EFFECT OF THE POTASSIUM ION ON INDUCTION OF NOTOCHORD FROM GASTRULA ECTODERM OF RANA PIPIENS


LUCENA J. BARTH AND Lester G. BARTH
Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Small aggregates of cells prepared from explants of ventral ectoderm of Rana pipiens gastrula can be induced to differentiate into a variety of cell types by altering the ionic composition of the medium in which they are treated or cultured (Barth and Barth, 1959, 1969). For example, nerve of two distinct types and pigment cells appear in experimental cultures, while epithelial sheets with ciliated patches or free-swimming ciliated masses differentiate from untreated control cell aggregates. These several types of differentiation are the end result of a sequence of steps beginning with an initial treatment period of 2-3 hrs, followed by a Na-dependent reversible period of 4-6 hrs and a final period of 5 or more days before visible differentiation (Barth, 1966; Barth and Barth, 1969).

The cations used in these studies (Na⁺, K⁺, Ca²⁺, and Mg²⁺) are supplied at concentrations well below the level where osmotic shock and cell injury occur (Holtfreter and Hamburger, 1955, page 273). The concentrations used can be applied continuously and the cells will live and differentiate. Thus we are working in vitro within a range of cation concentrations that could occur at local regions within the whole gastrula. The working hypothesis has been that during the course of normal gastrulation and later differentiation, release of cations from bound or sequestered states, followed by binding at new sites within the embryo, leads to conditions similar to those provided in cell cultures and found effective in switching cells to a new type of differentiation (Barth and Barth, 1974).

In addition to the naturally occurring cations, we have used the lithium ion as a tool for bringing about the initial step in induction. In the experiments reported below gastrula ectoderm cells were treated for 2-3 hrs in standard solution to which 71 mM Li⁺ was added. Following this treatment the cells regularly differentiate into nerve and pigment cells when cultured in standard solution containing 1.3 mM K⁺. In the course of experiments elsewhere reported (Barth and Barth, 1974) it was discovered that increasing the concentration of K⁺ in the culture medium causes lithium-induced ectoderm cells to differentiate into notochord. The purpose of

---

1 This work was supported by a grant from the National Science Foundation (GB 23026) to the Marine Biological Laboratory, Woods Hole, Massachusetts 02543.
the present paper is to describe and discuss these experiments and their implications in relation to ionic regulation of cellular differentiation.

**Material and Methods**

Eggs of *Rana pipiens* obtained by pituitary injection were used at mid-gastrula stage (S11, Shumway, 1940). The basic methods of obtaining the cells used for experiments and the composition of standard solution have been published earlier (Barth and Barth, 1959 and 1969) and have been summarized more recently (Barth and Barth, 1974).

**The cells**

Small explants consisting of approximately 125 cells were prepared from larger explants of prospective epidermis of the gastrula (ventral ectoderm). The outer pigment coat layer of the large explant was loosened by means of brief treatment with EDTA, peeled off and discarded. The inner layer of ectoderm cells was teased into small explants by means of hair loops. The aggregates of cells thus obtained are small enough to permit ready access to treating solutions without having been subjected to complete dissociation and possible damage to cell surfaces that isolated embryonic cells at this early stage might suffer from the EDTA solution.

Six large explants yield approximately 150 small explants containing about 125 cells per explant. These cell aggregates were treated with test solutions in 15 ml tenter dishes and are cultured on coverglasses in 5 ml tenter dishes. More than 5,000 small explants, or cell aggregates, were used in the experiments reported presently.

Permanent preparations of cells adhering to coverglasses are made by washing out the culture medium with standard solution lacking serum and replacing with Bouin's fluid for a minimum of 120 min. Bouin's solution is replaced by successive tap water rinses and the preparations are stained for 10 min in Ehrlich's hematoxylin (50% in distilled water). The coverglasses with adhering cells are passed through increasing concentrations of EtOH into 2.5% Eosin Y in 100% EtOH for 1–2 min. Dehydration in 100% EtOH and clearing in xylene require 2–3 min in two changes of each solution. The coverglass preparations are inverted and mounted on glass slides for observation and photographing.

**Standard solution**

The medium used for operation and culture is a modification of both Holtfreter's and Niu-Twitty's solutions (Barth and Barth, 1959, 1969). This standard solution is varied in the present experiments to contain increasing amounts of $K^+$. The final solution is prepared from three component solutions made with doubly glass distilled water. *Component A*: NaCl (Biological Grade), 5.150 gr; KCl, 0.075 gr; MgSO$_4$$\cdot$7 H$_2$O, 0.204 gr; Ca(NO$_3$)$_2$$\cdot$4 H$_2$O, 0.080 gr; CaCl$_2$$\cdot$2 H$_2$O 0.060 gr; H$_2$O to 500 ml. *Component B*: NaHCO$_3$, 0.200 gr; H$_2$O to 250 ml; *Component C*: Na$_2$HPO$_4$, 0.0300 gr; KH$_2$PO$_4$, 0.375 gr; H$_2$O to 250 ml. The final solutions are prepared by mixing aliquots of 50 ml of A, 25 ml of B, and 25 ml of C. 100 mg of calf serum (Nutritional Biochemical Corporation) were added to B before mixing. When $K^+$ was varied, calculated amounts of a standard solution of KCl
were added to C before autoclaving and mixing the components. The pH of standard solution is 7.9–8.1. The ionic composition of standard solution expressed in terms of mM is: 90.8 mM Na⁺ (2.8 mM from buffers); 1.3 mM K⁺; 0.83 mM Mg²⁺; 0.74 mM Ca²⁺.

**EDTA solution**

As used in our system EDTA loosens the outer from the inner layers of prospective epidermis cells, but does not dissociate the gastrula into individual cells. The concentration of EDTA is 10 mg/100 ml of calcium- and magnesium-free buffered salt solution (Barth and Barth, 1959, 1969).

Sterile solutions, glassware and operating instruments are used throughout, and operations are made within a sterile operating cabinet.

**Table I**

<table>
<thead>
<tr>
<th>Concentration of K⁺ mm added to standard solution</th>
<th>Types of cellular differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3</td>
<td>Radial and ganglionic nerve + pigment cells (1–2)</td>
</tr>
<tr>
<td>2.3</td>
<td>Radial and ganglionic nerve; pigment cells (1)</td>
</tr>
<tr>
<td>3.3</td>
<td>Pigment cells (1–2); networks (2); notochord + radial and ganglionic nerve (2)</td>
</tr>
<tr>
<td>4.3</td>
<td>Networks (2); notochord (2); nerve + pigment cells (1)</td>
</tr>
<tr>
<td>5.3</td>
<td>Networks + notochord (3); radial nerve (1); pigment cells (1)</td>
</tr>
<tr>
<td>7.3</td>
<td>Radial and ganglionic nerve; pigment cells (1)</td>
</tr>
<tr>
<td>9.3</td>
<td>Radial and ganglionic nerve (3); pigment cells (1) to rare</td>
</tr>
</tbody>
</table>

**Results**

**Effect of external K⁺ concentration on induction of notochord**

Three of the types of cellular differentiation listed in Table I have been photographed and described earlier (Barth and Barth, 1959, 1962, 1963). Nerve appears in cultures in two principal forms. Radial nerve consists of a central mass of neuroblasts from which long nerve fibers radiate outward. Ganglionic type nerve results when neuroblasts migrate outward and accumulate in depots connected by a complex network of nerve fibers. Pigment cells develop from aggregates that first spread out peripherally in a flat, thin sheet and then separate to become single cells. A granular pigmented ring appears next to the nucleus and by about five days of culture the cells become dendritic. Typical pigment cells develop melanin and may live for several weeks in our non-nutrient culture medium. Radial nerve, ganglionic nerve and pigment cells are induced in that sequence, as intensity of the treatment used to bring about the shifts of differentiation is increased (concentration of ionic inductor or length of treatment period), as reported in Barth and Barth (1964).

The new cell type introduced in Table I is notochord, which was recognized when the extensive networks of cells mentioned in Table I were observed to evolve
into masses of highly vacuolated cells. The gradual transformation of networks of pre-notochord cells into recognizable masses of notochord cells will be described, together with photographs, in section 2 of the present "Results."

For the experiments summarized in Table I, aggregates of cells prepared from explants of ventral ectoderm were treated with 71 mM Li⁺ in standard solution for 2.5–3 hrs. Following this treatment the aggregates were cultured in standard solution in which K⁺ was varied from 1.3 mM–9.3 mM. Cultures were observed through 10–20 days; 5.3 mM K⁺ is shown to be optimal for notochord differentiation. The table is a summary of results obtained from approximately 5,000 cell aggregates, each containing about 125 cells. Numerals in parentheses indicate intensity of induction of a given cell type as (1) sparse, (2) extensive, or (3) very extensive.

Table I shows that at 1.3 mM K⁺, the concentration present in standard solution, and at 2.3 mM K⁺, ectoderm cells from the gastrula, induced by lithium treatment of 2.5–3 hrs, differentiated as observed in the past into nerve and pigment cells. When, however, the lithium-induced cells were cultured in standard solution in which the K⁺ concentration had been raised to 3.3–5.3 mM, networks of vacuolated cells were formed, which fused to form notochord-like masses. Optimal concentration of K⁺ for producing this shift in differentiation is 5.3 mM. At 7.3 and 9.3 mM K⁺, the cells differentiated into nerve and pigment cells, just as in 1.3 and 2.3 mM K⁺. There is a gradual shift from pigment cells with few notochord cells at 3.3 mM K⁺ to approximately 98% notochord with sparse pigment cells at 5.3 mM K⁺.

From the experiments summarized in Table I it could not be determined whether K⁺ exerted its transforming effect immediately after the initial lithium induction period, which we know to be Na⁺-dependent and reversible (Barth, 1966), or whether the potassium ion acted during the days-long period preceding visible differentiation of induced cells. This question was resolved by the following type of experiment. Gastrula cell aggregates prepared as usual from explants of the ventral ectoderm were treated for 3 hrs with 71 mM Li⁺ added to standard solution. All of the aggregates were transferred for post-treatment to standard solution in which K⁺ was raised to 5.3 mM. After 4 hrs half the aggregates were transferred to culture dishes of standard solution containing 1.3 mM K⁺. After 18 hrs of post-treatment in high K⁺ the remaining aggregates were cultured in standard solution containing 1.3 mM K⁺. The former group formed radial and ganglionic nerve and pigment cells with no networks or notochord. The 18-hr post-treated group formed networks of pre-notochord as well as extensive notochord, just as though they had been cultured continuously at 5.3 mM K⁺. Thus it may be concluded that K⁺ has its effect during the post-induction period and that the high level of potassium is required for more than 4 hrs but possibly less than 18 hrs if differentiation is to be shifted into the pathway that leads to notochord.

The significance of these observations on the effects of K⁺ added to the medium at concentrations from 1.3–9.3 mM resides in their demonstration that the post-induction period of 4–18 hrs following the relatively brief initial "induction" treatment is a period during which shifts in ion ratios can play a decisive role in determining the path of differentiation a cell will take—whether toward ganglionic nerve, melanocyte, notochord, mesenchyme, etc. This post-induction period therefore becomes a focal point for further manipulation of ion ratios in an attempt to
open up still other pathways of differentiation to the “induced” or “activated” cells.

Although K⁺ is the key factor in completing the initial induction by lithium in these experiments, it was found that Ca²⁺ can modify differentiation of induced cells. As reported in Barth and Barth (1974) the differentiation of pigment cells is dependent upon the normal amount of Ca²⁺ in standard solution. Experiments cited in the 1974 paper proved that calcium can be lowered from the standard concentration of 0.74 mM to 0.2 mM in complete absence of Mg²⁺ for up to 18 hrs following lithium induction, and nerve and pigment cells differentiate in standard solution following such drastic post-induction treatment. On the other hand, if lithium-induced cells were treated continuously with the same low concentration of divalent cation (0.2 mM Ca²⁺ and 0 Mg²⁺), pigment cell differentiation was inhibited. The amount of sodium in the culture medium was the standard concentration of 90.8 mM (2.8 mM from buffers). K⁺ was present at 3.3 mM, which leads to notochord differentiation at standard concentrations of 0.74 mM Ca²⁺ and 0.83 mM Mg²⁺.

Inhibition of pigment cell differentiation is caused specifically by the 0.2 mM Ca²⁺ and 0 Mg²⁺ medium. Cells thus arrested in their differentiation contain a sharp, dense ring of slate-gray granules which persist without further change for as long as the cultures remain alive (up to 2-3 weeks).

Some preliminary experiments in which th Na/K ratios have been more drastically altered should be mentioned here. It is remarkable that the Na⁺ concentration of the culture medium following lithium induction may be drastically lowered (from 90.8 mM to 41.8 mM), with simultaneous increase in K⁺ (from 1.3 mM to 39 mM), and the aggregates survive and differentiate for at least a week. Lithium-induced cells cultured in standard solution form nerve and pigment cells, whereas lithium-induced cells cultured in the 1:1 ratio of Na⁺ to K⁺ form no nerve and the pigment ring cells are atypical and are probably pre-notochord cells. The ratio of Na⁺ to K⁺ in standard solution is 90.8:1.3.

Differentiation of notochord cells in cultures of gastrula ventral ectoderm

In standard solution, which contains 1.3 mM K⁺, lithium-induced cells form sharp, clear pigment rings (Barth and Barth, 1959, Fig. 7) and differentiate into melanocytes. The first stages of this type of differentiation are apparent by the third or fourth day of culture, when the aggregates have become massive sheets of about 100 contiguous large cells. Within the next day or two most of these cells show an accumulation of pigment granules in the form of a ring near the nucleus in the cytoplasm. From this point on, beginning first in cells at the edges of the large sheets of “ring cells,” there occurs a progressive transformation into dendritic cells with pigment rings dispersed to give uniform pigmentation. These large cells with amoeboid processes were identified as melanocytes (Barth and Barth, 1959).

When lithium-induced cells are cultured in standard solution with K⁺ raised to 3.3, 4.3 or 5.3 mM, again pigment rings form; but the rings are from the beginning faint and diffuse. The following description of the subsequent behavior of this kind of pigment ring cell is based upon cultures in which K⁺ in standard solution was raised to 5.3 mM, since this concentration gave the highest percentage of the typical high potassium effect. Frequently more than 95% of the cell aggregates followed this pattern of differentiation.
FIGURE 1. Cell aggregates treated with 71 mM Li$^+$ in standard solution for 3 hrs, were cultured in standard solution with high potassium (5.3 mM). Eight-day culture shows networks of cells containing small vacuoles. Scale bar represents 20 microns.

FIGURE 2. Same slide as Figure 1. Scale bar represents 20 microns.

FIGURE 3. Cell aggregates treated with 71 mM Li$^+$ for 4 hrs, were cultured in standard solution with 5.3 mM K$^+$. Nine-day culture shows fusion of vacuoles to form large vacuoles, interference contrast. Scale bar represents 20 microns.

FIGURE 4. Li$^+$ treatment for 4 hrs; fixed on day 11 of culture in standard solution with 5.3 mM K$^+$; fusions among cells with large vacuoles. Scale bar represents 20 microns.
The diffuse pigment rings disperse by about the sixth day of culture; cells become dendritic and the cytoplasmic extensions of individual cells make contact with adjacent cells. The result is an extensive network of cells. After about 8 days in culture the cells composing the network become filled with clear vacuoles, usually 10–12 or more per cell. Figure 1 is a low power view of this stage of early vacuolization within the network. A day or two later most of the cell bodies comprising the network are filled with vacuoles. Figure 2 shows at higher magnification two vacuolated cells fusing, and also shows cytoplasmic extensions to surrounding cells within the network which have not yet become vacuolated.

Within the next 24–48 hrs the small vacuoles coalesce so that the average cell now contains 4–6 large granules (Fig. 3). Continued fusions among vacuolated cells give rise to small masses, which become detached from surrounding masses. Figure 4 shows several vacuolated cells fused to give one small mass, as well as several nearby vacuolated cells in the process of joining the group. Figure 5 shows these vacuolated cells at higher magnification.

These small masses of cells filled with large vacuoles were highly suggestive of notochord. However, the vacuolated cells were smaller than those found in notochord differentiating in situ in the neurula, and the masses formed by spontaneous fusions among adjacent vacuolated cells were themselves too small to be identified with confidence as notochord.

The next step was to direct notochord from the archenteron roof of stage 14 (early neural fold) embryos and stage 16 (neural tube). A center strip was excised that included not only prospective notochord but also the tightly adhering neural layers above. This larger explant was treated with EDTA briefly in order to loosen the pigment coat layer and the nervous layers. Dissection of small aggregates of cells from the notochord region then was possible. The samples contained also some nerve tissue and some lateral mesoderm from the archenteron roof. Cultured in standard solution containing 5.3 mM K⁺, these aggregates differentiated to give some nerve and twitching muscle, but also formed masses of notochord with large vacuole-filled cells (Fig. 6). In some instances, individual vacuolated cells migrated away from the central mass and formed networks (Fig. 8). The similarity is striking between these networks of vacuolated cells obtained by dissection of normal embryos and the networks observed in lithium-induced gastrular ectoderm (compare Figs. 4 and 8).

An obstacle in positive identification of lithium-induced notochord cells was the small size of the masses formed by spontaneous fusions of vacuolated cells in the relatively thin population of cells migrating outward from an attached aggregate consisting of approximately 125 ectoderm cells per aggregate. In order to achieve a more normal cell mass and morphology, composites of 10–15 aggregates were prepared. This was done simply by swirling the dish to concentrate the aggregates after 2 hrs in the lithium treatment dish and pushing aggregates together by means of hair loops. Left for an additional hour in the lithium solution, the composites were sufficiently cohesive to be passed through a wash of standard solution and transferred to coverglasses in culture dishes containing standard solution with K⁺ raised to 5.3 mM.

Figure 7, is an example of the differentiation observed in a composite of 10 fused aggregates on day 10 of culture. Within the main mass of cells one observes an inner curved, elongate group of vacuolated cells. The resemblance to notochord of this group of cells was unmistakable under the microscope.
Figure 5. Same as Figure 4, showing large vacuoles within Li⁺-treated aggregates cultured in high K⁺ medium. Scale bar represents 20 microns.

Figure 6. Group of notochord cells derived from cells taken from archenteron roof at early neural fold stage; seven-day culture. Scale bar represents 20 microns.

Figure 7. Elongate mass of clear notochord cells within a mass of ectoderm cells produced by fusion of 10 cell aggregates. Before fusion, cell aggregates were treated with 71 mM Li⁺ in standard solution for 3 hrs. The composites of 10 lithium-treated aggregates were cultured in standard solution with 5.3 mM K⁺ for 10 days. Scale bar represents 20 microns.
The transformation of gastrula ectoderm cells into notochord by means of external K⁺ extends the range of types of cellular differentiation already demonstrated to be subject to regulation by cations (Barth and Barth, 1969). In previous investigations various patterns of nerve and of neural crest derivatives have been obtained in a stepwise process in which the initial “induction” period is followed by a period of 4–18 hrs during which induction is reversible and depends upon the concentration of sodium in the medium. It is during this second period that a 5-fold increase in external K⁺ causes later differentiation of notochord. While potassium at concentrations which can be applied continuously without killing the cells is not effective as an inductor, the same concentrations are very effective in modifying cells already induced by lithium. While Li⁺ induces nerve and pigment cells in standard concentration (1.3 mM K⁺ in standard solution), when K⁺ is raised to 5.3 mM, notochord is induced but pigment cells are rare to absent (Table I). The third and final period preceding visible differentiation, although independent of K⁺, is dependent upon the external concentrations of Ca²⁺ and Mg²⁺ (Earth and Earth, 1974).

Many years ago (1938) Holtfreter explanted small pieces of presumptive notochord from urodele and anuran gastrulae and found that they formed nerve and epidermis as well as notochord. It is possible that Holtfreter’s solution lacks the required concentration of K⁺ and that contact with the ectoderm that normally supplies the K⁺ is necessary for notochord differentiation.

In a separate publication (Barth and Barth, 1974) we present a general theory with the objective of relating the considerable information thus far obtained from cell cultures (Barth and Barth, 1959, 1962, 1963, 1964, 1969) and from studies of whole embryos (Morrill, Kostellow and Murp hy, 1971; Kostellow and Morrill, 1968; Stableford, 1967) to ion regulation of normal embryonic induction. The general theory postulates a normal mobilization of cations from internal compartments. These cations would be trapped within the roof of the archenteron and the presumptive neural plate by the relatively impermeable outer surfaces of cells carried in during gastrulation. Concentration of cations then could result in local internal amounts and ratios of ions similar to those found to induce nerve, pigment cells and notochord when applied to small aggregates of gastrula ectoderm cells in culture.

The mechanisms whereby cations regulate cellular differentiation still are unknown. Numerous studies have provided evidence for a probable link between K⁺ and regulation of protein synthesis in general, although the mechanism is not yet agreed upon. For example, Tupper (1973) in experiments on the sea urchin egg finds that increased K⁺ exchange and protein synthesis closely parallel each other. He suggests that increases in K⁺ permeability and K⁺ decompartmentalization normally occur at fertilization and are reflected in change in membrane potential as well as in Na/K ratios. McDonald, Sachs, Orr and Ebert (1972), using external K⁺ similar to those of the experiments reported here, attribute the effect

*Figure 8.* Small masses of archenteron roof cells removed at neural tube stage were cultured in 5.3 mM K⁺ in standard solution for 9 days. In addition to coherent masses of notochord cells as in Figure 6, other notochord cells migrated away from central masses and formed networks of vacuolated cells. Compare with Figure 4, which shows similar networks of vacuolated cells in cultures of lithium-induced gastrula ectoderm cells. Scale bar represents 20 microns.
of K⁺ on DNA synthesis and growth to changes in membrane potential. Lubin (1967) reported that in mammalian cells, when amphotericin was used to cause loss of cell K⁺, there was a parallel depression in rates of protein and DNA synthesis, reversible by adding K⁺ to the medium. R. Steinhardt (personal communication to Dr. J. D. Ebert) suggests that the controlling factor in the changes in protein and DNA synthesis brought about by membrane depolarization by means of K⁺ may be intracellular Ca²⁺ activity. Depolarization may result in increased permeability to Ca²⁺ as well as an inhibition of the Ca²⁺ pump. Renewed interest in ion changes at fertilization (Mazia, 1934; Heilbrunn, 1943) and early cleavage has involved application of electrophysiological methods to events at fertilization and early cleavage (Monroy, 1965; Epel, 1972; Weissenseel and Jaffe, 1972; Steinhardt, Shen and Mazia, 1972; Morrill et al., 1971). Extension of similar methods to the gastrula stage should provide important clues to link changes in membrane potential to the kinds of changes in cation concentrations we now know are able to regulate cellular differentiation.

Studies on the regulation by ions of cell differentiation as distinct from general synthetic activity are still relatively meager. Lash, Rosene, Minor, Daniel and Kosher (1973) report an increase in cartilage formation and chondroitin sulfate synthesis in chick somites in vitro when the K⁺ of the medium is increased from 2.69 mM to 4.68 mM. The stimulation persists even after the cells are returned to 2.69 mM K⁺. This K⁺ effect resembles ours in that the induction of notochord is obtained by changing the K⁺ from 1.3 mM to 5.3 mM. Also the cells may be returned to 1.3 mM K⁺ after 18 hrs and notochord differentiation will occur. Evidently some irreversible change occurs during 24 hrs exposure in Lash’s system and during 18 hrs in ours.

Ionic regulation of chromosome activity in insect larval salivary glands is well known (Kroeger, 1963; Lezzi, 1970). The work of Kroeger and of Lezzi, in fact, provided impetus for the present experiments, since the high potassium effect reported here was an unexpected offshoot of an ongoing investigation of various Na/K ratios as related to differentiation of gastrula ectoderm cells. Beritashvili, Kvavilashvili and Kafiani (1969) have proposed that in the fish embryo the increase in K/Na ratio may play a part in switching over nucleic acid synthesis from replication to transcription paths.

Independent of mechanism, the experiments reported in the present paper provide definite proof that K⁺ can switch differentiation from ectoderm to notochord, and further suggest that the potassium effect will most profitably be studied during the period following the initial induction. Whether the effect of K⁺ is a direct effect of potassium concentration upon synthesis of new kinds of proteins or whether potassium acts indirectly through alteration of ATP utilization, through altering the Na/K ratio or still other mechanisms is unknown.

A final word as to the differentiation of K⁺-induced notochord in cell cultures: The relatively small size of the cells and of the masses formed by fusion of these vacuolated cells observed in the present studies agrees with observations reported by Mookerjee, Deuchar and Waddington (1953). These workers isolated urodele notochord cells from gastrula and neurula stages by treatment with alkaline solutions at or above pH 7.6. They observed that isolated cells proceeded to become vacuolated but did not “attain as large a size as they would do within the embryo” (page 406). As reported in the present paper, larger masses of K⁺-induced noto-
chord cells could be prepared by fusing lithium-treated cell aggregates and culturing the composites in 5.3 mM K⁺. The result was readily identifiable as notochord.

After the present work had been completed, the recent work of Takaya (1973) came to our attention. This investigator reports the formation of small numbers of *transient* vacuoles in differentiating cells of the newt embryo—ectoderm, muscle, cartilage, liver, *etc.* Only in notochord cells, however, Takaya notes that vacuole formation continues and vacuoles persist, with fusion of small vacuoles to form the larger vacuoles which characterize the differentiated notochord. Takaya's work on vacuolation during notochord formation in the embryo thus is in good agreement with the findings presented in the present paper concerning the induction of notochord from presumptive epidermis by means of the K⁺ ion.

This work was supported by a grant from the National Science Foundation (GB 23026) to the Marine Biological Laboratory, Woods Hole, Massachusetts 02543.

We are indebted to Dr. James D. Ebert, Director of the Marine Biological Laboratory, for his encouraging interest in the research and for extending our knowledge about related investigations in progress in other laboratories.

Dr. Allan D. Dingle provided the photographs, as well as stimulating and critical comments, for which we express warm gratitude. To Miss Martha Coneybear we owe thanks for faithful and reliable technical assistance.

**Summary**

Small aggregates of cells prepared from explants of ventral ectoderm of *Rana pipiens* gastrula were treated for 3–4 hrs in 71 mM Li⁺ added to standard solution. When cultured in standard solution containing 1.3 mM K⁺, the aggregates of ventral ectoderm cells differentiated into nerve and pigment cells. As the K⁺ concentration in the culture medium was raised through 2.3, 3.3 and 5.3 mM, the frequency of pigment cells declined and networks of vacuolated cells were formed, which later fused to form small notochordal masses. Raising the K⁺ to 7.3 and 9.3 mM gave nerve and pigment cells again, as in the lower potassium concentrations. The effect of high K⁺ on lithium-induced cells becomes irreversible during the first 18 hrs. Cells may be returned to 1.3 mM K⁺ after 18 hrs and notochord differentiation will occur.

Induced notochord cells first appear as somewhat larger cells which become filled with very small vacuoles by 7–8 days of culture. These small vacuoles then coalesce to form larger and larger vacuoles (9–12 days). The vacuolated cells aggregate into small clumps. Cultures were fixed and stained at various intervals for photography.

Cultures of small explants of notochord in 5.3 mM K⁺ were observed to undergo the same type of differentiation. In both cases the notochord cells and vacuoles were smaller than in the whole embryo. Larger explants of notochord from archenteron roof gave large masses of notochord cells about the same size as in the whole embryo. When lithium-treated aggregates of the ventral ectoderm were fused together in 5.3 mM K⁺ to form a composite of 10–15 aggregates, the notochordal masses were larger and the notochord cells were about the same size as obtained in comparable sized explants of notochord from the roof of the archenteron.
These findings are discussed in relation to the effects of high potassium on membrane depolarization, protein synthesis, DNA synthesis, and increase in intracellular Ca²⁺.

LITERATURE CITED


View This Item Online: https://www.biodiversitylibrary.org/item/17301
DOI: https://doi.org/10.2307/1540407
Permalink: https://www.biodiversitylibrary.org/partpdf/30572

Holding Institution
MBLWHOI Library

Sponsored by
MBLWHOI Library

Copyright & Reuse
Copyright Status: In copyright. Digitized with the permission of the rights holder.
Rights Holder: University of Chicago
License: http://creativecommons.org/licenses/by-nc-sa/3.0/
Rights: https://biodiversitylibrary.org/permissions

This document was created from content at the Biodiversity Heritage Library, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at https://www.biodiversitylibrary.org.