Localization of Amnion-Forming Areas in the Early Chick Blastoderm[1,2]

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The 'lymph vesicle', as it is commonly called, that appears quite frequently in the chorio-allantoic grafts, was interpreted by the author ('42) as representing an amnion. This interpretation was indicated by several findings when the transverse strips of chick blastoderms of somite stages were transplanted to the chorio-allantoic membrane. These were 1) the fact that the vesicle made its appearance only when an extra-embryonic part of the blastoderm was included in the transplants; and 2) the fact that the wall of the vesicle not only consisted of a similar histological structure to the amnion, but also exhibited in most cases peristaltic contractions peculiar to it.

Potential organ-forming areas in the chick blastoderms were already mapped out in detail by Willier and Rawles ('35) and Rawles ('36), but no information has been forwarded regarding the amnion-forming areas. The present study was planned, first to ascertain whether the above interpretation on embryos in somite-stages could hold true even in the early chick blastoderms (head-process stage), and further to acquire some definite knowledge concerning the localization of these areas in the same stage. An abstract of the results given in this paper has been published previously in Japanese (Kume, '47).

Material and Methods

For the convenience of comparison with the results by Rawles ('36), experimental procedures used by her were adopted with slight modification throughout the experiments. Materials were exclusively blastoderms of so-called head-process stage (having the process 0.30–0.73 mm in length). For the purpose of confirming the previous interpretation, transplantation of transverse strips of blastoderms was performed at first. These strips were obtained by dividing the blastoderms by three transverse cuts (fig. 1), one cut passing through the primitive pit, another being made anterior to this 0.43–0.86 mm, and the third posterior to the first 0.56–0.77 mm.

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The second strip (designated as BC) and the third strip (designated as DE) thus made correspond respectively almost with the levels B plus C and D plus E in Rawles' case ('36; fig. 5, p. 276).

For amnion-forming areas, each strip was again separated into the median (M), the left (L) and the right (R) pieces respectively, and these were transplanted. The separation was made longitudinally parallel along the median axis of the blastoderm 0.15–0.18 mm to the left and to the right. Before these procedures, the blastoderms were stained lightly with neutral red solution, which was very useful for identification of stages and for measurements. Methods of transplantation and of securing grafts were as usual.

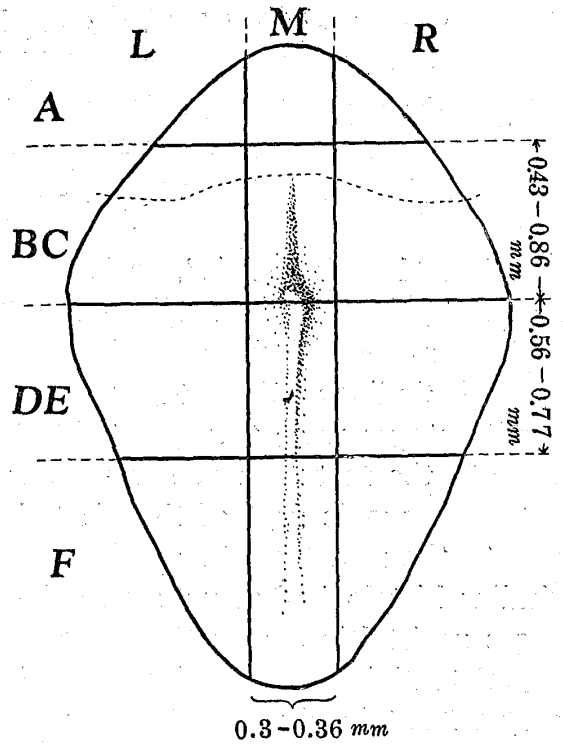


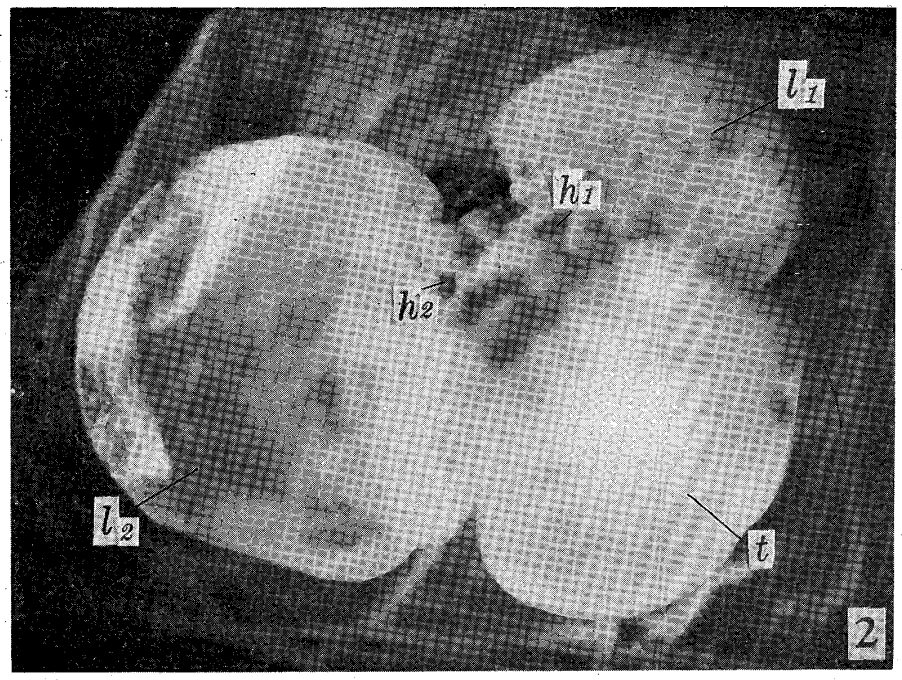
Fig. 1 Illustration of the method of sectioning a blastoderm. Letters A, BC, DE and F indicate the four strips divided by three transverse cuts. Each of these strips is again separated into three pieces, a median (M), a left (L) and a right (R).

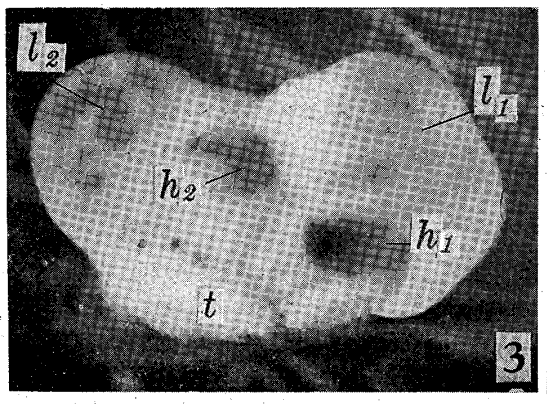
Results

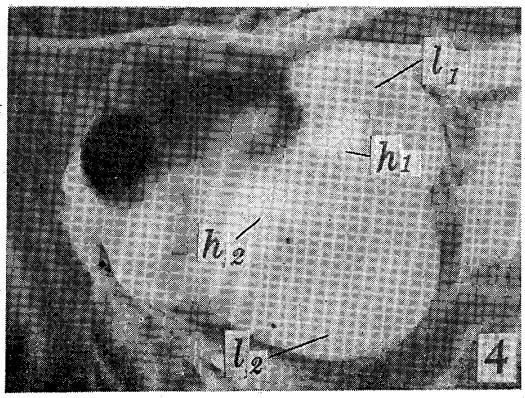
Results of transplanting strips BC and DE To begin with, the results of transplantation of strips BC and DE are considered. A total of 32 grafts, one half of them coming from BC and the other from DE, was obtained. Of these grafts, 10 cases each had a pair of vesicles separated from each other (figs. 2 and 3), 7 cases each had a pair of vesicles fused together, 7 cases each had only a single vesicle, and in the remaining 8 cases no such structure appeared. Peristaltic contraction of the wall of vesicle was observed in 11 cases, which, however, was less active (5~11 contractions per minute) than that observed in the previous study.

Situations brought out by cases with two separate vesicles are strikingly similar to those of the analogous cases in the previous study, which have given a hint as to the origin of vesicles. Cases shown in fig. 2 and fig. 3 are the most typical of these, both coming from the transplants of strip DE.

As can be seen in fig. 2, the case 266–11 has two large vesicles (1_1 and 1_2) that are developed just at the opposite sides of a mass of tissues lying between. When observed in a living state, no contraction of their walls was noticed, but the hearts, two in number (h_1 and h_2), each facing one of the vesicles, showed a regular rhythmic pulsation. By the side







Figs. 2 and 3 Phtographs, respectively, of cases 266-11 and 264-14, each obtained from a transplant of strip DE. Note two vesicles (l_1 and l_2) lying at the opposite sides of a mass of tissues (t). h_1 and h_2 denote hearts. ×6

Fig. 4 Photograph of case 269-5 obtained from a transplant of strip BC, in which two vesicles (l_1 and l_2) are fused together. A pair of eyes (black circular areas) and hearts (h_1 and h_2) are noted. ×6

of each heart, there were noticed small but greenly coloured areas, which were identified as liver tissues after sectioning. Fig. 5 is a drawing of a section through the vesicles and the hearts of this graft. This shows how the various structures such as vesicles, hearts and livers are placed symmetrically with reference to the centrally situated nervous tissues and intestine, and shows how closely their whole arrangement resembles that of the normal.

In the case 264-14 shown in fig. 3, the vesicles are far smaller when compared with the case 266-11, but contraction of their walls was noticed when observed in a living state. Relations between various structures in the graft are alike in both of these cases and we have nothing to add.

As has been the case in previous study, what is shown by cases with a pair of separate vesicles is that there exists a certain definite relation between the vesicles and the various structures developed in the tissue mass. And, as this relation is represented by the facts that the vesicles are observed always to be laid down outside the tissue mass and are placed symmetrically with reference to the structures such as the nervous tissues or the intestine, this seems to suggest that the vesicles are originated from the extra-embryonic portion of the blastoderm.

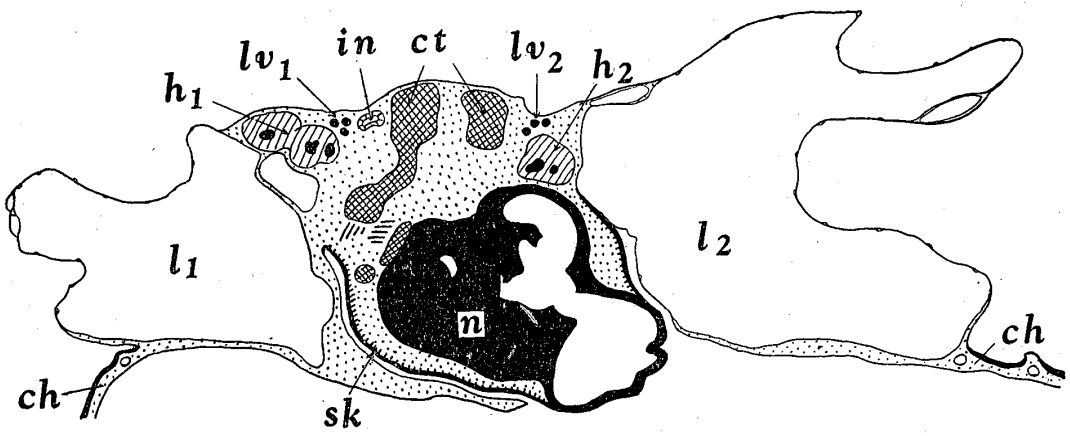


Fig. 5 A section of the case 266-11 through vesicles and hearts to show arrangement of organs in the graft. ch, chorio-allantoic membrane; ct, cartilage; h_1 and h_2, hearts; in, intestine; l_1 and l_2, lymph vesicles; n, nervous tissues; sk, skin.

Results of transplanting the divided pieces of BC and DE We are next to consider the results obtained by divided pieces of strips BC and DE, which will throw further light upon the question of the origin of vesicles. From strip BC, 30 grafts (9 median, 10 left, 11 right) out of 33 transplants (10 median, 10 left, 13 right), and from strip DE, 19 grafts (10 median, 5 left, 4 right) out of 32 transplants (13 median, 10 left, 9 right) were recovered. General results are in good agreement with those by Rawles: the graft frequency is the highest in the median and higher in the left than in the right, and regarding the amount of tissues produced in the grafts also, the same relation is observed, but the kind of tissues differentiated in them is different according to the different regions. Thus, from strip BC, tissues such as brain, notochord, cartilage, muscle and entodermal tubes are produced in the median, while, heart, liver, cartilage, muscle, entodermal tube and vesicle are produced in the laterals. It is of interest to note that in cases in which the vesicle appears in the laterals, it is only in a form of a single and not in that of a pair as in the former cases (figs. 6-11). As shown in table 1, vesicles appear in 5 out of 10 grafts from the left piece, and in 2 out of 11 grafts from the right piece. Contraction was observed in 2 from the left and none from the right. These results indicate that the vesicle is a peculiar production of the laterals, and not of the median, that is, it is originated from the extra-embryonic portion of the blastoderm.

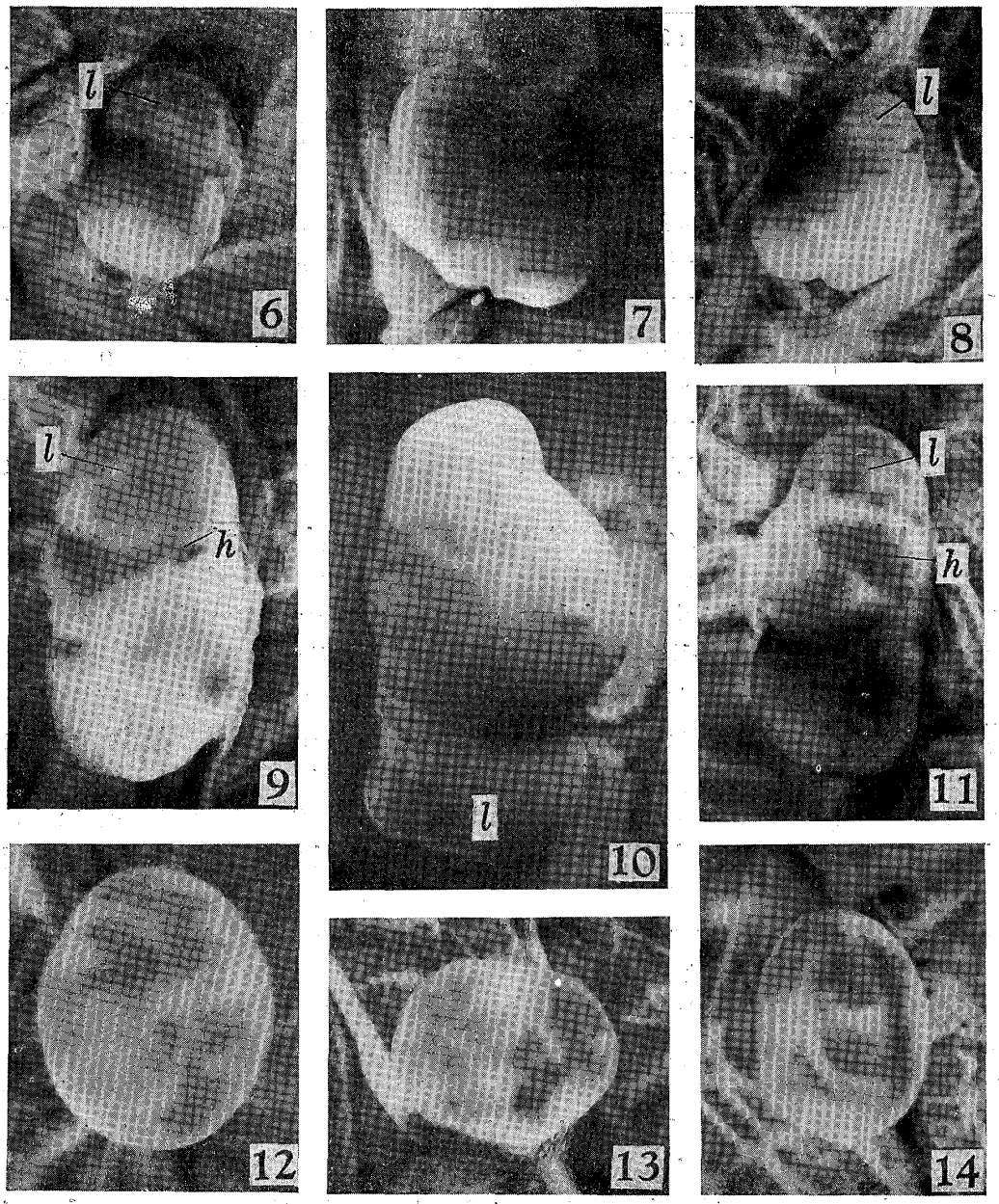
Table 1

		A	BC	DE	F
Left	No. of transplants	12	10	10	13
	Grafts obtained	0	10	5	4
	Vesicles appeared	0	5	5	4
	Vesicles contracted	0	2	2	2
Median	No. of transplants	10	10	13	14
	Grafts obtained	3	9	10	5
	Vesicles appeared	0	0	1	5
	Vesicles contracted	0	0	0	4
Right	No. of transplants	12	13	9	14
	Grafts obtained	0	11	4	2
	Vesicles appeared	0	2	4	2
	Vesicles contracted	0	0	3	1

In strip DE, as in strip BC, the median piece yields grafts more frequently and larger ones than either of the laterals. Mesonephros, cartilage, entodermal tube and less quantity of nervous tissues and notochord are the tissues differentiated in the median; while, heart, liver, cartilage, muscle and entodermal tube, but not mesonephros, are the tissues differentiated in either of the laterals. As to the vesicle, it is produced in 100 per cent of the grafts in either of the laterals, contraction being observed in 2 out of 5 grafts in the left and in 3 out of 4 grafts in the right. In the median piece, it is observed only in one (244–24) out of 10 grafts obtained.

As this last one was exceptional and came from the transplants whose posterior transverse cut was made 0.77 mm posterior to the pit, it must have contained the region usually included in the median piece of the following F level. Therefore, some median pieces of strip DE were obtained by cutting further behind than this, and these were again subdivided into anterior and posterior pieces for the purpose of testing their capacity to produce vesicles. From the anterior piece thus made, vesicles appeared in none of the 4 grafts obtained, while they were produced in 3 out of 4 grafts from the posterior piece. These results indicate that at least the anterior portion of median DE has no capacity to produce the vesicle, and somewhere near 0.77 mm posterior to the pit is the probable anterior-most portion of the vesicle-forming area in the median.

Results from strips A and F, and their divided pieces Strip A comes from the most anterior portion of the blastoderm which is still lacking the mesoderm. This portion, as shown in detail by Rawles ('36), has only a slight capacity to grow on the membrane. Actually, no transplants of this strip or its divided pieces yielded anything worthy to be called grafts, and only a bit of nervous tissues was noticed in 3 cases in the median.



Figs. 6, 7 and 8 Photographs, respectively, of cases 248-6, 248-1 and 249-7. These grafts were obtained from the transplants of the left, the median and the right pieces of strip BC respectively. Note that a vesicle (1) is developed in the grafts of the laterals, but not in the graft of the median. ×8

Figs. 9, 10 and 11 Photographs, respectively, of cases 245-20, 244-24 and 244-29. These grafts were obtained from the transplants of the left, the median and the right pieces of strip DE respectively. Vesicle (1) is developed in every case, but the one in the case 244-24 is exceptional (see text). Hearts (h) are developed in the grafts from the laterals. ×8

Figs. 11, 12 and 13 Photographs, respectively, of cases 245-11, 245-3 and 249-17. These grafts were obtained from the transplants of the left, the median and the right pieces of strip F respectively. Vesicles are almost only products from strip F. ×8

Strip F, which includes a rear half of the primitive streak, also represents another portion that has only a feeble capacity to produce grafts; their frequency is less and the amount of tissues differentiated in them is scarce. However, the most striking feature of the grafts obtained from this strip or its divided pieces is that the vesicle which always appears together with each of them is rather large. From the median piece, 5 grafts were obtained out of 14 transplants, the vesicle being accompanied in every case and contraction observed in 4 of these. As was the case in other strips, the right piece was inferior in graft-frequency to the left. But, the vesicle was produced in every case together with the graft obtained, and contraction was observed in one of the 2 grafts. From the left, the vesicle was produced in all of the 4 grafts obtained, and contraction was noticed in 2 cases.

Considerations

The conclusion reached by the previous study on embryos in somite-stages is also supported by the present experiments on blastoderms in earlier stage, namely, the median head-process stage, showing that the so-called 'lymph vesicle' represents an amnion that is differentiated in the chorio-allantoic membrane. Evidences for this interpretation are nothing different from those presented in the previous study. There are, in the first place, good reasons to believe that the vesicle originates from the extra-embryonic region. These are furnished by the facts that when the transverse strips of pre-nodal (BC) or post-nodal (DE) regions are transplanted, a pair of vesicles is produced in most of the cases, and in cases in which these are separated, they are arranged bilaterally in symmetry with reference to the various tissues in the grafts; and further, when the strips are divided into three pieces and used as donors, the vesicle appears only in the laterals and in a single form only instead of a pair.

Further evidences are furnished by the histological structure as well as the functional activity shown by the vesicle. The wall of the vesicle is a thin membrane lined by a layer or layers of smooth muscles and can exhibit a peristaltic contraction quite similar to that of the amnion. Although contractions exhibited by the vesicles obtained in this experiment were less active than those in the previous study, no characteristic differences between them are found. Besides, the fluid in the vesicle resembles that in the amniotic vesicle, and this fact may be taken as an additional evidence.

It is next proposed to discuss how far the vesicle-forming areas are extended in the blastoderm, and how frequently the vesicle is produced in each of the areas. When the results described in the above section are surveyed as a whole, it is seen that the regions which have the

capacity to produce vesicles cover the hoof-shaped areas lying at the posterior two-thirds of the marginal zone in the blastoderm; whereas, the regions that have no or only a slight connection with vesicle-formation are the most anterior portion of the blastoderm (strip A) and the median regions that are supposed to become most of the embryonic body proper (M. BC; M. DE). The regions capable of producing vesicles almost coincide with the extension of the lateral mesoderm, and this fact is of interest to suggest that the existence of mesoderm is a necessary factor for the formation of vesicle, namely, amnion.

Among the regions that have the capacity to form vesicles, those that have the highest frequency are the hindermost portion (strip F) of the blastoderm and both of the lateral pieces of strip DE. In these regions, vesicles occur in 100 per cent of the grafts obtained. Among these, particularly the median piece of strip F, which includes the rear half of the primitive streak, can produce comparatively larger and more actively contracted vesicles (frequency of occurrence of contracted vesicles being 80 per cent). As the region is more removed from this in anterior direction, the frequency of vesicle formation and of occurrence of contracted vesicles declines: thus, in the left lateral, vesicle-frequency is 50 per cent and frequency of occurrence of contracted vesicles is 40 per cent in strip BC, and comes to zero in strip A: in the right lateral, the former is 18 per cent and the latter is zero in strip BC, and comes to zero in strip A. In the median, the decline is quite rapid and vesicle-frequency is only 10 per cent in strip DE and zero in either of strips BC and A.

Localization and manner of gradation of vesicle-forming potencies not only furnish a peculiar example of organ-forming areas in general, but also give some clue as to the manner of amnion-formation. According to Willier and Rawles ('35) and Rawles ('36), organ-forming areas in the early chick blastoderm take generally an ovarian form, their developmental potencies diminishing peripherally from a center within each area. But, as to the vesicle-forming area, situation is somewhat different. This extends over an area of horse-shoe-like form, its horns directing anteriorly along the lateral sides of the blastoderm. And, as to its developmental potency, there is a center where the posterior middle half of the primitive streak lies, and from where it is graded anteriorly, gradually along the lateral sides of the blastoderm, but quite abruptly in the median.

When we consider these results together with Wetzel's conclusion ('29) based on vital staining, that the lateral mesoderm originates in the posterior half of the primitive streak which acts as a center of morphogenesis, we are led to an assumption on the formation of amnion. If the lateral mesoderm is a necessary factor for the formation of the vesicle, and again, if the vesicle represents an amnion differentiated in the chorio-allantoic membrane, the posterior half of the primitive streak, and not

the so-called 'pro-amnion' which is devoid of mesoderm, may act as a centre for the formation of the amnion.

Summary

1. Conclusion reached by the previous study on embryos in somite-stages ('42) that the so-called 'lymph vesicle' represents an amnion differentiated in the chorio-allantoic membrane, was also supported by the present experiments on blastoderm in head-process stage.

2. Localization of vesicle-forming areas in the head-process stage was tested by transplanting divided pieces of the blastoderm (fig. 1).

3. Results show that the regions capable of forming vesicles almost coincide with the extension of the lateral mesoderm. Regions which have no or only a feeble capacity to produce vesicles are the foremost portion of the blastoderm and the median portion that is supposed to become the embryonic body proper.

4. There was noted a gradation of developmental potency as manifested by the frequency of vesicle-formation and of occurrence of vesicle that has shown peristaltic contraction. This was the highest in the median part of the hindermost portion of blastoderm, where there is the posterior middle half of the primitive streak, and was graded off anteriorly from this centre along the sides of the blastoderm.

5. Some necessary factors for the formation of amnion were suggested.

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