Limited axial structures in nodeless chick blastoderms

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INTRODUCTION

The author has been using post-nodal segments of early chick blastoderms as an experimental system to study early morphogenesis and differentiation. The segment is produced by a transverse cut at about 0.4 mm from the primitive pit and is known for its very limited potencies in chorio-allantoic transplantation or in culture, in contrast to anterior parts containing Hensen's node (Hunt, 1931; Willier & Rawles, 1931; Rudnick, 1938; Waddington, 1933; Spratt, 1952; Butros, 1962). One of its characteristic features is the appearance of a concavity in the middle of the anterior border in the shape of a 'V' (Waddington, 1935; Rudnick, 1938), but it is not established whether this is due to regression movements of the primitive streak or to retraction of lateral parts (Jacobson, 1938). Experiments attempting to clarify this point are reported in this paper.

Another feature noted upon explanting the post-nodal segment is the accumulation of mesoderm in the midline, forming columns that seem to supplement the primitive streak gradually. This mesoderm is not sufficiently organized to form somites and notochord. It was hoped to obtain better mesodermal organization and induction of neural tubes with the increase of lateral mesoderm effected by leaving an anterior extension to the post-nodal piece in the form of lateral 'arms'. True to expectation, as the experiments reported here will indicate, columns of mesoderm both somitic and lateral accumulated in the midline and as a result neural folds or tubes developed in these explants.

MATERIALS AND METHODS

White Leghorn eggs were obtained from the University Farm and stored at 16 °C for a few days until used. Incubation directly from storage lasted for 23 h at 37.5 °C, and gave blastoderms ranging from definite streak (DS) to head process (HP) and head fold (HF) stages. The culture media used were Spratt's albumin-agar or whole egg-agar (Spratt, 1947) placed in watch-glasses supported

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on cotton rings in Petri dishes (Fell & Robison, 1929). Sterile technique was used throughout.

Post-nodal segments. Blastoderms were transected transversely at 0.35 mm or as indicated posterior to the primitive pit and the posterior segment cultured for about 24 h.

Marking. Carmine marking was accomplished by applying fine particles, previously wetted with Ringer’s solution, with glass needles to the explants. Glass needles were pressed into the culture medium to serve as fixed landmarks from which measurements could be made.

Fig. 1

Text-fig. 1. Outline of blastoderm, representing all stages used, showing ‘U’ cut.

Text-fig. 2. Control blastoderm (see text).

‘U’ cuts. A large area including Hensen’s node, head process or head folds when present was removed by two lateral cuts parallel to the axis of the blastoderm and a third, transverse cut, at the 0.35 mm level posterior to the pit, joining the lateral cuts (Text-fig. 1). It should be noted that the lateral cuts reached out to the anterior borders of the blastoderm and hence the top of the blastoderm was left open. The area opaca was left intact. This deaxiated or nodeless and processless blastoderm differs from the post-nodal segments (above) by the two lateral extensions or ‘arms’ anterior to the level of the transverse cut. For controls lateral cuts were made as in the ‘U’ blastoderm but extending along the whole blastoderms, the middle part of the blastoderm was discarded and the two ensuing lateral strips were cultured (Text-fig. 2).
I. **Marking post-nodal explants and whole blastoderms**

(A) Carmine marks were placed 0-3 mm to the inside of the anterior (cut) border. In all sixty specimens examined the marks were found off the explant on the medium as though the border had slipped from under them and was displaced posteriorly. Marks on whole blastoderms at the level from which ‘post-nodals’ are removed showed regression of the central marks. A line joining the new positions of the marks gives a V-shaped curve.

(B) Marks were placed 0-6 mm to the inside of the anterior border. Table 1 gives the distances from fixed needles for representative specimens of thirty-five explants, from which it is clear that there was a substantial posterior displacement of the marks, especially when the marks were close to the midline.

Table 1. *Distances in micrometer units (10 units = 0·33 mm) from carmine marks (a) and (b) to a horizontal line parallel to anterior border of the explant*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Stage</th>
<th>Mark (a): lateral</th>
<th></th>
<th>Mark (b): medial</th>
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<td>2</td>
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II. **Superimposing camera lucida drawings of explants**

A study made by superimposing camera lucida drawings of post-nodal pieces at the beginning of the culture period (Text-figs. 3, 4, 5), and at the time of the ‘V’ concavity (about 6 h later) shows the posterior displacement of the cut edge very strikingly. A region of 0·6 mm behind the anterior (cut) edge of the explanted post-nodal pieces recedes backwards in a proportionately increasing amount as one approaches the midline from both sides, thus describing a smooth symmetrical V-shaped concavity. With this movement of the inner cells, the anterior edge itself follows suit in a sort of a compression wave that causes marks placed near the edge to remain out. Another evidence of this compression
is seen in rounding off at the upper, originally square corners of the explant. It should be noted that in these specimens no marks were placed in the midline (which recedes most). The slight displacement of the marks shown in the Text-figs. 3–5 occurs because they were placed laterally. A study of Text-figs. 3–5 and the data of Table 1 suggests this interpretation: the ‘V’ concavity of the post-nodal fragment is an indication of the continuation of the normal activity of that area in the intact blastoderm, i.e. regression or some form of posterior displacement. The explanted post-nodal piece proceeds as though it is an integral
part of the blastoderm for the first 10 h or so, but later proceeds to regenerative activity, mobilizing the mesoderm to close the 'V' gap.

III. Nodeless blastoderms

The appearance of bulging columns of mesoderm in the posterior region of partly transected blastoderms suggested the possibility of enhancing morphogenesis by increasing the amount of mesoderm in post-nodal pieces. This was accomplished by cutting blastoderms in such a manner as to leave anterior, lateral extensions as explained under Methods (Text-fig. 1).

Series A. Horizontal cut 0.35 mm from the primitive pit; lateral cuts 0.8 mm apart

In this series a total of 19 U-blastoderms (HF) were cultured out of which 8 developed neural tubes and some form of somites. The controls of this series were 6 post-nodal explants and 9 lateral extensions or arms (Text-fig. 2), none of which had neural tubes or somites. No notochords appeared in this or any other series.

Morphology of U-blastoderms in culture. The two arms proliferated mesially and within 8–10 h filled the extirpated area except for a thin slit. The transverse, cut border receded posteriorly, forming a narrow depression in the midline that was quite different from the wide V-shaped concavity of the post-nodal isolates (Butros, 1962). In the regenerative phase there was no central strip projecting anteriorly as in the case of post-nodal segments. Small pulsating heart primodia appeared in both of the arms in most specimens. The U-shaped blastoderms differed in another very significant manner from post-nodal explants: the rise of lateral body folds or elevations in the posterior region flanking a central strip. As seen with the dissecting microscope, each elevation seemed to consist of a vesicular, transparent outer chamber and a core of thick, cylindrical mesoderm. The central, tubular strip seemed to include a neural tube. At their posterior ends, the two elevations joined to form a flask-shaped bottom that resembled the receding node region (tail bud) of normal embryos.

Histology of U-blastoderms. All specimens were similar in their histological character and in the order of appearance of structures as seen in serial sections starting anteriorly.

(a) The most anterior part consists of the tips of the lateral extensions or arms that have approached each other medially and partly filled the cut space with loose tissue. (The gap between the arms increased during handling of the explants.) The primitive cell-layers of this vesicular region were interrupted by typical but small heart primodia. The mesial edges of the arms were definitely not neuralized at this level (Plate 1, fig. A).

(b) A little lower, the arms consisted of spaces; the mesoderm was hardly noticeable. There were nothing but coelomic vesicles in two consecutive rows of serial sections (Plate 1, fig. B).
(c) Thickened mesoderm began to appear more posteriorly in each arm and was unmistakably lateral plate mesoderm with splanchnic and somatic layers and a typical coelome. Blood vessels appeared in the midline (Plate 1, fig. C).

(d) Further below, somitic mesoderm in the form of hollow knobs, cups or spheres began to appear mesial to the lateral plate of each arm. At this level the inner margins of the arms showed various degrees of neuralization and if the two parted arms were to be brought in contact, they would show neural grooves whose forms and symmetries varied from one specimen to the next (Plate 1, fig. D).

(e) More posteriorly, the lateral plate mesoderm approached the midline more closely, and the central knobs developed into somites in some areas of each specimen. These had typical dermatomes, radiating cell columns and limited myocoeles (Plate 1, figs. E, F).

(f) Moving further down in every specimen the neural folds approach each other and, depending on the type of symmetry, constitute a neural groove or tube. Y-shaped, roughly rectangular, round, oval, slit-like or double neural grooves or tubes have been encountered. The abrupt and striking transition from ectoderm to neural tissue is recognizable. The segmental mesoderm becomes diffuse at this level and a space intervenes between its right and left components (Plate 1, fig. G, Plate 2, figs. R, S, T).

(g) At this level lateral body folds, typical of 2-day embryos, that were gradually appearing as elevations of ectoderm on both sides, become very conspicuous. They rise half-way across the blastoderm on each side and course in a

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**Plate 1**

All figures magnified × 100

Fig. A. Anterior part of an 'arm of the U-blastoderm showing a heart primordium.

Fig. B. Region in the U-blastoderms which shows no structures other than coelomic spaces.

Fig. C. Posterior to the region represented in fig. B, thickened lateral mesoderm begins to appear in the 'arms' of U-blastoderms. Initial neural plate thickenings are apparent. In this specimen the arms are much parted.

Fig. D. Posterior to the level presented in fig. C, somitic mesoderm become apparent together with neural folds and grooves.

Fig. E. A specimen showing flattened neural plates and somites in U-blastoderms.

Fig. F. Another specimen showing neural plate and somites in U-blastoderms.

Fig. G. At a more posterior level than represented in figs. E and F a complete neural tube can be seen somewhat ventrally placed, and flanked by somites.

Fig. H. The body folds on both sides of the neural tube in a U-blastoderm. The folds involve ectoderm as well as extensions of mesoderm flanking the neural tube dorsally.

Fig. I. Control of U-blastoderms produced by complete longitudinal cuts and explantation of the lateral strips; absence of thickened mesoderm and neural tissue is noticed.

Fig. J. Control of U-blastoderm in the form of post-nodal explant, showing invaginated ectoderm but lacking thickened mesoderm and neural tissue.
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curved manner above and around the middle part of the body carrying the mesoderm with them. The neural tube is in the centre of these folds as in normal embryos and blood vessels appear in the usual positions (Plate 1, fig. H).

(i) As the neural tube is vanishing gradually, transition to primitive groove and knot is noticed. Confluence of the three germ layers in the midline occurs as though the explanted blastoderm had a node that later receded posteriorly. Blood islands were abundant below this region.

Control specimens of series A. Ordinary post-nodal pieces and ‘arms’ were used as controls. The ‘arms’ were from blastoderms given two complete lateral cuts like those used in preparing the U-blastoderms (Text-fig. 2).

Examination with the dissection microscope showed two separate heart primodia in the anterior regions as was the case in the U-blastoderms. No axial structures of any type were noted in any of the specimens. Serial sections showed no mesodermal accumulation, nor body folds, nor neural folds. Other than in the heart primordia, mesoderm was hardly noticeable in the sections. Rare spots of primitive, limited proliferation in lateral mesoderm appeared but revealed no axial organization. Blood islands formed in the extra embryonic areas (Plate 1, fig. I). The post-nodal explants used as controls showed only a transition from ectoderm to weakly neuralized tissue at the invagination site. Segmental mesoderm was poor as compared with the organized somites of the experimental specimens (Plate 1, fig. J).

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Plate 2

All figures magnified × 100

Fig. K. An anterior region of a U-blastoderm from series B, showing a heart primordium and the end of neural vesicle.

Fig. L. Region in ‘arms’ of U-blastoderm in series B that lacks axial mesoderm and neural tissue.

Fig. M. At a more posterior level than that presented in fig. L (series B), neural plate and slightly dispersed somitic mesoderm are noted.

Fig. N. Controls of series B in the form of post-nodal explants, showing primitive ectodermal invagination and a slightly thickened mesoderm.

Fig. O. A specimen from series C showing neural tube and mesenchyme in a U-blastoderm. The body folds are in the form of solid invaginations.

Fig. P. Control specimen from series C in the form of a lateral strip explant indicating absence of axial mesoderm and neural tissue.

Fig. Q. A specimen from series E, showing a U-blastoderm that had developed body folds and a neural tube.

Fig. R. Another U-blastoderm from series A, showing the development of a neural tube in connexion with the lateral plates and somitic mesoderm.

Fig. S. Another specimen from series A, showing axial structures as noted in fig. R.

Fig. T. One more U-blastoderm from series A, showing body folds, axial organization of the mesoderm and neural tube.
Series B

The cuts were the same distance from the primitive pit as for series A but lateral cuts were closer to the midline, i.e. 0.7 mm wide between the two vertical cuts. Eighteen U-blastoderms were cultured out of which eight developed neural tubes and somites. The U-blastoderms (HP and HF) in this group developed small neural vesicles at the extreme tips of the ‘arms’ but in other respects they were similar to series A. Serial sections showed the same sequence of structures along the antero-posterior axis as in series A. Hearts appeared at the anterior regions of the ‘arms’ (Plate 2, fig. K), and as they vanished from view the neural vesicles also thinned into flat plates and then disappeared completely. There was a stretch of two rows of sections on the slides that had no neural tissue nor thickened mesoderm (Plate 2, fig. L). Following this, lateral plate mesoderm appeared as in series A. Close contact of mesoderm with the ventral side of the ectoderm, reversing the usual polarity, induced neural plates de novo at this lower level (Plate 2, fig. M). The neural tissue further on grew into neural tubes of various shapes and approached the dorsal side progressively. Mesoderm aggregated in the form of somites in some areas, but was mesenchymal at the level of the neural tube. The control explants of this series consisted of 6 post-nodal and 8 lateral or ‘arm’ segments. There were no fully organized axial structures in the post-nodal explants (Plate 2, fig. N). The ‘arms’ showed neural vesicles at the extreme anterior tips. This was followed by coelomic spaces devoid of thickened mesoderm. Some ‘arm’ explants developed heart primordia in the anterior regions that vanished further below and had no more thickened mesoderm of any type. There were no neural tubes.

Series C

Same age and same U-cut as series B except that the anterior tips of the ‘arms’ were cut away before explantation to eliminate the neural vesicle anteriorly. Except for lack of anterior vesicles, there was no difference in the results from those in series B, as all of eight U-blastoderms developed the same structures and in the same sequence, i.e. the arms start with open spaces with thin membranous lining, then lateral plate and somitic mesoderm appear followed by neural folds and tubes (Plate 2, fig. O). The eight lateral strip-controls of this series did not differ from the controls of series B (Plate 2, fig. P).

Series D

Similar to series A as far as the dimensions of the U-cut are concerned but of earlier, definite streak stage. From the 19 U-blastoderms of this DS stage, 6 developed neural tubes, a decrease as against series A. In other respects, including order of arrangement of structures, there was no difference from series A. The controls of series D were post-nodal explants taken from DS stage; none of these fifteen explants developed axial structures.
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Series E

U-blastoderms of DS stage, with a more posterior (0.5-0.6 mm) transverse cut of the same width as in series D. None of the fifteen explants developed neural tubes but rather showed weakly neuralized ectodermal invaginations; somitic aggregations were also weak. The sequence of appearance of structures, however, was not different from the other series (Plate 2, fig. Q). The fifteen 'arms' serving for control explants showed no axial organization.

DISCUSSION

The 'V' depression

Being part of a blastoderm, the post-nodal segment has the inertia of continuing functions that it normally performs in the intact blastoderm. The dominating event at this stage is the regression of the node and therefore it would not be far-fetched to look for an activity of the post-nodal isolate that reflects this process. Several lines of evidence have shown this to be true. The agreement in the contour of the cut border of the post-nodal explant (a 'V' curve) and the pattern of movements of marks placed on the intact blastoderm at the level of transection of post-nodal explants (also a 'V' curve) point to the possibility that the isolated posterior region is retreating to allow room for the 'nodal regression', even in absence of a node. Measurements of distances of carmine marks from a fixed horizontal line (Table 1) also indicate displacement that may be interpreted as regression of points that are near the midline. It should be emphasized, however, that this does not contradict Jacobson's idea of 'some retraction of the lateral parts' that 'causes the split to gape very widely' (Jacobson, 1938). The present author has noted above that in the early hours of explantation a peculiar slipping away of the anterior border occurs, resulting in its displacement posteriorly, leaving some peripheral carmine marks out on the plate. Superimposing drawings of the explants taken at intervals showed that along the center the displacement is much deeper than at the lateral edges. This can be explained by a smooth and symmetrical retraction of the anterior border. The displacement or regression movements demonstrated by objective measurements (Table 1), however, occur a few hours later. Bellairs (1963) has described a 'V' concavity lateral to and independent of the primitive streak and considered this as evidence against regression, but the ring technique of culturing used by her may account for unsymmetrical tension that may have caused this depression.

Axial structures in the U-blastoderms

It is evident from studies of presumptive areas and maps based on chorio-allantoic grafts of the chick blastoderm (Pasteels, 1937; Rawles, 1943; Rudnick, 1938, 1948; Spratt 1952, 1955) that the U-cut in this work extirpated the presumptive neural, notochordal and perhaps a large part of the somitic areas.
Heart primordia appeared in control, lateral segments and U-blastoderms as expected because the presumptive heart region was not totally extirpated. Similarly, neural vesicles were present in the anterior regions of the U-blastoderms and the control, lateral segments; in post-nodal controls neither hearts nor neural vesicles appeared. Rudnick (1955) has described the unique situation whereby anterior brain areas appear in explants that were never exposed to head-process induction.

A. Somites and neural tube. The appearance of somites and neural tube in the U-blastoderms that have only a posterior region of the primitive streak is not unexpected. Waddington found the anterior two-thirds of the streak capable of inducing neural and axial tissues; he cites several cases in which the neural plates developed from tissue located lateral to the midline (Waddington, 1932, 1933). Fraser (1960) finds that neural plate can arise from tissue located lateral to the midline and states that mesodermal cells of different prospective fate can form somites and that grafts of neural tube can induce condensation of somitic mesoderm.

The most posterior blastodermal level from which isolated strips can give neural tissue is not agreed upon but varies between 0.2 and 0.7 mm posterior to the pit, depending on the procedures, the authors, and what they call neural tissue (see Waddington, 1935; Dalton, 1935; Rudnick, 1938; Spratt, 1952; Fraser, 1960). However, we are not concerned here with small areas of neural tissue but with an organized neural tube which did not appear in post-nodal controls transected at or below 0.35 mm. Neural tubes also did not appear in lateral strip controls but they as well as somites did appear in the U-blastoderms. The anterior part of the U-blastoderms was mostly coelomic space, and lateral and somitic mesoderm appeared somewhat posteriorly. In every case there was a contact between the somitic mesoderm and the initial ectodermal thickening forming the neural folds in the ‘arms’ of the U-blastoderms. One cannot decide whether the neural folds induced the somites or vice-versa. Examination of the sections suggests that cells may have moved from the ‘arm’ region to condense in lateral plate and somitic masses posteriorly, because such masses were not observed in the lateral strip controls which lacked the primitive streak. If this is the case then some movement like the one Bellairs (1963) describes, but spreading from ‘arms’ to streak, must have been instrumental in forming the somites and the neural tube.

B. Notochord. No notochords appeared in any U-blastoderms, nor in any control specimen. Regeneration of notochord through the activity of the node is possible in stages younger than definitive streak (Waddington, 1930; Spratt, 1957). The notochord field in the chick is strictly delimited and is localized quite early in development (Rudnick, 1955). Shoger (1960) found that mechanical injury or chemical injury of the notochord center did not prevent its regeneration in blastoderms grown in vitro. Extirpation of the presumptive notochord area (notochord center in the node) and the head process as was done in this
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work should naturally result in total loss of the notochord. No cells at the stages used can replace the notochord center.

CONCLUSION

It seems the U-blastoderm offered more mesoderm and space for the interaction of the mesoderm and ectoderm than the post-nodal segment. Morphogenetic movements, shown to be the essence of early development (Spratt & Hass, 1960), produced an accumulation of mesoderm in the region of the primitive streak that resulted in the formation of somites and the induction of neural tubes.

SUMMARY

1. Definitive streak to head-fold blastoderms were given two lateral cuts parallel to the streak and a third transverse cut at 0.35 mm posterior to the pit, thus removing a rectangular piece around the node 0.8 x 0.35 mm in area. Upon explantation many of these U-blastoderms developed somites and neural tubes, but notochords were absent.

2. Two types of controls were used: posterior segments of blastoderms produced by transection at 0.35 mm posterior to the pit, and lateral regions of blastoderms explanted from outside the central 0.8 mm zone. These controls did not develop true somites or neural tubes but did form ectodermal invaginations and mesodermal aggregations in the midline.

3. The results are interpreted as manifestations of an increase in the amount of mesoderm and of the space available for morphogenetic movements.

RÉSUMÉ

Structures axiales dans des blastoderms de poulet dépouvrus de nœud de Hensen

1. Dans des blastoderms de stades Ligne Primitive définitive à Repli céphalique transverse on pratique deux sections latérales parallèles à la Ligne Primitive et une section transversale à 0.35 mm du nœud de Hensen. On enlève ainsi un fragment de blastoderme entourant la région du nœud d'une surface de 0.8 x 0.35 mm.

En culture, plusieurs de ces blastoderms en forme de U acquièrent des somites et des tubes neuraux, mais pas de notocorde.

2. On utilise deux sortes de témoins: des fragments postérieurs de blastoderms obtenus par une section transversale à 0.35 mm en arrière du nœud, et les régions du blastoderme situées de part et d'autre de la zone centrale de 0.8 mm. Ces témoins ne forment pas de vrais somites ni tubes neuraux mais présentent des invaginations ectodermiques et des amas mésodermiques sur la ligne médiane.

3. On interprète ces résultats comme étant des manifestations de l'augmentation de la quantité de mésoderme ainsi que de l'espace disponible pour les mouvements morphogénétiques.
REFERENCES


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