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Kinetic Properties of the Marginal Zone of the Amphibian Egg in Relation to the Histological Differentiation

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In the normal course of development of the amphibian embryos there occur various kinds of kinetic phenomena, such as epibolic and embolic movements of the surface materials at the time of gastrulation and tubulation and inward migration of the neural plate at the time of neurulation. On these kinetic phenomena of embryonic tissues and cells, HOLTFRETER ('39, '43, '44, '48) has reported several precise investigations in which it is shown that the ectodermal cells which are exposed to the inductive action from the archenteron roof aquire a tendency to elongate into the cylindrical bodies and to move from outside inwards, whereas the cells which cannot receive the inductive stimuli remain spherical, and spread out on a proper substratum. It may be inferred from his findings that the different kinetic properties appear in the cells endowed with the different histogenetic tendencies.

However, it should be impossible to expect that the similar situation is always found in various embryonic tissues in various developmental stages. At the time of gastrulation the marginal zone, particularly in its dorsal half, shows a marked morphogenetic movement of stretching (VOGT, '22, '29). This stretching occurs not only in the intact embryo, but also in the transplant and even in the explant from the early gastrula (SPEMANN, '31; TÖNDURY, '36; HOLTFRETER, '38, '39; SCHECHTMAN, '42). Moreover, it has been stated that the stretching in the various sectors of the marginal zone is an inherent and active process which manifests itself independently of the whole (VOGT, '22; SPEMANN, '38).

On the other hand, it is also true that the histological differentiation of the isolated piece of the marginal zone proceeds quite differently from what it would follow when left intact in the embryo. A small piece taken from the presumptive notochordal area gives rise not only to the notochord but also to the somites and even to the neural and epidermal structures (HOLTFRETER, '38). The developmental fates of the presumptive somite, pronephros and blood material in the early neurula stage are likewise switched each other by the environmental factors (YAMADA, '39, '40). It may be stated, therefore, that the various parts of the marginal zone at the commencement of gastrulation are not definitely determined with respect to their differentiating tendency, but are in a labile state of determination. Comparing these histogenetic tendencies with the former kinetic ones, a clear disparity in the time

of determination is pointed out in the marginal zone. From this disparity it will be difficult to expect that the differences in the kinetic tendency correspond exactly to the differences in the histogenetic tendency in this area. It will be better to anticipate that the two tendencies manifest themselves quite independently. In this line of considerations the present experiments were attempted to ascertain whether the kinetic and the histogenetic phenomena proceed independently in the marginal zone.

Before going further, the author wishes to express his sincere thanks to Prof. M. ICHIKAWA for his encouragement throughout the course of this study. He is also deeply indebted to Prof. H. TAKAYA of the Kônan University for his valuable criticisms.

Materials and Methods

For the purpose of tracing the exact movements of cells, the tissues isolated from the early gastrula were cultured on the inorganic substratum. Various inoragnic substrata, such as glass, cellophane film and collodion membrane were tested, and the last one proved it the most adequate for this purpose. The tissue could adhere to it very firmly and survived for more than two weeks, showing the marked transformation of size and shape on it.

Procedures preparing the substratum were as follows. A few drops of ether solution of collodion were put on the slide glass and stretched out by a glass-rod.

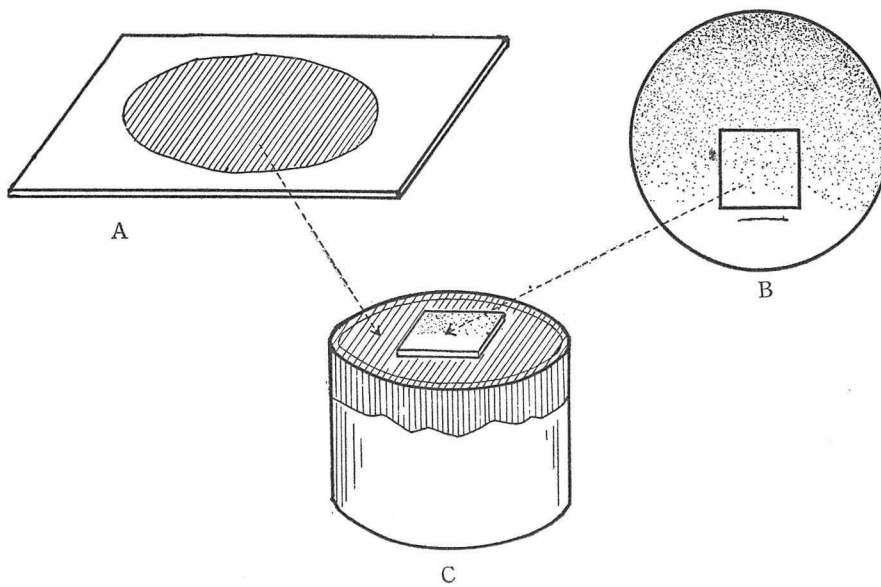


Fig. 1. Schema of preparation of collodion-substratum and the explantation of the tissue from the embryo.
A···collodion-film on the glass
B···explant from the dorsal part of the marginal zone.
C···glass-cylinder with the collodion-film used for the culturing of the explant.

When ether had perfectly evaporated in 2 or 3 hours, they left a very thin film of collodion on the glass. The film was stripped off from the glass with a needle, brought to a cut end of the glass-cylinder which was 15 mm in diameter and 10 mm in height, and sticked to it by the same collodion solution like a drum membrane, as shown in Fig. 1. The glass-cylinder thus equipped with the collodion membrane on one end was sterilized by steam. It was immersed in the full strength HOLTFRETER solution in the culture dish.

The eggs of *Triturus pyrrhogaster* and *Hynobius nebulosus* were collected from the fields near Kyoto. From the early gastrula in which the blastoporal groove just appeared, the dorso-median part of the marginal zone was cut apart in square. The isolated piece was to include the presumptive materials of the prechordal plate, notochord and small quantities of both somite and neural plate. Square pieces of the equal size were also isolated from the dorso-lateral, lateral and ventral parts of the marginal zone, and also from the ectodermal region at the animal pole. The former two pieces were always isolated from the left side of the embryo. These are shown in Fig. 2. The explant was put on a collodion substratum in the culture

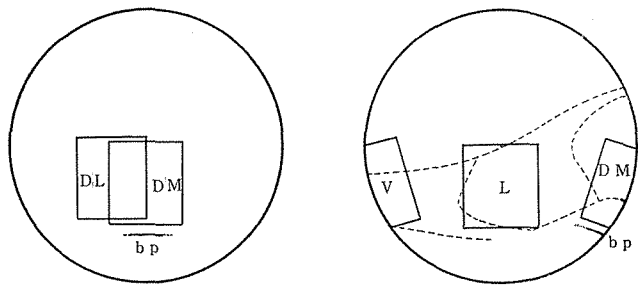


Fig. 2. Schema showing the isolation of the various sectors in the marginal zone of the early gastrula.
left···dorsal view
right···lateral view
bp···blastopore.

medium and pressed repeatedly with hair-loop to facilitate their adhesion. The tissues of *H. nebulosus* were adhesive and sticked itself to the collodion very firmly, while those of *T. pyrrhogaster* were less sticky and hardly adhered to it. The isolates always curled up and detached themselves within 24 hours. Consequently, the former tissues were mainly used.

The configuration of the explanted tissues was observed once a day and drawn with camera lucida. In some cases the vital staining with neutral red and Nile blue was done.

After cultivation for about 2 weeks the tissues together with collodion membrane were fixed with BOUIN's solution, embedded in paraffin and sectioned at 10μ thick. They were stained with MAYER's haemalaum and eosin.

Part I. Kinetic Process

Series DM—*The tissue taken from the dorso-median part of the marginal zone*

In this series, 18 specimens were available. In 17 out of them, the kinetic processes proceeded with quite similar pattern. Namely, the lower, paler part of the piece which had been situated just dorsal to the blastopore became narrower and thicker in 24 hours after cutting, while the upper, darker, pigmented part showed no marked change. In the following 1 or 2 days, the upper part first began to spread, while the lower part became narrower and narrower, and stretched at the same time in the lower direction. Consequently, a compact ridge-like protrusion was formed at the median of the lower edge. The configuration of the explants, as a whole, transformed from square into pentagon which was bilaterally symmetrical to their median line.

In 3 cases the lower halves of both lateral edges were previously stained with Nile blue as shown in Fig. 3. The colour marks stretched markedly lengthwise,

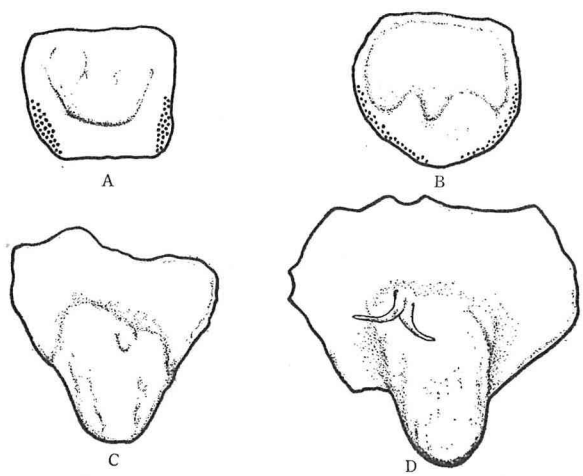


Fig. 3. Configuration of the explant taken from the dorsal part of the marginal zone (DM-12).
A···immediately after explantation
B···24 hours later
C···on the 6th day
D···on the 16th day
Dotted area indicates the colour mark.

but did not spread laterally, they became rather narrower than before. Judging from these transformation of colour marks, it may be stated that in the first 2 or 3 days of cultivation there occur two simultaneous movements of tissues in the lower part of the explant, one is the congregation toward the median part and the other is the stretching toward the lower direction.

In further days of cultivation, the upper part of these 17 specimens spread

continuously, eventually forming a thin epithelial tissue. The ridge-like protrusion of their lower part also became thicker and stretched further. Thus, they sustained the bilateral symmetrical configuration throughout cultivation.

In the remaining one case, the stretching and the congregation of the lower part were obscure. It spread centrifugally in a few days after isolation and eventually formed a thin epithelial tissue with a circular contour.

Series DL—*The tissue taken from the dorso-lateral part of the marginal zone*

In this series 6 specimens were available. The right edge of the explant always corresponded to the median line of the embryo from which it was isolated. The lower part of the explants congregated toward the median of the explant and stretched toward the lower direction, forming a ridge-like protrusion in the first 2 or 3 days after isolation, while their upper part spread into a thin membranous tissue. Consequently, they always established a bilaterally symmetrical configuration.

In 3 cases the lower halves of lateral edges were stained with Nile blue and neutral red, as shown in Fig. 4. The colour marks stretched markedly lengthwise in the first 2 or 3 days of cultivation. They did not at all spread laterally, but became rather narrower. Thus, the kinetic process of the explants in the first 2 or 3 days of cultivation proceeded quite similarly to that in the explants of the previous series.

As to the transformation of the shape of the explants in further days, there was also no marked difference between this and preceding series.

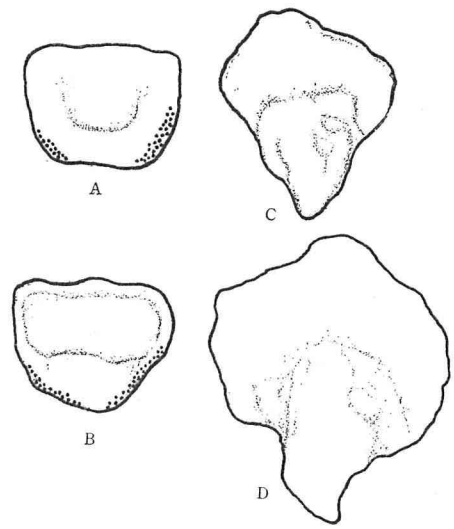


Fig. 4. Configuration of the explant taken from the dorso-lateral part of the marginal zone (DL-5).
A···immediately after explantation
B···24 hours later
C···on the 6th day
D···on the 16th day
Dotted area indicates the colour mark.

Series L—*The tissue taken from the lateral part of the marginal zone*

In 6 out of 11 available specimens the upper part spread out and the lower part congregated to the median line and stretched simultaneously toward the lower direction in the first 2 or 3 days after isolation. Then, the spreading and the stretching proceeded continuously till the end of cultivation. Therefore, there was no marked speciality in the kinetic phenomena in these cases.

However, in the remaining 5 cases the kinetic process occurred in the different pattern. In 2 explants the congregation and the stretching of the lower part coin-

cident with the spreading of the upper part occurred normally immediately after isolation. In the other 3 cases, the congregation and the stretching in the first 2 or 3 days were not obvious, although the upper part spread and the lower part became thick. In the following days all of these 5 explants spread in the centrifugal direction and eventually gave rise to a membranous structure with a circular outline.

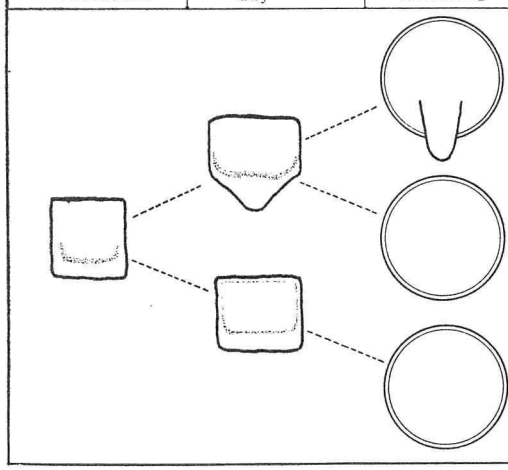
Series V— *The tissue taken from the ventral part of the marginal zone*

In this series were available 12 specimens, 9 out of which showed the congregation and the stretching of the lower part and the spreading of the upper part as in the case of the dorsal marginal zone. But in 5 out of these 9 cases the marked centrifugal spreading occurred from the second or third day after isolation and finally turned out a membranous tissue with a circular contour. In the other 4 cases, the spreading of the upper part and the stretching of the lower part proceeded continuously, although not so remarkable as in the explants taken from the dorsal part of the marginal zone.

In the remaining 3 cases a mere thickening, instead of the congregation and the stretching, was observed along the lower edge. The conspicuous, centrifugal spreading occurred first in the upper part and then extended to the thickened lower part with the resulting production of a thin membranous tissue.

The transformation of the shape of the explants taken from various sectors of the marginal zone can be divided into 3 types, as is shown in Table 1. The ex-

Table 1. Diagrammatic representation of the transformation of the shape of the explants taken from various sectors of the marginal zone.

Transformation of the Shape of the Explant			Series	DM	DL	L	V
soon after the isolation	on the 3rd day	at the end of culturing	Available	18	6	11	12
			Type 1	17	6	6	4
			Type 2	0	0	2	5
			Type 3	1	0	3	3

plants in which the ridge-like protrusion is formed in the early period of cultivation can be divided into two types according to their final form, one in which the protrusion persisted till the end of cultivation (Type 1), and the other in which the protrusion spread out into a thin membranous tissue (Type 2). The explants, the lower part of which did not congregate but thickened and finally spread out into a thin membranous tissue, represent Type 3.

Series DMT and DMS—*The square tissue taken from dorsal part of the marginal zone with its lower half divided into two or three parts*

In these series a relatively wide pieces were isolated from the dorsal part of the marginal zone, and their lower half was divided into two parts by a cut along the median line of the original embryos (DMT-series), or divided lengthwise into three parts (DMS-series). These explants were cultured on the collodion membrane as in the preceding series (Figs. 10, A and 11, A).

Seven in DMT-series and eight in DMS-series were available. In all of 15 specimens it was pointed out that the spreading occurred in the upper undivided part, while the congregation coincident with the stretching took place in every arm of the lower divided part.

Series E—*The tissue taken from the presumptive ectodermal area of the animal pole*

In this series 9 specimens were available. The tissue began to spread at 1 or 2 days after isolation. Then, many irregular folds were frequently formed, but became flattened again with the conspicuous centrifugal spreading. These explants established nothing but a very thin epithelial tissue each.

Discussion

As is enumerated above, the tissue taken from the various sectors of marginal zone indicated three kinds of kinetic phenomena in the first 2 or 3 days of cultivation. They are spreading, congregation and stretching. In the upper part of the explants the spreading always occurred. Comparing the spreading of this area to that of the ectodermal tissue, there is no marked difference. In the lower part of the explants, however, the congregation and the stretching proceeded markedly in the first 2 or 3 days of cultivation. As the invagination can not occur under the present experimental conditions, it is by no means easy to point out the correspondency between the congregation and the stretching in the explant and the kinetic process in gastrulation of the intact embryo. However, judging from the lapse of time after isolation, it seems probable that the stretching and the congregation in the explant correspond to the stretching and the confluence of the marginal zone in gastrulation. The congregation in the explant and the confluence in the intact embryo have a similarity in a point that both phenomena proceed always coincident with the

stretching of the tissue. On the other hand, a marked difference can be pointed out between them concerning the center of these movements. In the intact gastrula the confluence always proceeds toward the dorso-median line, whereas the congregation in the explant occurs always toward the median line of the explant itself, regardless of the original place in the embryo from which it is cut out.

In the present experiments it was also revealed that no marked qualitative difference was found in the kinetic properties of the explants coming from the various sectors of the marginal zone. This finding is not consistent with that obtained by SCHECHTMAN ('42). He isolated various parts of the marginal zone from the early gastrula of *Hyla* and cultured them in a saline solution. In his experiment it was shown that different sectors performed the respective kinetic processes. The explant taken from the dorsal part of the marginal zone always produced a club-shaped protrusion within 10 to 14 hours of culturing, while the explant isolated from the dorso-lateral or the lateral part of the marginal zone did not produce such a structure, but it became flattened and then stretched in the direction along the dorso-ventral axis of the original embryo. In the present experiment, however, such a marked difference could not be pointed out between them. This may be due either to the difference of the species used or to the difference of the experimental conditions employed or to both.

The kinetic phenomena did not come to the end in the first 2 or 3 days after isolation. In the majority of cases the spreading of the thin epithelial tissue and the stretching of the ridge-like protrusion continuously proceeded in the following days. However, it is doubtful that these kinetic processes in the later phase of cultivation correspond to the morphogenetic movements in the late gastrula. In the isolation experiments of the dorsal part of the marginal zone, HOLTFRETER indicated that mesodermal stretching became maximum on the second day after isolation, then the stretched explant recontracted into a more compact body, and still later it entered a new stage of axial elongation. He ascribed this elongation to the real growth process. In our present experiments also it is likely that the growth process takes part in the spreading and the stretching of the tissues in the later phase of cultivation.

Part II. Histological Differentiation

1. *General Remarks*

In the explants taken from the dorsal and dorso-lateral parts of the marginal zone, various kinds of tissues were obtained; the epithelial tissue in the upper part, and the ectodermal structures such as the neural and the epithelial, and the mesodermal ones such as the notochord and the muscles in the lower protrusion. The pronephric tubules were rarely found and the blood cells were hardly observed. The ear was formed very frequently, but the nose occurred only in one case. The eye was never met with. In three cases there were balancers at the upper end of the ridge-like protrusion (*cf.* Fig. 3, D). In one exceptional case which was described

in Table 1 as Type 3, only the epithelial structure and the undifferentiated cells-mass with plentiful yolk granules were observed.

In the explants taken from the lateral part of the marginal zone, various kinds of tissues were also found in 5 cases which remained their ridge-like protrusion till the end of cultivation. All of them belonged to Type 1 in Table 1, and the neural, epithelial, notochordal and muscular tissues were encountered in them. However, comparing them with the explants from the dorsal part of the marginal zone, these tissues were far less in quantity. In the remaining 6 cases, 1 in Type 1, 2 in Type 2 and 3 in Type 3, no more than the epithelial tissue was observed adjacent to an undifferentiated yolk-laden cells-mass.

The explants taken from the ventral part of the marginal zone never differentiated into any neural or mesodermal structure excepting the blood cells in one case. They always consisted of the epithelial membrane and the undifferentiated cells-mass with a plenty of yolk granules.

The results enumerated above are summarized in Table 2.

Table 2. Results of the histological examination.

Series	Available cases	Type of the configuration of explants (*cf.* Table 1)		Combination of the tissue differentiated				
		Type	No. of specimens	epithelial neural notochordal muscular	epithelial notochordal	epithelial neural muscular	epithelial blood	epithelial undifferentiated cells
DM	18	1	17	15	1	1	0	0
		2	0	0	0	0	0	0
		3	1	0	0	0	0	1
DL	6	1	6	6	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
L	11	1	6	5	0	0	0	1
		2	2	0	0	0	0	2
		3	3	0	0	0	0	3
V	12	1	4	0	0	0	1	3
		2	5	0	0	0	0	5
		3	3	0	0	0	0	3

The followings will be pointed out from this table. The notochord differentiated in nearly all explants taken from the dorsal and dorso-lateral parts of the marginal zone, 45 per cent of the explants from the lateral part and 0 per cent of the explant from the ventral part of the marginal zone. In a majority of cases in which the notochordal differentiation failed to occur, only epithelial tissue and the undifferentiated yolk-laden cells were observed. Moreover, it was a rule that in the explants of Types 2 and 3 the differentiation of the notochord, muscle and neural tissue always failed to occur.

2. *Organization of the Explants*

The various differentiated tissues occurred in the explants showing a definite organization.

DM 3 (dorsal part of the marginal zone : Fig. 7)

The upper spread part was the epithelial tissue, while the lower ridge-like protrusion consisted of the neural tissue, notochord and muscles. The notochord appeared at the median, attached to the substratum. The organization with respect to the various tissues showed a bilateral symmetry.

DM 12 (dorsal part of the marginal zone : Figs. 3, 8 A and 8 B)

From the upper epithelial tissue the balancers were protruded. At the basal end of the ridge-like protrusion two ears were found. Besides the well differentiated neural tissue, the notochord and muscles were arranged symmetrically in disposition in the protrusion. In the tip end of the protrusion the neural structure disappeared, while the mesodermal components were still observable.

DL 5 (dorso-lateral part of the marginal zone : Figs. 4, 8 C and 8 D)

The upper part consisted of the epithelial tissue alone. In the protruded portion, the well differentiated notochord, muscles and neural structures occurred symmetrically. There was no marked difference between this and explants in DM-series.

L 8 (lateral part of the marginal zone : Fig. 9)

This explant had the ridg-like formation till the end of cultivation. The cross section of this formation showed that the neural tube, notochord and a small amount of muscles differentiated in the bilaterally symmetrical organization.

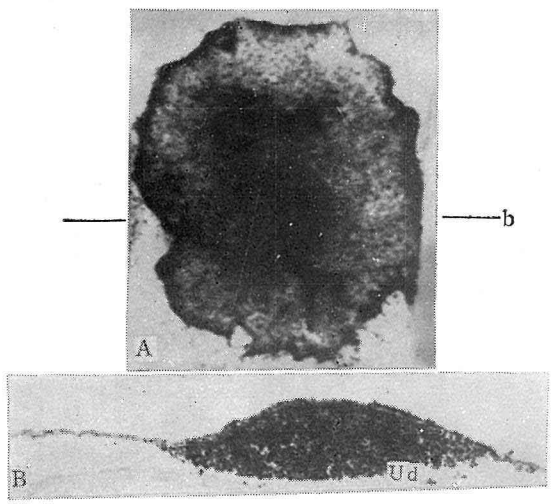


Fig. 5. Configuration and histological organization of the explant taken from the ventral part of the marginal zone (V-2).
A···configuration of the explant on the 16th day of cultivation
B···cross section at the level b in A
Ud : undifferentiated cells.

L 10 (lateral part of the marginal zone)

The ridge-like formation persisted in this explant till the end of cultivation. However, the cross section revealed that there were the undifferentiated cells alone in it.

V 2 (ventral part of the marginal zone : Fig. 5)

This explant spread out eventually into a thin membranous tissue. Mere epithelial tissue and the undifferentiated cells-mass occurred.

V 8 (ventral part of the marginal zone)

The ridge-like formation persisted till the end of cultivation, but there occurred no mesodermal differentiation in this formation.

DMT 16 (dorsal part of the marginal zone, the lower part was divided into two parts: Fig. 10)

Two notochords were formed independently, each in a divided arm. The respective arm formed a bilateral symmetrical organization. In relatively upper level the nose and in the following level the ears were found.

DMS 7 (dorsal part of the marginal zone, the lower part was divided into three parts: Fig. 11)

Three notochords were encountered, each in a divided portion.

Discussion

In all through the explants in which the notochord differentiated, it can be pointed out that the various tissues are arranged in a definite pattern along the longitudinal axis of the explant. Namely, in the most upper part only epithelial tissue appears, and in some cases balancer, too. In the following level, at the basal end of the ridge-like formation, the nose and the ear occur. The neural structures always appear from this level on. In the ridge-like formation which is produced by the congregation and the stretching of the tissue, the neural structure, the notochord and the muscles are always found, and the ear appears sometimes in this portion. In the tip end of the explants, the neural tissue and the notochord sometimes disappear and this portion is occupied by the undifferentiated cells. The distribution of these organs along the longitudinal axis of the explant is schematically shown in Fig. 6.

The definite pattern of the organization is, at the same time, pointed out in the medio-lateral direction of the explant. The notochord always differentiates just at the median of the ridge-like formation. The neural tissue occurs just above and the muscles, in general, on either side of the notochord. Consequently, it can be stated that the explants are organized each as a bilaterally symmetrical body. This bilaterally symmetrical organization is always established not only in the explant taken from

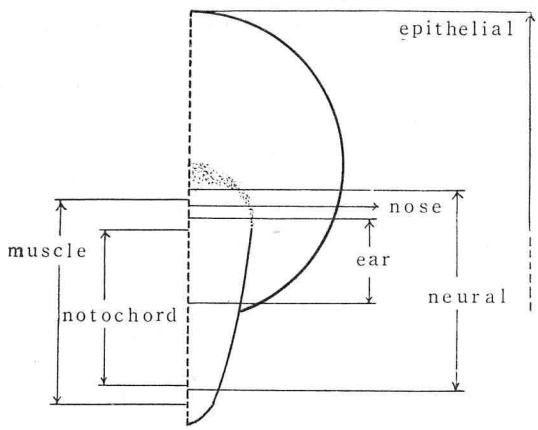


Fig. 6. Schematic representation of the distribution of various organs along the longitudinal axis of the explant.

the dorsal part of the marginal zone, but also in the explants coming from its dorso-lateral part or from its lateral part, in so far the notochordal differentiation takes place.

The bilateral symmetry in the differentiation of the axial mesoderm was previously reported by HOLTFRETER ('39) in the explantation of the lateral blastoporal region, as well as that of the dorso-median region, and also by MAYER ('35) in the transplantation of a half of the dorsal blastoporal lip and MINGANTI ('49) in the transplantation of the presumptive somitic area to the ventral part of the marginal zone. The present results agree well with these. It must be emphasized here, as HOLTFRETER stated, that the bilaterally symmetrical differentiation of the explants manifests itself most clearly under the conditions that they lie closely on the substratum.

General Considerations

In the present experiments it was revealed that in the explants taken from the various sectors of the marginal zone of the early gastrula, the kinetic processes such as spreading, stretching and congregation of the tissues occur in the very early phase of cultivation. These kinetic processes seem to be the manifestation of the spreading, stretching and confluence in the normal course of gastrulation. However, it is doubtful whether the various parts of the explant show the quite similar kinetic process to what they would do when left intact in the embryo. For instance, the congregation in the explant always proceeds toward the median of the piece, whereas the confluence in the intact embryo proceeds toward the dorso-median line of the embryo. Therefore, it should be stated that the kinetic processes in the explants are not always exactly the same as what they would show in the intact embryos, but modified to some extent according to the experimental conditions.

Moreover, a clear parallelism can be pointed out between the kinetic and histogenetic processes of the explants. The stretching and the congregation occurred in the lower part of the explant, and, as a consequence of these kinetic processes, a ridge-like structure was always produced. The histological observation revealed that, when the axial mesoderm occurred, it always differentiated in this ridge-like protrusion into the bilaterally symmetrical organization. In the region in which the stretching and the congregation were not observed, no axial mesoderm was formed at all. Therefore, so far as these cases are concerned, there is a clear parallelism between the kinetic process and the differentiation of the axial mesoderm. However, it is not permitted from this parallelism to consider that any sectors of the marginal zone always differentiate into the axial structure whenever they stretch and congregate. Because, some of explants taken from the ventral part and from the lateral one of the marginal zone could not differentiate into the axial mesoderm, notwithstanding the marked stretching and congregation of the tissue. Thus, although the parallelism is marked, in the cases in which the axial mesoderm differentiates, remains a question whether it indicates the causal relationship between the kinetic and histogenetic process or not. On this point further experiment is now carried on.

Summary

(1) For the purpose to ascertain whether or not the kinetic and histogenetic phenomena proceed independently in the marginal zone, the explants taken from the dorsal, dorso-lateral, lateral and ventral parts of the marginal zone of the early gastrula of *H. nebulosus* were adhered to the collodion membrane and cultured in HOLTFRETER's solution.

(2) The upper part of the explant always spread out, while the lower part stretched and congregated in the early phase of cultivation, forming a ridge-like protrusion. The protrusion persisted in some of the explants till the end of cultivation, but in the others it spread out into a membranous tissue in the later phase of cultivation.

(3) The axial mesoderm differentiated in nearly all of the explants taken from the dorsal and dorso-lateral parts of the marginal zone and in some of the explants from the lateral part. The axial mesoderm occurred always in the ridge-like protrusion, showing a bilateral symmetry.

(4) A parallelism between the kinetic and histogenetic processes was pointed out and discussed.

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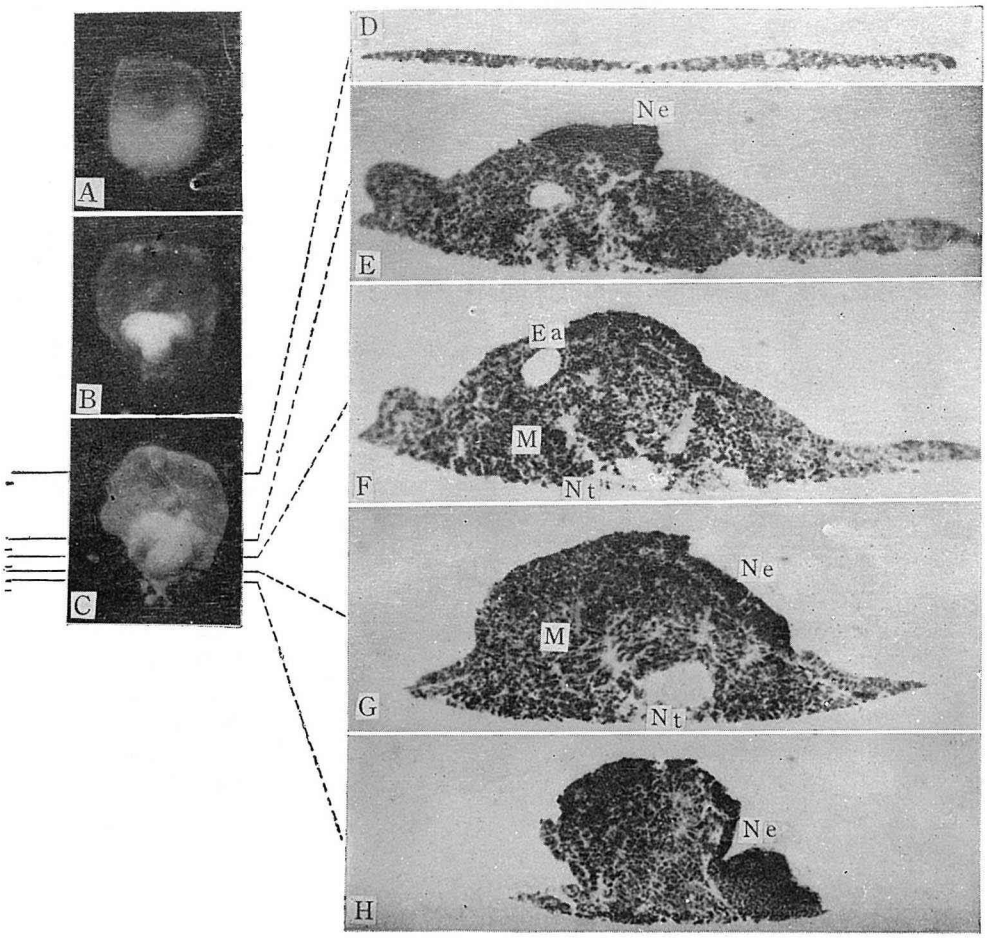

Fig. 7. Configuration and the histological organization of the explant taken from the dorsal part of the marginal zone (DM-3).

A···immediately after explantation

B···24 hours later

C···on the 14th day of cultivation

D, E, F, G and H show the histological organization of the explant at the levels indicated in C.

Ne: neural tissue, Ea: ear, Nt: notochord, M: muscle.

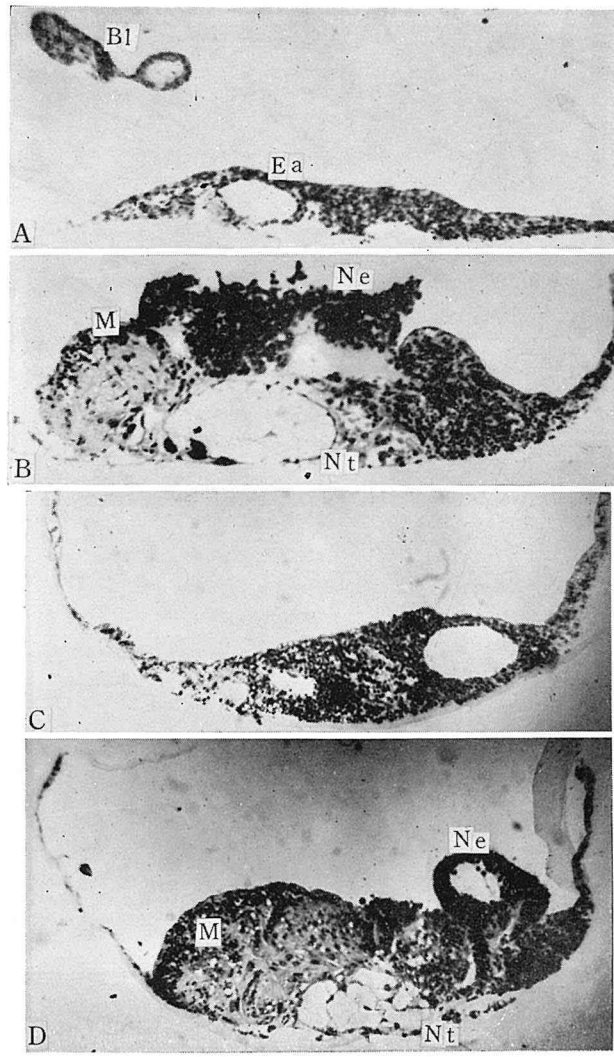

Fig. 8. Cross section of the explant taken from the dorsal part of the marginal zone (DM-12).

A···at the basal end of the ridge-like protrusion
B···at the middle of the protrusion

Cross section of the explant taken from the dorso-lateral part of the marginal zone (DL-5).

C···at the basal end of the ridge-like protrusion
D···at the middle of the protrusion

Ne: neural tissue, Ea: ear, Bl: balancer, Nt: notochord, M: muscle.

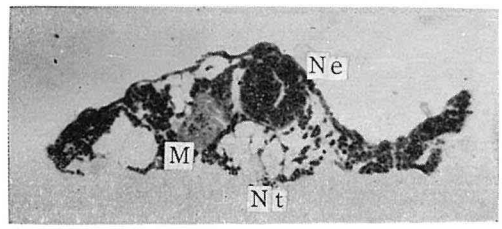

Fig. 9. Cross section of the explant taken from the lateral part of the marginal zone.
Ne: neural tube, Nt: notochord, M: muscle.

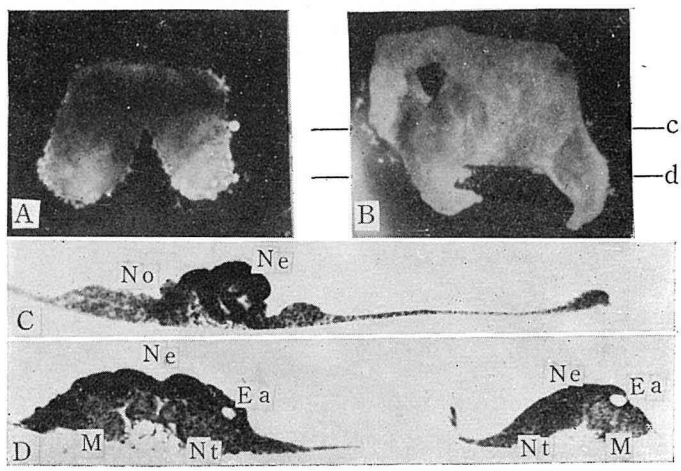

Fig. 10. Configuration and the histological organization of the explant taken from the dorsal part of the marginal zone and divided in its lower part into two (DMT-16).
A···immediately after explantation
B···on the 14th day
C···cross section at the level c in B
D···cross section at the level d in B
Ne: neural tissue, Ea: ear, No: nose, Nt: notochord, M: muscle.

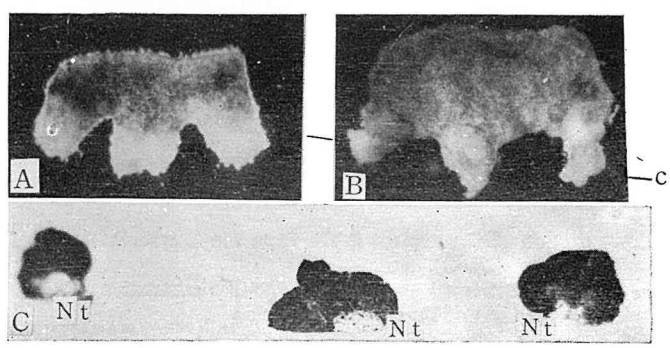

Fig. 11. Configuration and the organization of the explant taken from the dorsal part of the marginal zone and the lower part of which is divided into three (DMS-7).
A···immediately after explantation
B···on the 14th day of cultivation
C···cross section at the level c in B
Nt: notochord.