Culture of Dissociated Drosophila Embryos: Aggregated Cells Differentiate and Sort Out

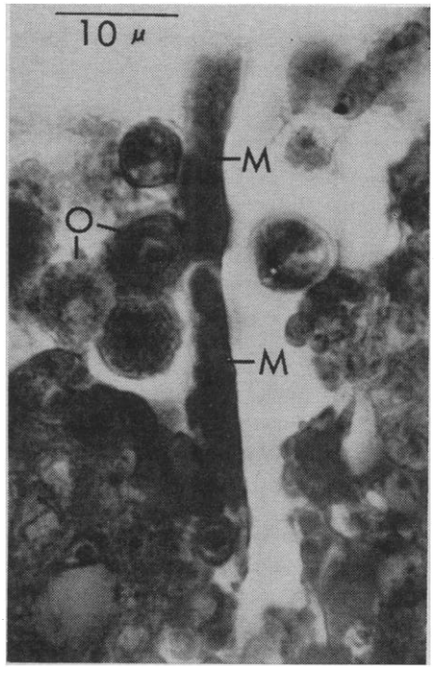
Abstract. *When the cells of* Drosophila *embryos are dissociated and cultured in vitro, aggregation of the cells begins rapidly and movements of the cells continue for at least 5 days. Oenocytes, hypoderm, nerve, muscle, and trachea cells take up characteristic positions in the aggregates. Cells from* X2 *mutant embryos sort out in identically the same way as do cells from normal embryos.*

An experimental technique frequently used in the study of morphogenetic movements and the mechanisms which control them is that of dissociating the tissues into a suspension of single cells and then allowing the cells to aggregate into a compact mass (*1*). Such experiments show that cells have selective affinities, since in aggregates of two or more cell types the different cells segregate from each other and reform tissues in characteristic positions within the aggregate. Changing cell affinities during development are thought to control the morphogenetic movements (*2*).

I have extended this technique to a study of the genetic control of cell affinities and morphogenetic movements in *Drosophila* embryos. This report is based on the study of 15 aggregates of cells from normal embryos and five from *X2* mutant embryos. The *X2* mutant was originally isolated by Auerbach (*3*) by x-irradiation of a *Drosophila melanogaster* stock; the *X2* gene is sex-linked and lethal. Male embryos hemizygous for the *X2* gene develop abnormally, never succeed in hatching, and die about 2 hours after the normal embryos have hatched. (Embryonic development takes 22 hours at 25°C.) Most of the normal cell types are differentiated in the mutant embryo, but all parts of the embryo are grossly disorganized because of abnormal morphogenetic movements.

The embryos were derived as follows. *X2*/FM4 females were mated to Oregon (Ore) R males (*4*); the *X2*/Ore R virgin females collected from this cross were mated to young Ore R males, and fertile eggs resulting from these matings were used in the experiments. Eggs were collected during a 1-hour period by the usual technique (*5*). After the embryos had developed for the desired length of time at 25°C the chorionic membranes were removed by immersing the embryos in 2.5 percent NaClO and rinsing them six times in saline. The mutants were then sorted out from the wild-type embryos, and the two groups of embryos were handled separately. After the vitelline membrane was slit with tungsten needles, the embryos were removed and transferred by pipet to the bottom of a small glass tube.

Dissociation of the cells was achieved by incubating approximately 15 embryos for 15 minutes in a Ca- and Mg-free saline containing ethylenediaminetetraacetic acid (0.12 percent)



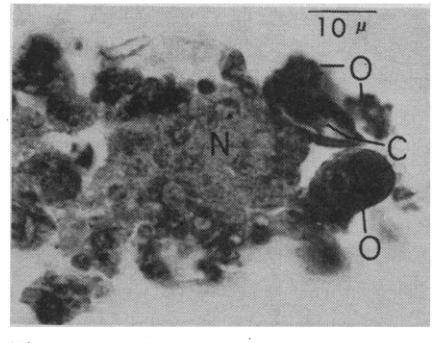


Fig. 1 (left). Section through an aggregate formed from 15 normal embryos, 7 hours old at the time of dissociation. The culture was maintained for 5 days before fixation. The long muscle cells (*M*) are surrounded by other cell types except where one muscle cell touches the surface of the aggregate. Loosely packed oenocytes (*O*) are external to the more tightly adhering inner cells (× 1300). Fig. 2 (above). Section through a different level of the same aggregate shown in Fig. 1. Oenocytes (*O*) are external, nervous tissue (*N*) internal. Between these two cell types is some blue-staining material (*C*), probably chitin secreted by hypoderm cells (× 900).

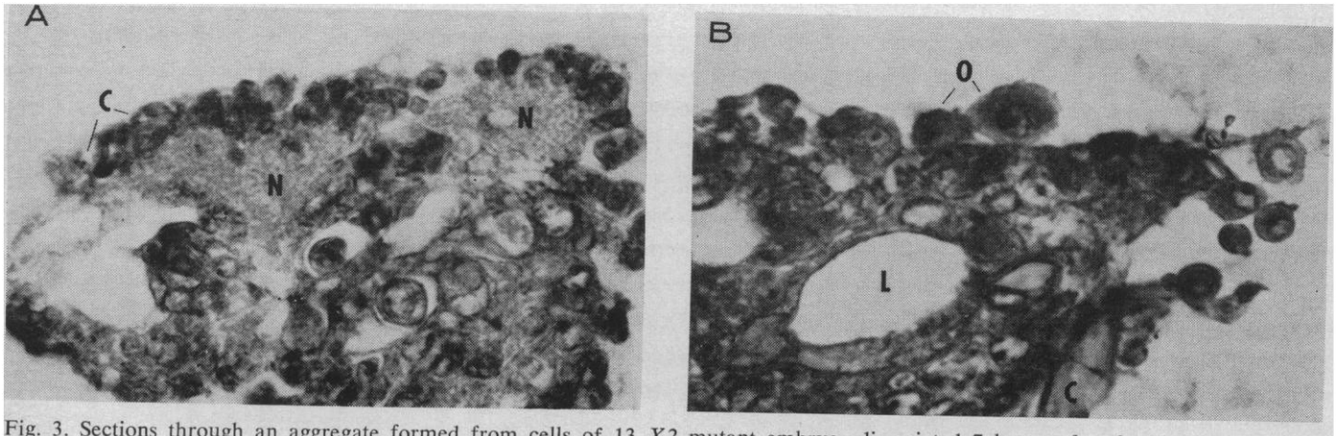


Fig. 3. Sections through an aggregate formed from cells of 13 *X2* mutant embryos dissociated 7 hours after fertilization and fixed after 7 days of culture. *A*, Nervous tissue (*N*) shows the typical arrangement of cell bodies external to nerve fibers. Blue-staining chitin (*C*) is outermost (× 1000). *B*, The lumen (*L*) may be a tracheal formation. Oenocytes (*O*) and a thick layer of chitin (*C*) form the outermost layer (× 1000).

and crude trypsin (0.15 percent) (*6*) and then rapidly vibrating the embryos in the tube for 15 seconds (*7*). The cells were washed three times in culture medium and then placed in a drop of culture medium (approximately 0.005 ml) on a siliconed cover slip, which was inverted over a vaseline-ringed depression. The culture medium contained TC Yeastolate 200 mg/100 ml, but in other respects was essentially the same as that developed by Schneider for culture of imaginal discs (*8*). Fetal bovine serum was added routinely to the culture medium, at a final concentration of 10 percent. The cultures were maintained at 25°C for 5 to 7 days without changing the medium, during which time the cells formed aggregates which were fixed in Bouin's fluid or in a mixture of formalin, acetic acid, and alcohol. Successful cultures were established from embryos that were from 6½ to 16 hours old at the time of dissociation.

The dissociation method yielded a uniform suspension of single cells only when young embryos, up to about 11 hours old, were used. Sometime between the 11th and 14th hours of development, changes occurred in the embryo which rendered it progressively more difficult to release single cells. When embryos 17 hours old were dissociated, a few single cells were released, but most of the cells remained in fairly large, undissociated groups.

In suspensions of cells from both normal and mutant embryos aggregation begins very rapidly. By about 2 hours after the hanging drop was set up the cells were already noticeably more tightly grouped. The cells continued to move about and pile up to form a sphere or thick disc, sometimes with irregularly shaped projections. The process continued throughout the culture period, as evidenced by the constantly changing size and shape of the aggregate.

Soon after the aggregate formed it began to show pulsations resulting from contractions of muscle cells. This continued throughout the culture period, whether normal or mutant embryos were used. Some of the contractions were very strong, moving the whole aggregate; other contractions were weaker but more rapid, beating about 80 to 120 times per minute in both normal and mutant aggregates. Such contractions were first observed about 10 to 20 hours after the culture was set up; the older the embryos were at the time of dissociation, the sooner the contractions were observed in the culture.

Figures 1 to 3 show Azan-stained (*9*) sections through aggregates of normal and mutant embryos. The sections of aggregates were compared with sections of normal larvae fixed and stained in the same way so that as many cell types as possible could be identified in the aggregates. Oenocytes, muscle cells, and nerve cells, in addition to chitin, were easily distinguished in the sectioned aggregates, but several other cell types remain unidentified.

Within the aggregates the various cell types separate from one another, at least partially, and take up characteristic positions. As shown in Figs. 1 to 3, the very loosely adhering oenocytes were outermost. Internal to these were hypoderm cells, which in turn covered nervous tissue. Tracheal cells (*10*) and large muscle cells were innermost, though the latter often reached to the surface of the aggregate.

No differences were observed between the positions of the different cell types within aggregates of cells from *X2* mutants and the positions within aggregates from normal embryos. Aggregates from mutant embryos remained alive in tissue culture 6 days longer than intact mutant embryos live within the vitelline membrane and chorion.

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References and Notes

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10. The identification of the tracheal cells is not definitive.
11. I thank Thomas Green and Roy Hemelt for valuable technical assistance during different stages of this work, which was supported by grants GB-307 and GB-2363 from the NSF. Part of the work was done in the biology laboratory of Loyola University, New Orleans, and part was done at Johns Hopkins University.

25 February 1965

Neoplasia in a Coral?

Abstract. *Anomalous growths of the colonial, deep-water coral* Madrepora kauaiensis *are interpreted as neoplasia. Since tissue was not preserved, evidence is derived solely from skeletal examination and consists of unusually rapid growth and progressively disordered growth of skeletal structures.*

Malformed corals are common, but in the great majority of such specimens that have been examined the malformations are directly attributable to the effects of predation or other physical injury. Pathologic morphologies other than those resulting from regeneration of injured tissues have generally been referred to under the term "abnormal" and not considered further.

The anomalous growths described here were found on the only known specimen of *Madrepora kauaiensis*. This oculinid coral was dredged in 1902 at "Albatross" Station 4136, off the Island of Kauai, Hawaiian Islands, from a depth of 529 to 635 m. Species of *Madrepora* are common deep- and cool-water colonial corals in the oceans of the world, usually occurring in depths below those in which the more widely known reef corals live. *Madrepora kauaiensis* was described by Vaughan (*1*), who noted the presence of at least one unusual corallite of the colony, interpreted it as an individual of another genus and superfamily of coral which had grown upon the *M. kauaiensis*, and suggested that this unusual portion was "*Mussa*? sp. young?": "a simple mussid coral was growing attached to *Madrepora kauaiensis* . . ." (*2*).

I believe Vaughan's interpretation of the specimen to be in error and suggest that the presumed mussid and two other corallites of the same degree of abnormality are the result of diseased growth of individuals of the colony. Because the specimen is unique and is a holotype, dissection of the skeleton is restricted.

Study of the soft tissues of the animal is not possible, for the specimen was dried after collection and the polyp tissue was destroyed. Information concerning the abnormal corallites is available from study of the skeleton, which tells much about the growth of the polyp, coral growth being accretionary, and developmental sequences are preserved in the structure of the skeleton.

The three individuals of *Madrepora*