which level it disappears. That portion which is going to form the dorsal wall of the archenteron passes laterad and upward to fuse again in the dorsal lip, while that portion which forms the floor of the potential archenteron will disintegrate in order to put this cavity into communication with the yolk.

These successive stages in the sinking of the streak behind the neurenteric canal, and its subsequent elevation and refusion anterior to this canal can be traced in a series of sections made through the streak of an embryo whose blastopore has become



Diagrams II.-VIII.

a crescentic opening with horns directed caudad. Diagrams II.-VIII. are intended to illustrate this process. Diagram VIII. passes through the streak behind the posterior extremities of the blastoporic horns. A primitive groove may be evident in this region, but in some cases only a fusion of layers exists. (See Mehnert, '92, Taf. XIX. Fig. 25; Mitsukuri, '86, Plate III. Fig. 8; Will, '93,

Taf. 35, Figs. 18^a-18^c; and my own Figures 18 and 19, Plate IV., and Fig. 48, Plate IX.) Whether there is on the streak an actual groove or not, one is forced to conceive of the existence of a potential archenteron in this region. When a primitive groove does exist, there is no difficulty in conceiving of an archenteron, — one, however, that is so compressed that it exists only as a fold or closed space between cell walls. Such a modified archenteron is seen to exist in Figure 23 (Plate V.). For the sake of emphasizing my view I have in Diagrams III.-VIII. drawn this archenteron as though it were open at the base of the groove. I have represented potential notochord by dots; the remaining portions of the several germ layers are so lettered (see *Abbreviations*, p. 54) as to be readily understood. A potential archenteron is represented as surrounded by the entoderm.

As we pass anteriorly along the streak, we come (Diagram VII.) into the region of the extreme posterior end of the backward-turned horns of the crescent, where the streak is beginning to sink below the level of the embryonic surface. The central portion of the streak is little if at all affected by this sinking; it often possesses along its axis a primitive groove. Mehnert's ('92) Figure 25 (Taf. XIX.), and my own Figures 47 (Plate IX.) and 56 (Plate XI.) illustrate this condition.

If we pass on anteriorly until we come near the posterior lip of the open portion of the blastopore, we meet the condition seen in Diagram VI. The two lateral depressions have sunk very deep, while the axis of the streak is little affected, and possesses oftentimes a primitive groove. Such a condition is represented by Mitsukuri and Ishikawa, '86, Fig. 18, Plate IV.; by Will, '93, Fig. 24^a, Taf. 36; and by my own Fig. 49 (Plate X.) and Figs. 55 and 58 (Plate XI.).

At a little later stage in the section from this region, or at this stage in sections a short distance in front of it, this whole central area (Diagram V.) is seen to have sunk some distance below the embryonic level, and to have changed to the form of a blunt cone. Mitsukuri's ('86) Figure 9 (Plate III.), and my Figures 50 and 51 (Plate X.), represent this condition. At the stage in which this plug area has been transformed into a cone all traces of a primitive groove have been obliterated. In earlier stages, however, the groove extends into this region.

At a point one or two sections farther forward (Diagram IV.)

the anterior or dorsal lips of the blastopore are approaching each other to fuse in the axial line of the embryo. (See Plate IV. Fig. 17, Plate V. Fig. 23, and Plate X. Fig. 52.) In a few series it is possible in the sections to trace the groove into this region, but in those instances in which the streak becomes depressed the groove is usually obliterated.

In Diagram IV. the ectoderm has made much progress in its journey laterad and dorsad. For the sake of clearness, I have represented this movement as occurring entirely in one plane. As a matter of fact, in consequence of the obliquity of the notochordal canal in early stages, caused by the uneven pace of the two lips in opening and closing, we must conceive of a migration not only laterad and dorsad, but also posteriad; for the ectoderm which adjoins the notochordal area in Diagram IV. will not only fuse in the dorsal lip at a point above, but in a plane posterior to, the one in which it now lies. When the canal comes in later stages to occupy a more perpendicular position, when, in other words, the ventral lip comes to open so rapidly that it has almost caught up with the dorsal lip in its process of closing, then the migration will be accomplished more nearly in the dorso-ventral plane, as is illustrated in my diagrams.

Mitsukuri's ('86) Figure 10 (Plate III.) represents a stage intermediate between those figured in my Diagrams IV. and III. The dorsal lip in Mitsukuri's figure is almost closed, while the ventral floor remains intact. In my Diagram III. the dorsal lip has fused in the axial line, but a differentiation into ectoderm and notochord has not yet been accomplished here. The condition figured in this diagram is illustrated in the sections shown in Figures 22 (Plate V.) and 46 (Plate IX.).

Mitsukuri's ('86) Figure 19 (Plate IV.) represents a stage again intermediate, so far as the differentiation of the notochord is concerned, between my Diagrams III. and II. In Mitsukuri's figure the ectoderm and notochord are differentiated as distinct layers, save for a very short space at the axis. The ectoderm and notochordal entoderm may become completely distinct from each other, as in Diagram II., for a space of several sections before the separation and disintegration of the entoderm is accomplished. Mehnert's ('92) Figure 24 (Taf. XIX.), or Mitsukuri and Ishikawa's ('86) Figure 11 (Plate III.), as well as my own sections, illustrate such a condition.

Diagram II. represents the condition found after the separation of the entoderm and the disintegration of that portion of it which forms the floor of the archenteron. In this manner, according to my conception, the whole streak anterior to the neurenteric canal comes to be converted into the axis of the embryo, and thus the length of the notochordal canal is greatly increased.

SUMMARY.

1. In turtles the notochordal canal is lengthened, in part at least, by the backward migration of its dorsal opening, — the neurenteric canal.

2. There is evidence that the lumen of the notochordal canal may in some cases be completed anteriorly and open towards the yolk ventrally before the dorsal or ectodermal opening is formed.

3. The lumen of the notochordal canal may be formed as an invagination from the dorsal surface, or by a yielding or disintegration of cells within a solid process.

4. The primitive groove exists on the streak before the horns of the open portion of the blastopore turn backward.

5. It may persist on the streak for some time after the horns of the open portion of the blastopore bend backward, and it is later obliterated.

6. The size of the open portion of the blastopore gradually diminishes.

7. The open portion of the blastopore migrates backward along the streak by a process of fusion of the halves of its anterior lip, and by a reopening of the halves of its posterior lip.

8. The anterior lip for a time fuses more rapidly than the posterior lip reopens, and thus the size of the open portion of the blastopore, or neurenteric canal, is diminished.

9. On account of this rapid fusion of the anterior lip and the tardy reopening of the posterior lip, a portion of the posterior lip or streak comes to lie temporarily ventrad and anterior to the anterior lip.

10. This depressed portion of the posterior lip separates in the median or axial line; each half passes laterad, dorsad, and then mesiad, to fuse again in the axial line of the anterior or dorsal lip.

11. This inequality in the rapidity of the fusion of the anterior or dorsal lip, and of the reopening of the posterior or ventral lip, causes the neurenteric canal for a time to assume an oblique direction.

12. Later, the posterior lip in its reopening more nearly keeps pace with the fusion of the anterior lip, and thus the neurenteric canal comes to assume a more nearly perpendicular position.

13. This process of posterior separation and anterior refusion of the lips is accompanied by the disintegration of that portion of the entoderm which corresponds to the floor of the notochordal canal.

14. The existence of such a potential canal in the streak region can be inferred from the presence of a primitive groove in the streak, from evidence of degeneration of the lower lying entoderm of this region, and, in rare instances, from the existence of this lower layer, or portions of it, as a distinct layer.

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kröte (*Cistudo lutaria* Gesn.). Zool. Jahrb., Abth. f. Anat. u. Ont.,
Bd. VI. pp. 529-615, Taf. 30-36.

EXPLANATION OF PLATES.

All figures on Plates I.-III. were outlined by means of a camera lucida, and shaded free hand.

ABBREVIATIONS.

<i>a</i>	Individual <i>a</i> of a pair of twins.
<i>β</i>	Individual <i>β</i> of a pair of twins.
<i>bl'po. a.</i>	Anterior lip of the blastopore.
<i>bl'po. p.</i>	Posterior lip of the blastopore.
<i>can. n'ent.</i>	Neurenteric canal.
<i>can. nt'cd.</i>	Notochordal canal.
<i>cl. vt.</i>	Yolk cells.
<i>ec'drm.</i>	Ectoderm.
<i>ec'en.</i>	Ectental line.
<i>en'drm.</i>	Entoderm.
<i>en'drm. a</i>	Entoderm of embryo <i>a</i> .
<i>en'drm. d. β</i>	Dorsal entoderm of embryo <i>β</i> .
<i>en'drm. v. β</i>	Ventral entoderm of embryo <i>β</i> .
<i>so'plu.</i>	Somatopleure.
<i>str. pr.</i>	Primitive streak.
<i>sul. pr.</i>	Primitive groove.
<i>sul. pr. a.</i>	Primitive groove anterior to the neurenteric canal.
<i>sul. pr. p.</i>	Primitive groove posterior to the neurenteric canal.

PLATE I.

All figures magnified 39 diameters.

- Fig. 1. Dorsal view of an embryo *Chrysemys picta*.
The concavity of the dorsal notochordal opening is directed posteriad.
- Fig. 1'. Ventral view of the same.
The jagged edges of the ventral notochordal opening show that the notochordal canal is in process of breaking through ventrally.
- Fig. 2. *Chelopus insculptus*, dorsal view.
The concavity of the notochordal opening is directed anteriorad.
- Fig. 2'. Ventral view of the same.
The notochordal canal has broken through at its anterior end.
- Fig. 3. *Chrysemys picta*, dorsal view.
The notochordal opening is a nearly transverse slit. The width of the broad notochordal canal is discernible on dorsal as well as ventral view.
- Fig. 3'. Ventral view of the same.
The anterior portion of the floor of the notochordal canal has broken through.
- Fig. 4. *Chelydra serpentina*, dorsal view.
The horns of the notochordal opening point posteriad. Along the axis of the embryo, reaching from the posterior lip of the notochordal opening to the posterior end of the shield, extends a well marked groove.
- Fig. 4'. Ventral view of the same.
Lateral portions of the shield have bent underneath during the process of hardening. The groove is discernible on the ventral surface as a light (unshaded) longitudinal band at the posterior end of the shield. The floor of the notochordal canal has broken through at its anterior end.

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PLATE II.

All figures magnified 39 diameters, except Figures 6 and 8, which are magnified 10 diameters.

Fig. 5. Dorsal view of a double embryo of *Chelydra serpentina*.

The horns of the notochordal canal of embryo β point anteriorad. The anterior lip of the notochordal opening is distinctly lobed, i. e. divided at the axial point by a median notch. Embryo β is growing forward for the greater portion of its length underneath embryo a . The axis of a lies anterior and oblique to the axis of β . A notochordal opening transverse to the axis, such as exists in β , is not discernible on a . At the point of union of a and β , the shields of both individuals are thrown more or less into folds.

Fig. 6. *Chelydra serpentina*, dorsal view.

The head fold and medullary folds are formed. The notochordal opening has assumed a distinct horseshoe shape, whose concavity is directed posteriorad. The external opening of the neurenteric canal is much reduced in size.

Fig. 7. *Chelydra serpentina*, dorsal view.

The dorsal notochordal opening is an almost transverse slit. The horns, however, have begun to bend slightly posteriorad. A groove reaches from the posterior lip of the dorsal opening to the posterior edge of the shield.

Fig. 7'. Ventral view of the same.

The notochordal canal has broken through below.

Fig. 8. Double embryo of *Chelydra serpentina*, dorsal view.

The axis of individual β is almost perpendicular to that of individual a . The horns of the dorsal notochordal opening of β are directed anteriorad.

NOTE.—By an oversight, the lettering of these embryos has been *interchanged* in Figure 8. (See also Diagram I. in the text, page 6.)

A long grooved streak leads posteriorad from the notochordal opening of a . Beyond the limits of the shield of a a groove or depression, continuous with the groove on the shield, is to be followed to the left border of the figure, where a break prevents its being traced further.

Fig. 9. *Ozotheca odorata*, dorsal view.

The outline of the notochordal opening is irregular. One pair of horns points posteriorad, and two pairs anteriorad.

Fig. 9'. Ventral view the same.

The anterior end of the notochordal canal has opened below, but seems to be closed again by a clump of tissue through which a narrow canal sinks perpendicularly from the dorsal to the ventral surface.

Fig. 10. *Chelydra serpentina*, dorsal view.

The dorsal opening of the notochordal canal is a nearly transverse slit. A median notch exists on the anterior lip.

Fig. 10'. Ventral view of the same, showing the ventral opening of the notochordal canal.

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PLATE III.

All figures are magnified 39 diameters, except Figures 12 and 12', which are magnified 10 diameters.

Fig. 11. *Chrysemys picta*, dorsal view.

The horns of the notochordal canal are directed posteriad. A slight pit is discernible at the anterior third of the shield.

Figure 11'. Ventral view of the same, showing narrow opening of the notochordal canal.

Fig. 12. Ventral view of an embryo of *Chelydra serpentina*.

Fig. 12'. Dorsal view of the same.

The medullary folds are beginning to close. The notochordal opening or neurenteric canal is reduced to a small horseshoe-shaped opening near the posterior end of the embryo.

Fig. 13. *Chelydra serpentina*, dorsal view.

The horns of the notochordal opening are bent far posteriad.

Fig. 13'. Ventral view of the same.

The notochordal canal opens ventrally.

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PLATE IV.

All figures on this plate are magnified 73 diameters. The position and direction of the sections, Figs. 14 to 21, are indicated in Diagram I., page 6, by the corresponding numbers.

- Fig. 14. Sagittal section of embryo β (by mistake lettered a) of Fig. 8, slightly oblique to the axis of β . The notochordal canal of β (*can. nt'cd.* β) has not opened ventrally. The notochordal canal (?) of a (*can. nt'cd.* a) is cut transversely. Consult text, page 7.
- Fig. 15. A section which passes through the left horn of the notochordal opening of β near its end.
- Fig. 16. Section cutting transversely the notochordal canal of a near the middle of its extent.
- Fig. 17. Seventh section in front of the anterior lip of the notochordal canal of a . The ectoderm and entoderm of this lip are not completely differentiated. A groove (*sul. pr. p.*) lies on the surface of the floor of the canal.
- Fig. 18. Cross section of a behind the blastopore. A primitive groove (*sul. pr. p.*), which is continuous with that of Fig. 17, is present.
- Fig. 19. Section showing the primitive streak (*str. pr.*), upon which, however, the groove does not extend as far backward as the place of this section.

14.

com. d. d.) com. pl. d. d. α

1

3

15.

α

16.

α

sul pra

sul p p

caplo.

17

sul p p

eu'dem

18.

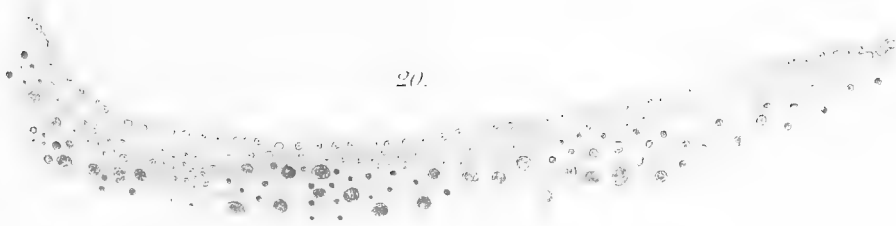
str pr

19

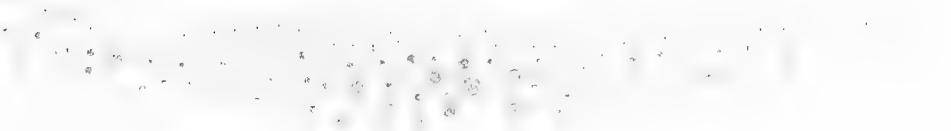
PLATE V.

- Fig. 20. The sixty-fourth section behind the dorsal notochordal opening of *a*. See Diagram I., page 6. $\times 240$.
- Fig. 21. The one hundred and twelfth section behind the dorsal notochordal opening of *a*. $\times 240$.
- Fig. 22. The region marked *sul. pr. p.* in Fig. 17, more highly magnified. $\times 420$.
- Fig. 23. The region marked *sul. pr. p.* in Fig. 18, under higher magnification. $\times 420$.
- Fig. 24. Section cut in the direction of the line 24-24, Fig. 5. The ectoderm of *a* is split at the axis of *a*. The asterisk marks the point of separation between the entoderm of *a*, and that of *β* . $\times 73$.
- Fig. 25. Section along the line 25-25, Fig. 5. The ectoderm of *a* is split at the axis of *a*. One half of the entoderm of *a* has disappeared together with the anterior floor of *β* . $\times 73$.
- NOTE. — By an oversight *α'* and *β''* have been interchanged in position in this Figure.
- Fig. 26. Section, the position of which is shown by the line 26-26, Fig. 5. The ectoderm of *a* is split at the axis of *a*. Part of the entoderm of *a*, and the anterior part of the floor of the notochordal canal of *β* , have broken through into the yolk. $\times 73$.

20.



21

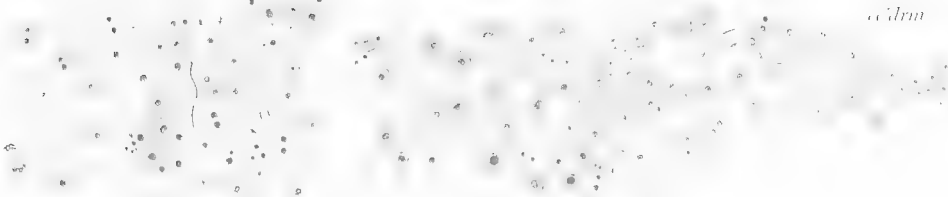


22

ad pr

25.

en'drm



24.

en'm

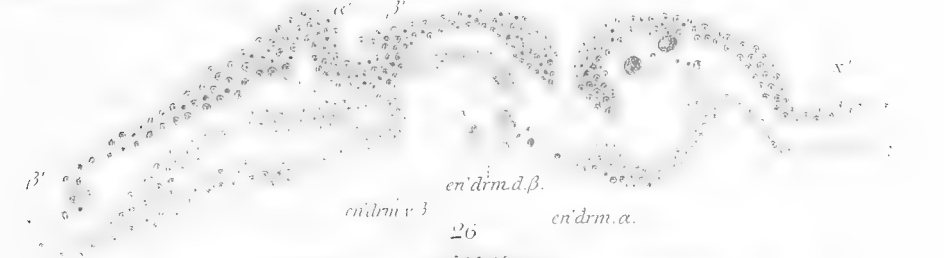
β''

β'

en'drm v. β

en'drm d. β

25.



α' β'

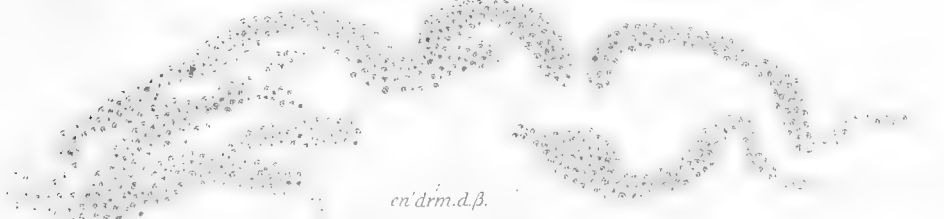
α'

en'drm d. β .

en'drm v. β

en'drm. α .

26



en'drm. d. β .

en'drm α en'drm v. β

en'drm. α .

PLATE VI.

All figures magnified 73 diameters, except Figures 30 and 31, which are magnified 240 diameters.

- Fig. 27. Section indicated by the line 27-27, Fig. 5. The ectoderm of *a* has fused in the axis. The entoderm of *a* (*en'drm. a*) has fused with the entoderm of *β* (*en'drm. v. β*) at the point marked by an asterisk. The notochordal canal of *β* is open at its anterior end.
- Fig. 28. Sagittal section through the remaining portion of *β*, indicated by the short line in the plane 28, Fig. 5.
- Fig. 29. Section lateral to Fig. 28, cut in the direction of line 29, Fig. 5. The point of union between individuals *a* and *β* is seen midway between the letters *a* and *β*.
- Fig. 30. Transverse section across the axis of embryo *a* in the region of line 30-30, Fig. 5. The ectoderm and entoderm are fused in the axis. A small canal, *can. n'ent. a*, sinks perpendicularly into this region of fusion.
- Fig. 31. Transverse section, cut along the line 31-31, Fig. 5, across embryo *a*. The ectoderm and entoderm are separated from each other at the axis, but both are grooved or constricted at that point.

27.

β^n en'drm d.p'

β'

en'drm v. β

en'drm. α .

en'drm v. β

28.

en'nt ed. β

29.

α

en'drm
en'drm d. β

en'drm v.

en'drm α

30.

en'n'ent. α

31

en'drm

en'drm

PLATE VII.

All figures magnified 73 diameters.

- Fig. 32. Sagittal section through embryo shown in Fig. 7, Plate II. The notochordal canal is open below for the greater portion of its extent.
A dagger marks the supposed anterior end of the notochordal canal.
- Fig. 33. Section lateral to, and parallel with, that of Fig. 32. The notochordal canal is opening at the point marked by an asterisk.
- Fig. 34. Section lateral to, and parallel with, that shown in Fig. 33. This section passes near the lateral margin of the notochordal canal.

52.

A

53.

P

54.

*

*

PLATE VIII.

All figures magnified 73 diameters, except Figures 35, 42, and 44, which are magnified 420 diameters.

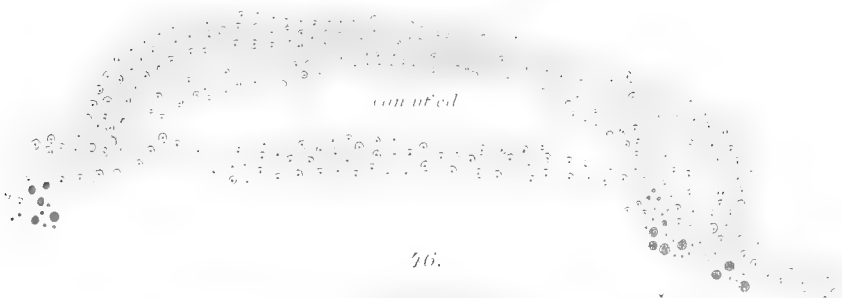
- Fig. 35. Transverse section in the region of the notochordal canal, taken at the position of line 35, Fig. 44. Surface views of this embryo are seen in Figs. 3, 3', Plate I.
- Figs. 36-43. Transverse sections of the streak taken at the points indicated in Fig. 44 by corresponding numbers.
- Fig. 39. A layer of yolk, *cl. vt.*, is spread out over the ectoderm.
- Fig. 40. The ectoderm is separated at the points *ec'en.* The yolk, *cl. vt.*, is streaming through this gap.
- Fig. 41. The ectoderm is widely separated at the points *ec'en.* The yolk is spread out over the ectoderm.
- Fig. 42. Cells and yolk globules outlined under higher magnification by means of the camera lucida; *ec'en.* are points at which the ectoderm is proliferating nuclei ventrally and laterally.
- Fig. 43. The ectoderm is differentiated entirely across the dorsal surface, but it is mixed with, and seems to be feeding on, the yolk cells within and above it.
- Fig. 44. A reconstruction in the sagittal plane from cross sections. The sagittal plane falls at the points marked 44 on the transverse sections, Figs. 36-43. The lumen of the notochordal canal is filled for the posterior third of its extent with a substance which I interpret as degenerating cells.

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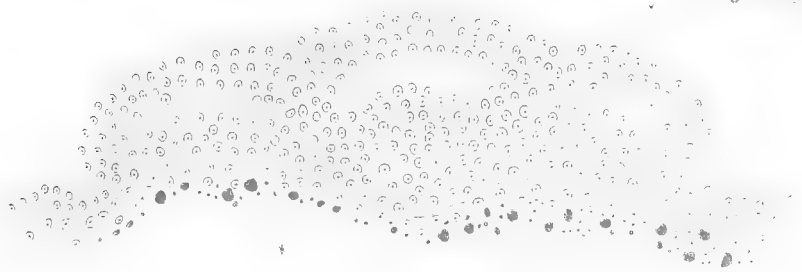
PLATE IX.

- Fig. 45. Transverse section of the notochordal canal of embryo shown in Figures 1 and 1', Plate I. The section here figured falls just posterior to the anterior end of the floor seen in Fig. 1', Plate I. $\times 73$.
- Fig. 46. Transverse section of the same embryo passing through the anterior lip of the dorsal notochordal opening. $\times 73$.
- Fig. 47. Transverse section (the third behind the notochordal opening) through the streak region of Figs. 2, 2', Plate I. $\times 240$.
- Fig. 48. Transverse section, the sixth behind the dorsal notochordal opening of the embryo shown in Fig. 2, Plate I. $\times 240$.

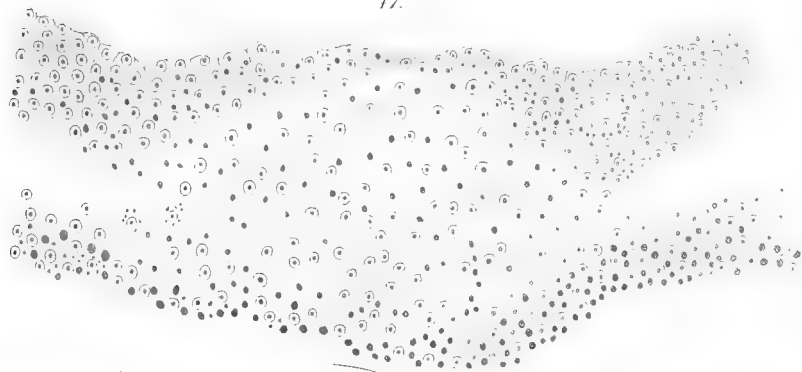
45



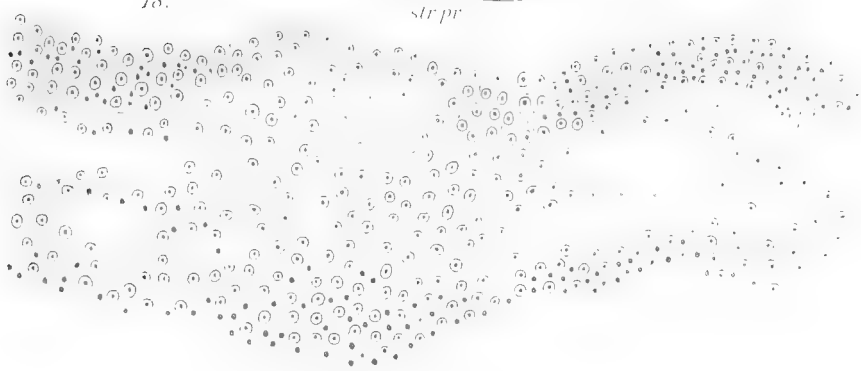
46.



47.



48.



str pr

PLATE X.

Figures 49-51 are magnified 73 diameters; Figures 52 and 53, 240 diameters.

- Fig. 49. The tenth section behind the dorsal notochordal opening of an embryo which closely resembles in surface view Fig. 7, Plate II.
- Fig. 50. Third section behind the dorsal notochordal canal of the same embryo. The so called plug region is grooved.
- Fig. 51. Representation of the third section behind the dorsal notochordal opening of the embryo seen in surface view in Fig. 13, Plate III.
- Fig. 52. Third section anterior to the dorsal notochordal opening of the embryo seen in surface view in Fig. 11, Plate III. The halves of the anterior lip are still fusing.
- Fig. 53. Third section behind the dorsal notochordal opening, from the same embryo as the preceding figure.

49

salp p p

50

salp p p

51

salp r t

52

55

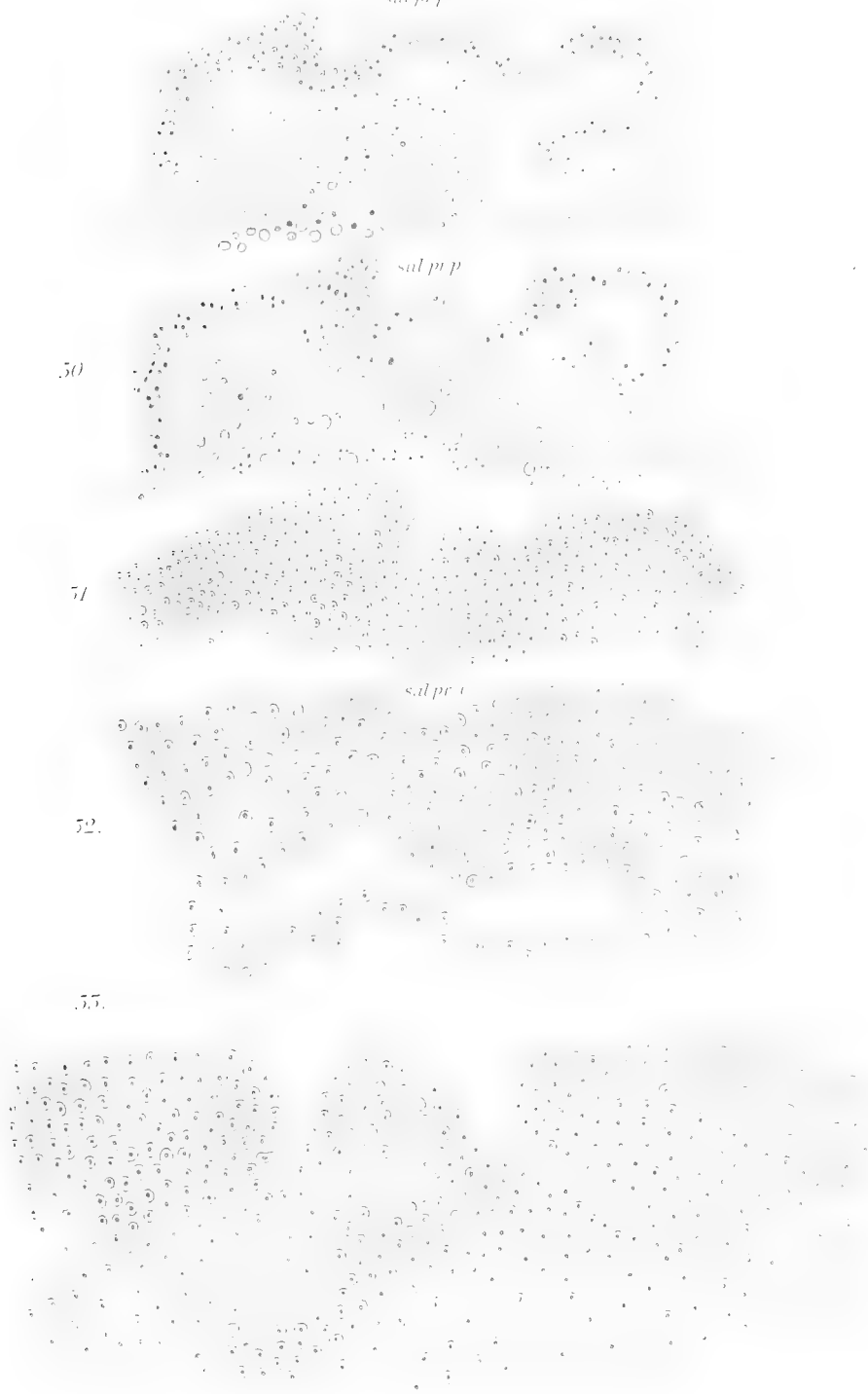


PLATE XI.

All figures magnified 73 diameters, except Figure 58, which is magnified 420 diameters.

- Fig. 54. An oblique section through the notochordal opening of the embryo seen in surface view in Fig. 9, Plate II., cut along the line 54. This section passes through a lobe of the opening on the left, and cuts through the edge of a lobe on the right side.
- Fig. 55. The third section behind the dorsal notochordal opening. The so called plug region is grooved.
- NOTE. — By a mistake the primitive groove is marked as neurenteric canal, *can. n'ent.* It should have been *sul. pr.*, as in Figure 56.
- Fig. 56. Sixth section behind the dorsal notochordal opening. The groove still persists.
- Fig. 57. A section near the anterior margin of the shield. A blind canal (*can. n'cd.*) exists within the entoderm of this region.
- NOTE. — The letters *sul. pr.* should have been omitted.
- Fig. 58. Represents under a high magnification the so called plug region seen in Figure 55.

54.

bl'po a

bl'po p

can. n'ent

55

56

sul pr

sul pr

58

sul pr

57.

can n'ed

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