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Development of the Organized Embryo from the Temporarily Disaggregated Gastrula in Amphibia

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It has been demonstrated by various investigators that the early gastrula of Amphibia has a marked capacity of regulation to differentiate into a normally organized embryo against the various experimental interferences. In the present experiments, it is questioned whether or not the early gastrula, which is temporarily disaggregated into free cells, can differentiate into a normal embryo with respect at least to the axial organs.

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Material and Method

It was introduced by HOLTFRETER (1943) that an amphibian embryo could be disaggregated into free cells without serious, injurious effect by the alkali treatment. The adequate value of pH for this treatment was shown to be 9.6 or 9.8 (TOWNES and HOLTFRETER, 1955). In the present experiments, for the purpose of preventing the loss of free cells, it was attempted to disaggregate a gastrula within the vitelline membrane. In this condition, the early gastrula of *Triturus pyrrhogaster* could not be disaggregated completely into free cells in the solution of pH 9.6 for more than one hour. To accomplish more complete and rapid disaggregation, it was treated with the solution of higher alkalinity.

The early gastrulae were sterilized in 60% alcoholic solution for 30 seconds. The jelly coat was removed and the embryos with the vitelline membrane were then put into the alkali-solution. The solution was prepared by dissolving 0.1 g of NaOH in 100 ml of the HOLTFRETER solution. Immediately after immersion, the prospective ectodermal area began to disintegrate from the animal pole, and the blastocoel diminished gradually and disappeared as the disintegration proceeded. The free cells piled up in the vitelline membrane.

Within 10 to 20 minutes, the piled-up cells tumbled down by a gentle rolling of the mass by means of a needle. The rolling was repeated till the disaggregation of the gastrula into free cells seemed to be complete. This rolling of the mass was simultaneously effective in intermingling the various types of cells. Time required for this treatment varied case by case from ten to thirty minutes. Then, the mass of cells in the vitelline membrane was rinsed in the HOLTFRETER solution with pH 7.2. The vitelline membrane was carefully taken away after 24 hours, when the free cells had perfectly been reaggregated into a compact body in a majority of the cases. However, it was frequent occurrence that cloudy accumulation of the fine granules was marked under the top of the vitelline membrane. These embryos were discarded, because this material seemed to be the debris of broken cells. After the removal of the vitelline membrene, the naked bodies were cultured each in a separate dish filled with the HOLTFRETER solution of full strength. The bed of the dish was equipped with agar-agar plate. During the cultivation it occurred in some specimens that the cells became free again. They were also omitted from the further cultivation. After about two-weeks' cultivation they were fixed with BOUIN's fixative, embedded in paraffin and sectioned at 10μ thick. Sections were stained with MAYER's haemalum and eosin.

Experimental Results

1) *Change of the configuration of the reaggregated masses.*

At the end of alkali treatment, the surface of the disaggregated gastrula was a mosaic of various types of cells. However, within 24 hours' culturing in the optimal pH condition, it reaggregated into a compact body with two regions. As is shown in Fig. 1, A, the brownish, pigmented region occurred in the upper part of the body, and the remaining surface, which was larger than two-thirds of the whole, was occupied with large and white cells. Judging from the size and colour of the cells, the former region was probably composed of prospective ectodermal cells and the latter area consisted of prospective endodermal cells. The distribution of the prospective mesodermal cells could not be determined exactly from outside. The upper margin of the white area spread a little over the surface of the pigmented region. This fact seemed to agree well with HOLTFRETER's finding that the epibolic movement of the prospective ectoderm was inhibited by subjecting the early gastrula to alkaline media for a brief period (1948). It was also frequently observed that a small furrow occurred in the upper pigmented region just above the border line. However, it was impossible to detect if the inward migration of the surface material occurred through this furrow.

Even in further cultivation, no neural plate was ever observed in all of the 9 specimens. However, a marked change of form, as a whole, was observed in 5 specimens; i.e., one could not remain spherical but became triangular (Fig. 1, B), while the other four established a tail-like protrusion (Fig. 1, C). The remaining four out of nine cases indicated no conspicuous change of shape; i.e., they were spherical till the end of cultivation (Fig. 1, D).

On the other hand, it was common that the brownish pigmented region spread out gradually to cover the upper half of the embryo till the end of cultivation, although the remaining lower half was still occupied with endodermal cells. The axial elongation, as in the normal embryo, was hardly observed, except the protrusion of the tail-like structure in the aforesaid four specimens.

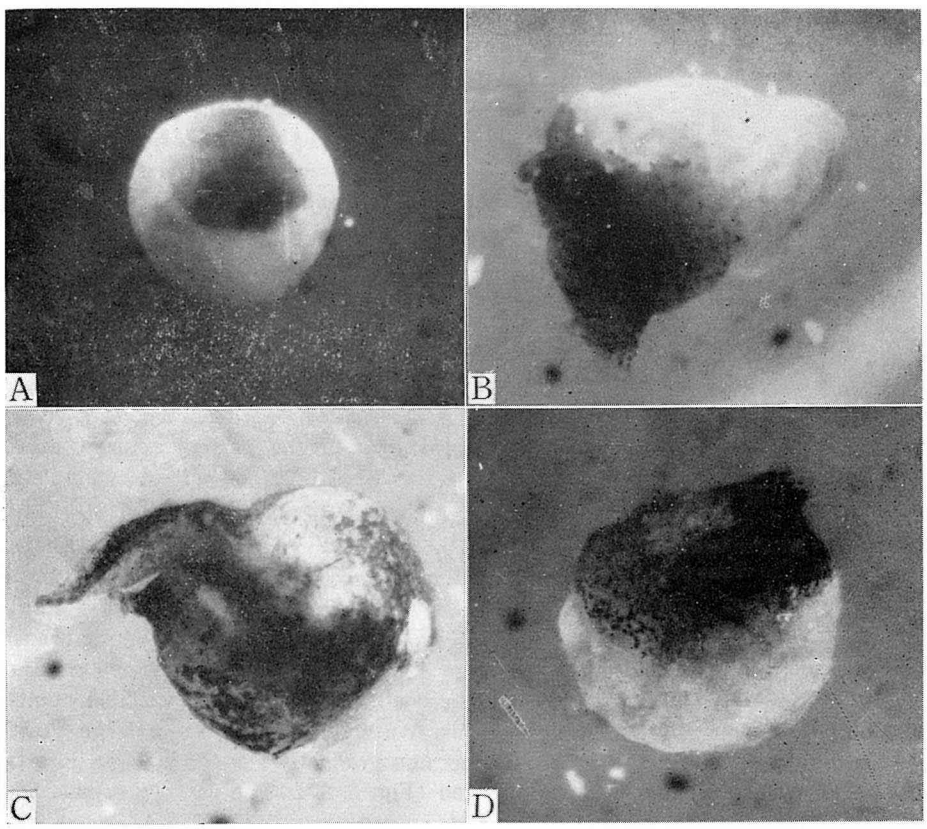

Fig. 1. Configuration of the reaggregated mass of cells of a gastrula at 24 hours after the end of the alkali-treatment (A), and in the 14th day of cultivation (B, C, D).
A···Completely reaggregated mass in which two different regions, pigmented ectodermal and white endodermal one, could be distinguishable.
B···Triangular shaped embryo.
C···Embryo with a tail-like protrusion.
D···Spherical embryo.

2) *Histological observation.*

The microscopical observation has revealed that the marked necrotic or pycnotic figures were hardly observed. On the contrary, well differentiated structures such

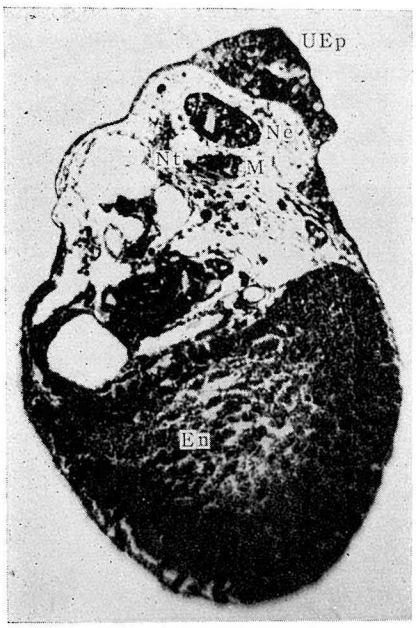

Fig. 2. Cross section of an embryo, showing that various tissues are arranged from top to bottom in a similar pattern to the dorso-ventral organization of the normal embryo.
UEp···mass of undifferentiated ectoderm,
Ne···neural tube, Nt···notochord,
M···muscles, En···endoderm.

as epidermis, notochord, muscles, pronephric tubules as well as neural and mesenchymatous tissues always occurred in every case. The melanophores were also of general occurrence. The endodermal cells, however, remained an undifferentiated mass of cells, except for a peripheral membraneous epithelium which differentiated in some cases. The location of the endodermal mass was quite the same as in the exogastrulae. It should be noticed that the above mentioned organs and tissues did not occur at random, but appeared rather in a normal arrangement. As is shown in Fig. 2, the upper part of the embryo was composed of ectodermal structures and the lower part, endodermal cells. The mesodermal organs occupied the part between the two. The pattern of these structures derived from three germ layers showed little deviation from the dorso-ventral organization of the normal embryo, except that the whole surface was not completely enveloped by epidermis.

Ectodermal derivatives — The neural tissue occurred as a tubular structure, but it did not so much stretch as in the normal embryo. It frequently exhibited the branching. Moreover, it had not always a single neurocoel, but frequently a variable number of it. The one end of the tube became massive or expanded into a large vesicle with a thin wall, while the other end became gradually slender and got into the tail-like protrusion whenever it was formed (Fig. 3, A). The sensory organs such as nose or eye were never observed. Ear-like structures were found, though very rarely, adjacent to the massive or vesicular part of the neural tube.

The epidermal tissues did not cover the whole surface of the embryo. An undifferentiated mass of ectodermal cells was always formed at the dorsal side of the body (Fig. 2). In one exceptional case, a neural tube was formed in the epidermal mass without any adjoining mesodermal components.

Mesodermal derivatives—Notochord was formed without fail in every specimen. Its configuration was neither a straight rod nor an amorphous mass. It occurred frequently as a single but a tortuous or gnarled cord at the middle region of the embryo and occupied an intermediate position between the upper ectodermal and the lower endodermal tissues (Fig. 3, B). In extreme 3 cases, two or six fragments

of notochord appeared in one embryo. Even in these cases they occurred not separately but together.

Generally speaking, the muscles were very poor in quantity and the segmentation was scarcely observed. On the other hand, typically segmented somites occurred in the tail-like protrusion. A large quantity of mesenchymatous cells appeared in lieu of muscles. Sometimes they scattered loosely, and sometimes they lined up in a layer of one cell thick lining a number of cavities.

Well differentiated pronephric tubules were always found, while the blood cells appeared only in one case.

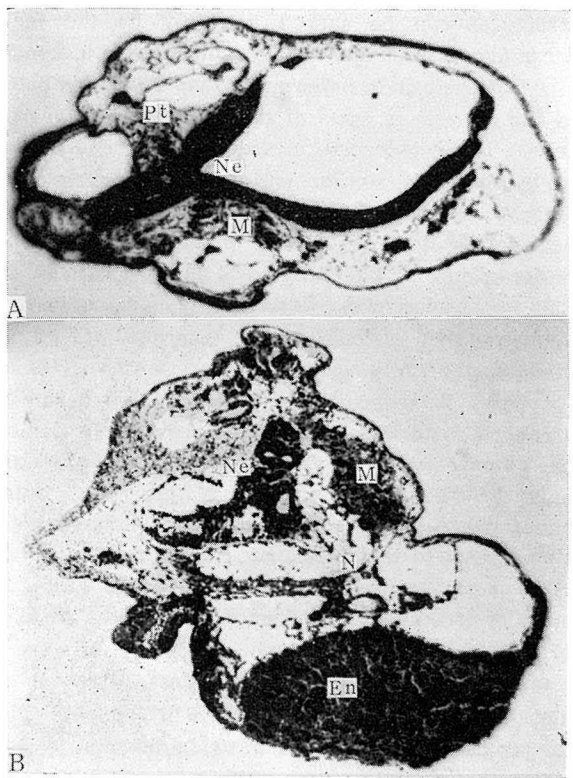

Fig. 3. Sections of embryos showing the axial structure of a neural tissue (A), and a notochord (B).
A···Vesicular (right) and tubular (left) structure of a neural tube.
B···A tortuous notochord appears at the intermediate portion between the ectodermal and endodermal tissues.
Ne···neural tissue, N···notochord, M···muscles, Pt···pronephric tubules, En···endoderm.

Considerations

It was shown that the temporarily disaggregated and then reaggregated gastrula could produce a well differentiated embryo, although various kinds of defects or malformations were pointed out. These anomalous structures seemed to be produced owing mainly to the collapse of the original cell arrangement. However, under the present experimental conditions, it was not neglected that the other kind of agent simultaneously took part in producing them. As was indicated above, the spreading tendency of the prospective ectodermal area was markedly inhibited after the treatment of alkali, moreover the differentiation of a neural tube without adjoining mesoderms was observed. Consequently, it cannot be denied that the alkalinity used in the present treatment was so high that it exerted noxious and presumably sublethal effects upon the prospective ectodermal cells in addition to the disaggregating effect (HOLTFRETER, 1945; YAMADA, 1950). Therefore, it seems probable to state that the formation of the anomalous

structures can be caused not only by the mere collapse of the original cell arrangement, but also by the subnormal differentiation of individual cells.

However, it is noteworthy that a definite pattern of organization is established in the embryo in spite of the temporary disaggregation of normal arrangement of cells. The ectodermal, mesodermal and endodermal tissues are arranged from top to bottom in a similar pattern to the dorso-ventral organization of the normal embryo. The configuration of the terminal, massive or vesicular portion of the neural tube does not provide distinct criteria to compare them with normal division of the brain. But, judging from the formation of the ear-like attendants, it is likely that this structure is a deuterencephalon. If so, it may be stated that the temporarily disaggregated gastrula can differentiate into an embryo, in which the basic pattern of dorso-ventrality and antero-posteriority is established distinctly.

Here, it arises a question how this organization is established from the disarranged material. Although it seems true that the regulation capacity which has been demonstrated by various investigators plays an important role, the other factors must be taken into account in this case. Unfortunately, in so far the present experiments are concerned, the data obtained are too poor to give any satisfactory elucidations to the question. However, it is worthy of discussing a point for the sake of further investigation. It is that within 24 hours after disaggregation, the mass of free cells could recover a definite pattern of organization in the optimal pH condition, namely the upper part of the mass was occupied with the prospective ectodermal cells and the lower part, with the endodermal cells, not showing a mosaic pattern of cells arranged at random. This fact seems to indicate that the temporarily disaggregated gastrula restores to some extent its original organization along the animal-vegetal axis, by means of segregation and sorting of different types of cells. The disaggregation of a body and the subsequent restoration of the original organization have already been studied in sponges (GALTSOFF, 1925, 1926, 1929; WILSON, 1932), and recently in neurulae of Amphibia (TOWNES and HOLTFRETER, 1955). Through these studies it becomes clear that the segregation is performed owing to the tissue specific, kinetic tendencies such as directed cell movements and selective cell adhesions. Since the segregation actually occurs in the present experiments, it should be anticipated that the cells of a gastrula have specificities in kinetic properties.

It has been demonstrated in various explantation and transplantation experiments that the developmental fate of various parts of a gastrula is not yet strictly determined. Therefore, it may not be expected that in gastrulae the difference of the kinetic specificities of cells appears in accordance with the difference of their prospective significance. The detailed analysis on the regional differences of the kinetic specificities should be investigated further.

Summary

1) The early gastrulae of *Triturus pyrrhogaster* were disaggregated into

free cells within each vitelline membrane by subjecting them to alkaline medium for 10 to 30 minutes.

2) When returned to the neutral medium, the disaggregated mass of cells reaggregated into a compact body. Distinct segregation of the ectodermal cells from the endodermal ones occurred in this body.

3) In the course of two-weeks' cultivation, the body developed into a well differentiated embryo, in which a basic pattern of dorso-ventral and antero-posterior organization was established, notwithstanding the formation of various anomalous structures.

4) From these results it is anticipated that the specificities in kinetic properties exist even in the gastrula cells.

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