

THE LOCATION OF THE CHICK EMBRYO UPON THE BLASTODERM.

BY

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WITH 2 PLATES AND 15 FIGURES IN THE TEXT.

An experimental study of the avian egg has led me to examine the following points:

1. The location of the embryo in the material of the unincubated blastoderm.
2. The direction of growth before, and after the appearance of the primitive streak.
3. The origin of the material from which the later embryo arises.

According to Kopsch¹ this third point has been definitely settled. He concludes that nearly all of the embryo develops from the primitive streak. I quote his own words: "Somit entsteht der Embryo, mit Ausnahme des praechordalen Teils, des Kopfes, durch Umwandlung des Primitivstreifens."

I² have already mentioned some experiments, and will describe others, in the following pages, which seem to prove that only the trunk and caudal regions of the embryo arise from the material of the primitive streak.

The methods used by Assheton, myself, and Kopsch are practically the same, and therefore require little explanation. I have

¹Kopsch, Fr. Ueber die Bedeutung des Primitivstreifens beim Hühnerembryo. Leipzig, 1902.

²Peebles, F. A Preliminary Note on the Position of the Primitive Streak, and its Relation to the Embryo of the Chick. Biolog. Bulletin, Vol. IV, No. 4, 1903.

again used the method described in my earlier work.¹ A small window was made in the shell just above the blastoderm, and the operation performed, after which the opening was closed by a piece of shell, sealed with strips of the shell membrane. All instruments used in the experiments were carefully sterilized, and the shells of freshly opened eggs used for closing the windows. The loss of eggs through infection was small. In most of the experiments one egg in each set was opened and then sealed again, without operating upon it, in order to have a check with which to compare the eggs upon which experiments were made. In this way it was possible to determine roughly, after further incubation, whether abnormalities were due to opening the egg or to the operation performed upon the blastoderm. In general it was found that the development of eggs in which windows were made was delayed about two to four hours.

I. THE LOCATION OF THE EMBRYO IN THE MATERIAL OF THE UNINCUBATED BLASTODERM

In 1896, Assheton² described some experiments that he made on the unincubated blastoderm of the chick. Sable hairs were inserted at various points and their position determined after periods of incubation varying from eighteen to forty hours. Assheton proved that Duval's³ theory of the formation of the primitive streak is incorrect, that instead of forming by the confluence of the posterior margin of the blastoderm, the primitive streak appears in the region of the unincubated blastoderm which lies between the center and the posterior margin of the area pellucida. I have repeated Assheton's experiments, making the injuries with a hot needle instead of a hair, without removing the egg from the shell. The results agree with those of Assheton, as the following experiments show:

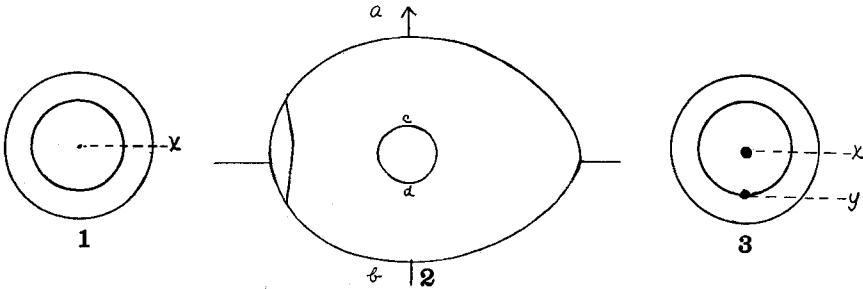
¹Peebles, Florence. Some Experiments on the Primitive Streak of the Chick. *Archiv. für Entwicklungsmech. der Organismen*. VII Band. 1898.

²Assheton, R. An Experimental Examination into the Growth of the Blastoderm of the Chick. *Proceedings of the Royal Soc.*, Vol. 63, 1896.

³Duval. De la Formation du Blastoderme dans l'Oeuf d'Oiseau. *Annales des Sciences Naturelles, Zoologie*, Vol. 18.

Experiment I. A small window was made in the shell of an egg a few hours after it was laid. The blastoderm measured 2.8 mm. in diameter and the area opaca and area pellucida were faintly defined. A hot needle (No. 12) was inserted in the center of the blastoderm (Text-fig. 1, x) and quickly withdrawn. The shell was sealed and the egg put in the incubator, the temperature of which varied from 37° – 39° Centigrade. At the end of twenty hours the egg was opened, and the blastoderm killed, removed and stained. The primitive streak was clearly defined (Pl. I, Fig. 1) extending from the posterior margin of the area pellucida to the point of injury (x). The cells around the wound seemed greatly increased in number and showed evidence of forward growth which must have been stopped by the injury.

Other eggs, injured in the same way (Text-fig. 1) were left in



the incubator for a longer period, from thirty to forty-eight hours. Pl. I, Fig. 2 is a surface view of one of these embryos after forty-eight hours' incubation. The embryo is well developed, fourteen pairs of somites are present, and the heart is forming. The injured area lies dorsal to the heart on a level with the anterior somites. The brain region has failed to develop.

From Assheton's results, and from these just described, we must conclude that the primitive streak and the greater part of the later embryo form from that region of the unincubated blastoderm which lies behind the center, between it and the posterior margin of the area pellucida. The question arises whether or not the posterior margin of the area pellucida is a fixed region in all eggs, and what the relation of the long axis of the embryo is to the long axis of the shell. In Text-fig. 2, an egg is represented

as opened above the blastoderm. The air chamber, which lies in the blunt end of the shell, is at the left, and the pointed end at the right. The chalazæ extend on each side of the yolk in the long axis of the shell. In this position the blastoderm may be divided into right and left halves, the arrow *a-b* indicating the median plane of the bi-laterally symmetrical embryo. We then speak of the region *c* as the anterior border, and *d* as the posterior border of the blastoderm.

Assheton, in another series of experiments, has made two injuries in the unincubated blastoderm (Text-fig. 3) one in the center (*x*) and the other in the posterior border (*y*) of the area pellucida. He found that the primitive streak appeared later between these two injuries, and he concluded from this that the point (*y*) marks the posterior end of the embryo.

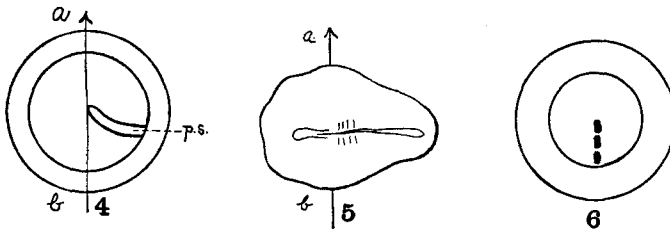
In order to distinguish the region of the primitive streak from the rest of the area pellucida, I shall call it the radius *x-y*. This radius with the corresponding one anterior to the center make the diameter which represents the median longitudinal axis of the embryo. In order to determine the constancy of the occurrence of the embryo in this position I have kept the record of 100 eggs. The eggs were taken from the nest on the same day that they were laid. They were placed in the same position in a basket from which they were transferred to the incubator. After incubation for eighteen to forty-eight hours the embryo in every fertile egg, with the exception of two, was found in the median line (Text-fig. 2 *a-b*). The two exceptions are shown in Text-figs. 4 and 5. The first embryo (Text-fig. 4) was incubated eighteen hours. At the end of this time the primitive streak had formed, but instead of lying on the radius *x-y* it extended from the center to the right side of the blastoderm and was bent towards the posterior margin. The second egg (Text-fig. 5) was incubated for a period of twenty-eight hours. The normal embryo lay at right angles to the line *a-b*. In both of these eggs the chalazæ were found in abnormal positions, and the yolk membrane was wrinkled in many places, showing that the yolk had been abnormally twisted in its passage through the oviduct.

After I had discovered that the position of the normal embryo, when undisturbed by twisting or shaking, is constant, I deter-

mined to find out, if possible, whether the embryo would form on any other part of the blastoderm if development on the radius $x-y$ was prevented.

Experiment II. The blunt end of the egg was held in the left hand so that the blastoderm lay on top of the yolk; a small window was made immediately above it, and a series of injuries were made with a hot needle in the radius $x-y$. The number of injuries was dependent upon the size of the area pellucida. Usually there is space enough to insert the tip of a No. 12 cambric needle in three places between x and y (Text-fig. 6) before all the cells are destroyed.

At the end of eighteen hours the eggs were killed, but no trace of primitive streak in another region was found. About 60 per cent. of the blastoderms showed a large hole where the area pellucida had stretched apart in the growth of the blastoderm. No



evidence of the formation of the embryo around the margin of the hole could be found.

The experiment was repeated and the eggs were incubated from thirty to forty hours. An examination of these embryos showed no development around the margin of the wounded area, but in front of it and posterior to it some development had taken place. In Pl. I, Fig. 3, a surface view of an embryo of forty hours' incubation is given. The brain is abnormal, but shows no lack of material, the notochord is present, but greatly reduced in length. There is some trace of the heart lying on each side of the notochord, but back of it none of the embryo has formed. There is no evidence of growth of the area pellucida in a posterior direction, but anteriorly it is the normal size and shape.

Another embryo incubated thirty hours is shown in Pl. I, Fig. 4. In this embryo no brain developed but growth from the heart

region caudad is evident. The injured area (*w*) is surrounded by thickened ridges and back of the hole made by the wound the notochord is present. Behind the notochord lies the posterior end of the primitive streak. No mesoblastic somites are present.

The object of these experiments was to prevent development along the radius *x-y* by killing the cells in the region where the primitive streak develops. In this way it was hoped that the primitive streak might be formed in some other part of the area pellucida. The results show very clearly that no other part of the blastoderm is capable of forming the primitive streak. They also show that the region of the unincubated blastoderm along the radius *x-y* is the region from which the mesoblastic somites develop, *i. e.*, the trunk region of the embryo. From these experiments it seems evident that the position of the embryo upon the blastoderm is determined before the egg has been incubated, and probably before segmentation is completed, for some of the eggs which I used were operated upon within two hours from the time that they were laid.

II. THE DIRECTION OF GROWTH OF THE EMBRYO BEFORE AND AFTER THE FORMATION OF THE PRIMITIVE STREAK.

Marshall¹ describes the growth of the blastoderm from the beginning of incubation as follows: "After incubation has commenced, the blastoderm spreads rapidly, retaining its circular shape. By the end of the first day of incubation it is about the size of a sixpence, and by the end of the second day it has extended nearly half way round the egg."

According to Duval² the edge of the blastoderm advances over the egg at every point except at the posterior margin, and the edges on each side of this point meet each other in the middle line to form the primitive streak ("plaque axiale"). Assheton's³ experiments have proved, however, that the growth is symmetrical as Marshall states.

¹Marshall. Vertebrate Embryology.

²Duval. *Loc. cit.*

³Assheton. *Loc. cit.*

While the margin of the area opaca is symmetrical, that of the area pellucida is not. During the first few hours of incubation the two areas increase uniformly, but towards the fifteenth hour the area pellucida begins to extend posteriorly, the anterior region remaining spherical in outline.

Experiment I. The uniformity of growth in the anterior half of the blastoderm can be seen in the following experiment. The unincubated blastoderm was injured at three points (Text-fig. 7, x , p and o); the needle was inserted at the center (x) at the middle point of the anterior margin (p) and at the right margin of the area pellucida (o). The injuries in the margin were at equal distances from the center. After eighteen hours' incubation the distance between x and p was the same as the distance between x and o (Pl. I, Fig. 5) showing that the lateral and anterior growth were the same. The primitive streak was formed, but its posterior end (y) was much further from x than x was from p , while the distances before incubation were equal.

The results of earlier experiments¹ led me to believe that the region immediately in front of the primitive streak represents an area of rapid growth, because an injury made in this region did not affect one structure alone, but disturbed the organ covering a large area. This is also true when the center of the unincubated blastoderm is killed (Pl. I, Fig. 2).

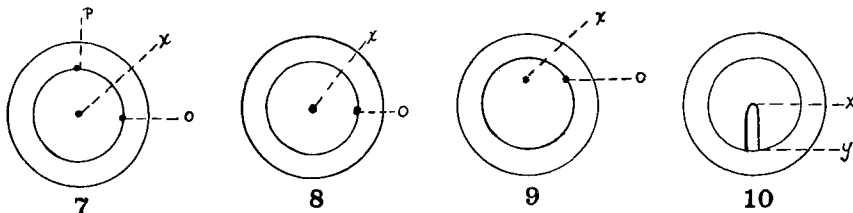
Experiment II. In order to determine the extent of growth in an interior direction from the center of the blastoderm I injured a point in the center (Text-fig. 8, x) and one on the same level at the side (o). The eggs were incubated thirty-six to forty hours. Pl. I, Fig. 6 is a surface view of an embryo at the end of thirty hours. The injury in the center of the blastoderm produced great disturbance in the development of the embryo anterior to the heart. No forward growth took place in the median line. The wound (o) at the side, which did not move forward, is at a level with the anterior somites. The normal growth of the margin of the area pellucida on the side of the injury did not take place. The margin is irregular and a peculiar rod of cells extends from the marginal wound to the median line of the embryo.

¹Peebles. *Loc. cit.*

Experiment III. In another set of experiments the two injuries were made about .5 mm. further forward (Text-fig. 9, x and o). The position of the injuries after forty hours is seen in Pl. I, Fig. 7. The wound at the side (o) has advanced with the growth of the blastoderm but the wound (x) in front of the somites has prevented the formation of the head, and the embryo is reduced in length anteriorly, the trunk and caudal regions are about the normal length.

From these experiments it seems evident that the region in front of the middle point of the area pellucida is the seat of active growth in an anterior direction.

Experiment IV. In order to determine the extent of growth posteriorly, two injuries were made, one in the center of the unincubated blastoderm and the other in its posterior margin (Text-fig. 3, x and y). The embryos were incubated thirty-six hours to



two days. They developed somites and medullary folds in the area between the wounds. Pl. II, Fig. 8, represents a surface view at the end of thirty-six hours. Notochord and somites have developed between the two wounds. The actual distance from x to y before the experiment was 1 mm. After incubation it was 3 mm. showing an increase in length of only 2 mm. The normal embryos at this age measure 4 mm. from heart to caudal end.

The results of these three sets of experiments show that the embryo may be greatly reduced in length by preventing growth anteriorly with the wound x and posteriorly with the wound y , and that the area pellucida grows less rapidly at the sides than in the median line.

Up to this time the experiments which I have described have been made upon the unincubated blastoderm. The change in the size and the shape of the area pellucida is comparatively

slight before the appearance of the primitive streak when the area becomes pear-shaped.

Kopsch¹ has found that when two wounds are made in an embryo of twenty-four hours' incubation, at a distance of 2 mm., one at the anterior, and the other at the posterior end of the primitive streak, the embryo does not reach its normal size in later development. The entire body is much shortened, and lies between the two wounds. I have repeated this experiment, and have obtained the same result, the primitive streak, in the eggs upon which I have worked, is much longer (3 to 3.5 mm.) in a twenty-four hour chick, and the anterior end is no longer visible, the head process and the notochord are present.

Another series of experiments was made by Kopsch when the primitive streak measured about 4 mm. A series of five injuries, at 1.5 mm. spaces, were made along the side of the primitive streak, and parallel with it. The embryos were incubated fifty and one-half hours, and at the end of this time the regions of the five wounds were located. Growth in length was greater in the region *back* of the anterior end of the primitive streak than it was in front of it.

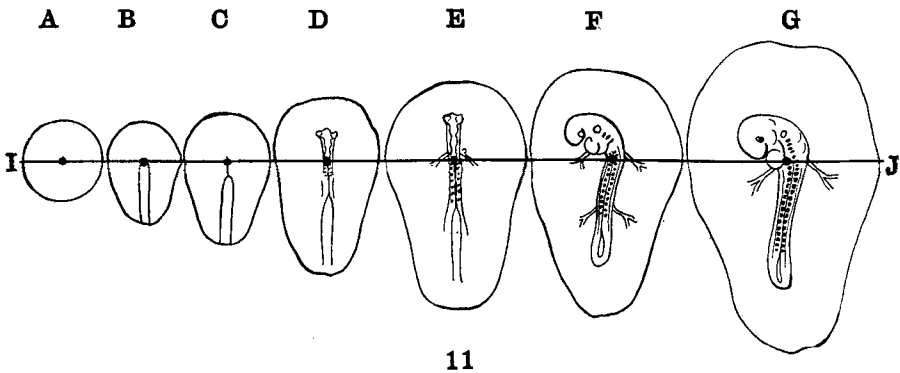
I have already described experiments which I have made upon the primitive streak and have tried to show that the anterior end of the primitive streak of sixteen to eighteen hours represents the region of the later embryo which lies back of the heart between the anterior somites.

Experiment V. These experiments were repeated with some modifications. Instead of injuring the anterior end alone, a second wound was made at the posterior end (Text-fig. 10, *x* and *y*). The embryo at the time of the operation was from sixteen to eighteen hours old. After forty hours a normal embryo developed but instead of extending posteriorly to the usual length it was shortened 2 mm. Another egg injured in the same way (Text-fig. 10) developed into an interesting embryo (Pl. II, Fig. 10). The posterior wound (*y*) healed so that no trace of it could be discovered, but the anterior wound (*x*), through the further

¹Kopsch. *Loc. cit.*

growth of the embryo, was left on one side. The only disturbance evident was in the medullary folds and somites on the side of the injury.

Experiment VI. A wound at the posterior end of the primitive streak is alone sufficient to shorten the embryo caudad. In Pl. II, Fig. 11, a surface view of an embryo of forty hours' incubation is shown. The wound (y) was made at the posterior end of the primitive streak of eighteen hours. Fourteen pairs of somites are present, and the embryo measures 3 mm. from the heart to the anterior border of the brain. This region is normal, but growth in a posterior direction has been stopped, by the wound, and the length is reduced 1.5 mm.



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Summary. The results from these experiments show that in the formation of the third-day chick neither head nor tail region can be taken as fixed points, indeed no one point on the blastoderm can be said to be fixed. In the series of diagrams (Text-fig. 11, A-G) I have indicated, in a schematic way, the method of growth from the beginning of incubation until the third day. The growth of the area opaca is symmetrical therefore it is not included in the diagram. The line I-J represents the plane dividing the unin-cubated blastoderm into anterior and posterior halves, and passes through the region in the older embryos which corresponds to the middle point of the area pellucida before incubation. From this point growth proceeds in all directions in the plane of the blastoderm. The growth from the first to the twelfth hour is sym-

metrical. From the twelfth to the eighteenth hour the area pellucida increases in length posteriorly. From the eighteenth to the twenty-fourth hour growth continues posteriorly and also proceeds in an anterior direction. From the end of the first day to the end of the second day it advances from the heart in both directions, more rapidly caudad than cephalad. After this time the tail and head are folded off from the surface of the blastoderm.

III. THE ORIGIN OF THE MATERIAL FROM WHICH THE LATER EMBRYO ARISES.

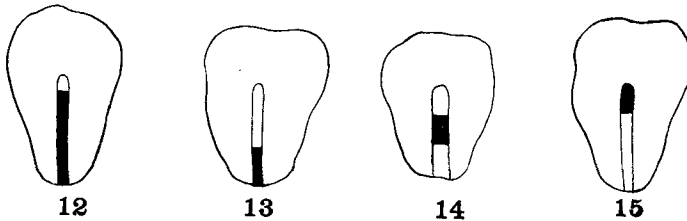
I have already spoken of Kopsch's conclusions as to the material from which the embryo arises, so that I shall merely mention my own results. If, as Kopsch says, the primitive streak represents the entire embryo with the exception of the pre-chordal head region, then the destruction of definite areas of the primitive streak should result in a failure to develop the parts which arise from the injured area.

Experiment I. The first experiment consisted in destroying all of the primitive streak except its anterior end (Text-fig. 12). This operation is very likely to kill the entire embryo as injury to so large an area usually results in a spreading apart of the margins of the wound. The further development of an embryo injured in this way may be seen in Pl. II, Fig. 12. The embryo is abnormal, but shows structures which indicate that when deprived of all of its material except the anterior end the primitive streak gives rise to the first few pairs of somites; and that the brain and notochord develop. The somites are much thinner than in the normal embryo.

Experiment II. In another series of experiments the posterior third of the primitive streak was destroyed (Text-fig. 13). The destruction of this region resulted in an embryo (Pl. II, Fig. 13), in which the entire caudal region was abnormal. The heart and brain, which are not represented in the figure, were normal, and fifteen to eighteen pairs of somites were formed in the anterior trunk region. This result agrees with Kopsch's view that the posterior third of the primitive streak represents the caudal region of the embryo from the twentieth somite to the posterior end.

Experiment III. In a third series of experiments the middle part of the primitive streak was killed (Text-fig. 14), leaving some of the material in front, and some back of the wound. According to Kopsch, in the later embryo the region from the first to the twentieth somites should be lacking.

Nearly all of the embryos which I operated upon, in this way, were so greatly disturbed by the wound that all development was checked. In Pl. II, Fig. 14, a surface view of the body region of one of the embryos which developed further is shown. The brain and heart were normal, therefore they are not included in the figure. Posteriorly the wound (*w*) stretched apart, but anteriorly medullary folds and ten or twelve pairs of somites are present. This result indicates that at least ten or twelve pairs of the first twenty somites come from the material in the anterior third of the primitive streak.



Experiment IV. Finally, the anterior third of the primitive streak was killed (Text-fig. 15). After further incubation the embryo developed a normal brain and heart in front of the wound. The trunk region (without the brain and heart) of one of these embryos is shown in Pl. II, Fig. 15. Back of the wound (*w*) eleven to fourteen pairs of somites are present. By comparison with normal embryos of the same age I conclude that these somites represent approximately, the tenth to the twentieth pairs, therefore all of the somites between the first and tenth pairs have been destroyed by injuring the anterior one-third of the primitive streak. The notochord is also lacking in these embryos.

It is evident from these results that the primitive streak of eighteen hours represents the material from which the trunk and tail regions of the later embryo develop; that the posterior third of the primitive streak represents the region back of the eighteenth

pair of somites, the middle third represents roughly, from the twelfth pair to the eighteenth, while the anterior third supplies material for those structures which lie *between* the heart and the twelfth pair of somites, but *does not* include the chordal region of the brain.

SUMMARY AND CONCLUSIONS.

1. The central point of the unincubated blastoderm represents the anterior end of the primitive streak, and later, the region just back of the heart; therefore, the greater part of the embryo develops in the posterior half of the blastoderm.

2. The region midway between the center of the unincubated blastoderm and its anterior border represents the head region of the later embryo.

3. The position of the embryo on the area pellucida is fixed. The long axis of the future embryo divides the unincubated blastoderm into right and left halves and a line drawn through the blastoderm in the long axis of the shell divides it into anterior and posterior halves.

4. Destruction of the material of the unincubated blastoderm between the center and its posterior margin does *not* result in the formation of the primitive streak on any other radius.

5. The growth of the blastoderm is uniform up to the eighth to tenth hour, and this uniformity is preserved in the later growth of the area opaca, but from the tenth hour the area pellucida begins to grow more rapidly in a posterior direction, then later it advances anteriorly until it assumes an oval form. Up to the third day the region immediately back of the heart (the anterior end of the early primitive streak) is the center of growth in all four directions, anteriorly, to the left, and to the right, and to a much greater extent posteriorly.

6. Injury to the center and posterior margin of the unincubated blastoderm results in a shortened embryo.

7. Injury at the posterior margin alone will shorten the embryo by preventing growth in a posterior direction.

8. Neither head nor tail region of the embryo can be taken as fixed points, the growth at each end proceeds until the head and tail become folded off from the blastoderm.

9. After destruction of all of the material of the primitive streak except its anterior end a small embryo with eight to ten pairs of somites develops.

10. The posterior third of the primitive streak furnishes the material for the caudal region of the later embryo. The middle third represents the trunk region, and the anterior third that part of the embryo which lies between the heart and the tenth to twelfth pairs of somites. The material of the primitive streak does not enter into the formation of the brain.

The Woman's College,
Baltimore, June 1, 1904.

EXPLANATION OF PLATES.

PLATE I.

- Fig. 1. Blastoderm 20 hrs. old. x , point of insertion of hot needle before incubation.
- Fig. 2. Ventral view of embryo 48 hrs. old. Injury made in center of unincubated blastoderm lies back of heart. Brain undeveloped.
- Fig. 3. Surface view of forty-hour embryo in which the material along the radius $x-y$ had been killed. Heart, brain and notochord are present.
- Fig. 4. Embryo 30 hrs. after operation described for Fig. 3. Heart and posterior body region present.
- Fig. 5. Primitive streak 18 hrs. old. The three black areas indicate the positions of the injuries made upon the unincubated blastoderm.
- Fig. 6. Surface view of embryo 36 hrs. old. The black areas indicate the wounds x and o made in the blastoderm before incubation.
- Fig. 7. Embryo 40 hrs. old. The openings x and o indicate the wounds made in the blastoderm.

PLATE II.

- Fig. 8. Embryo 36 hrs. old. The position of the wounds is indicated by the black areas x and y .
- Fig. 9. Embryo 40 hours after injuries were made in the anterior and posterior end of the 18 hr. primitive streak.
- Fig. 10. Embryo 12 hours older than that in Fig. 9 after the same operation.
- Fig. 11. Forty-hour embryo in which an injury had been made in the posterior end of the primitive streak of 18 hrs. The black region (y) indicates the wound.
- Fig. 12. Embryo incubated 36 hrs. after four-fifths of the material of the primitive streak was destroyed, leaving only the anterior end.
- Fig. 13. Fifty-hour embryo in which the posterior third of the primitive streak (w) was destroyed. The brain which was normal is not shown.
- Fig. 14. Surface view of embryo 30 hours after the middle part of the primitive streak was destroyed. The normal brain is not shown.
- Fig. 15. Embryo of same age as preceding one. The anterior third of the primitive streak was destroyed. Heart and brain which are not given are normal. w in these figures indicates region of injury.

