

INDUCTIVITY AND PLASTICITY OF THE VENTRAL BLASTOPORAL LIP CELLS

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INTRODUCTION.

In recent years our interest in the ventral blastoporal cells has been revived once again by the experiments of Yamada (1950). It is a common knowledge that the ventral blastopore cells are the counterpart of the dorsal blastopore of a gastrulae and in the process of neural induction they do not, as a rule, take part in this process of differentiation. The histological patterns that emerge out from the invagination of the ventral lip cells are the mesenchyme, blood islands and some other endodermal derivatives. But under a certain condition of alkalization, the ventral blastopore cells can be made to differentiate into notochord and somites as obtained by Yamada (1950). Keeping these remarkable developmental capacities in mind, translocation experiment has been conducted upon a gastrulae where the organizer has been replaced by its counterpart, the ventral blastopore. Interest will be focussed round these experiments, in order to know what happens when ventral blastopore replaces the dorsal blastopore and if there is any sort of normal induction resulting from the effects of the ventral blastopore upon the dorsal ectoderm. The ventral blastoporal cells have also been subjected to derivatives of the nucleic acids, citric acids and to some other conditions in isolation. There is reason to believe that these experimental designs would lead us to a better elucidation of the problem of the cellular plasticity and inductivity of the ventral blastopore of a gastrulae.

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MATERIAL AND METHOD.

The embryos of *Triturus alpestris* constitute the material of this study. Thirty-four successful grafting experiments have been done where the medio-ventral marginal zone of a gastrulae in the form of rectangular body has been placed in the vacated portion of the organizer of another gastrulae.

Another set of experiments concerns with the isolation of the ventral blastopore cells of the gastrulae which were subsequently cultured in 1/10th Holtfreter's solution containing some special chemicals. The Guanylic acid, citric acid, lithium choline and urea (for proportion see experimental results) have been used in the culture solution. Besides these, thermal exposure has also been used in the isolated cells of the ventral blastopore.

After the embryos and the isolates have been operated and kept alive for the desired length of time, they were fixed in Zenker's solutions. They were washed

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thoroughly in water before they were graded through alcohol and paraffin. The sections were prepared at 10μ thick and stained with Toluidine Blue.

EXPERIMENTAL RESULTS.

Doubling of the neural axis by the transplantation of the ventral blastopore in place of the organizer.

Whenever there has been a translocation of the ventral blastopore in place of the dorsal lip there has been always a tendency of doubling of the neural axis. The experimental animals, after being histologically analysed, reveal that the doubling nature is not an uncommon feature of most of them. Out of thirty-four cases, 25 are associated with some form of duplication of the neural axis. All are not of one category but in fact are representatives of a series of the neural duplication.

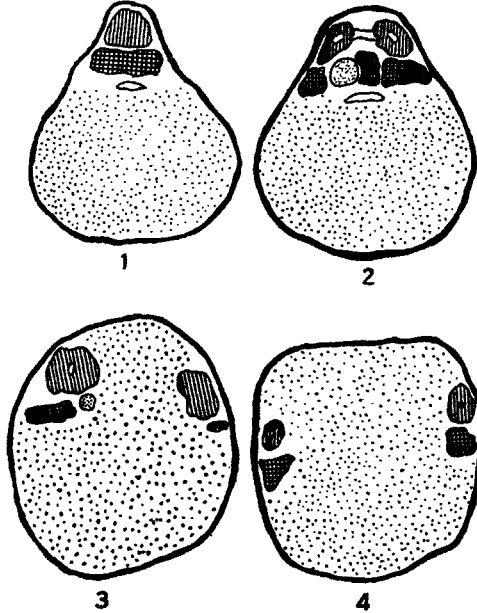
A case, typical of one class of doubling, encountered in this study has been depicted in Fig. 3. The interest in such cases lies on the fact that the two widely separated neural areas seem to occur on the two lateral extremities of the embryo. Out of the two neuralized area, the one which is seen on the left hand side, appears to be better formed to the stage of neural tube. The neurocoele within it is clearly visible. A comparatively smaller notochord lies beneath the neural tube with some mesodermal cells. The archenteron cannot be marked out definitely. The nature of the neural structure on the other side, occupying the right hand side, has been represented by a solid thickening of the ectoderm. No notochord can be traced out near the thickening ectodermal mass. This thickened ectodermal area seems to be slightly better developed than a pallisade condition but definitely underdeveloped of perfect neural nature. Significantly enough, the middle region where the graft has been put, is devoid of any other tissue except for some yolky endoderm. The place of normal appearance of the axis remains strangely endodermal. The presence of this dorsal endoderm makes a sort of a partition between the two laterally disposed neural areas. Another class of embryos slightly different in the nature of the doubling of the neural axis has been depicted in Fig. 4. There are two neural tubes with a neurocoele in each of them. Two tubes are of unequal size. The one on the left is much smaller than its counter part of the right. The absence of any notochordal cells in the vicinity of two neural tubes are facts of importance. The neural tubes are again widely separated by the intervention of yolky endoderm.

The other type of doubling, occurred only in a few cases may be discussed here. This type has been represented in Fig. 2. The special interest of this neural structure is that there are two canals observed in a single large mass of a neuralized area, i.e. two tubes occur in a fused condition within a common area. A tiny layer of the ectodermal tissue is interposed in between the two neuralised areas. The presence of this layer makes a discontinuity of the neural structure induced. The notochord is visible with a mass of mesodermal cells between the neural structures. It seems that yolky endoderm cells have not been represented here at this level of embryo.

Absence of any neural axis following translocation of the ventral blastoporal cells in place of the organizer.

Majority of the cases with our translocation of the ventral blastopore in place of the dorsal, have resulted in the doubling of the neural axis but only in a few cases the results also show certain other variations. These results are different in the sense that there is a tendency of suppression of neural structures. A representative case has been shown in Fig. 1. The figure renders the fact clear that there has been a very weak mobilization of the ectoderm cells at the mid-dorsal

side of the embryo, a place where normally the neural tube appears. The neuralization has failed to react in these circumstances and a condition of pallisade has resulted. The underlying tissue of the thickened area mostly are represented by loose mesodermal cells. Neither notochord nor somites are visible. In very rare cases distinguishable archenteron is observed. The fact becomes clear that the evocatory action in some way or other is hampered.



All are schematic representations of the operated embryos at tail bud stage.

- Fig. 1. Cross-section of an embryo in which the organizer has been replaced by the ventral marginal zone. Note the under-development of the neural tissue and the fusion of the mesodermal area.
- „ 2. Cross-section of a similar graft. Note the doubling of the neural axis interposed by a band of thickened ectoderm and three somital blocks and a chorda.
- „ 3. Cross-section of a double axis separated by the yolk endoderm. Note the large size of the neural tube on the left hand side with the chorda and one somite block; the pallisade condition of the neural on the right with a somite block.
- „ 4. Cross-section of the embryo with two axes. Note the presence of small neurocoele in both the neural tubes and absence of chorda under them.

Isolated ventral blastopore cells exposed to various physiological conditions.

(a) *In acidified Holtfreter solution.*

The ventral blastopore cells after isolation, have been cultured in 1/10 Holtfreter solution containing .3%, .5% and 1% of Guanylic acid. Cells have been kept in the acidified solution for varied length of time. The minimum time of exposure was $\frac{1}{2}$ a minute while some of them have been cultured continuously. Histological sections show that the development of these cells has not proceeded and has been blocked. Similar experiments with citric acid (pH 2.3 and 4.2) have also been done. Here the time of exposure is cut down to few seconds but the differentiation is blocked. Experiments with distilled water also give a similar picture of blocked development.

(b) *Holtfreter solution with lithium chloride and urea.*

In Holtfreter's solutions containing 4% lithium chloride and 1.25% of urea the ventral blastopore cells when cultured, the trends of differentiation is blocked. The deleterious effects hinder the process of development, even epidermal type of differentiation is also incapable of formation.

(c) *Thermal exposure to the ventral blastopore cells.*

The ventral blastopore cells, after isolation, have been exposed to 30°C. and 34°C. of temperature. The temperature shocks make the cells incapable of further differentiation. The development has been retarded completely.

DISCUSSION.

The main evidence emerged from these experiments will favour us to regard the ventral blastopore as an ineffective inductor in place of organizer. This seems to be true because straightforward induction seldom happens from the ventral blastopore upon the gastrular ectoderm. Great many cases of our experiments with the ventral blastopore have resulted in the production of two neural axes instead of one. This duplication of axis, however, does not account for any evidence of inductive capacity of ventral blastopore cells. Induction, seems to be always absent at the normal position of the embryo. But the translocated cells of the ventral blastopore, may be credited that it has managed to fragment the one organizer action into two. In a previous study, Bautzmann (1932) has been able to show that the 'Randzone' when transplanted in place of the organizer, duplication of the axis may result. Parallel results in chick embryos, have been encountered in the experiments of Abercrombie and Bellairs (mentioned by Waddington, 1952).

The presence of the ventral blastopore cells in the organization centre, appears to break up the unitary action of evocator by being itself non-inductive. Moreover, the translocated cells possibly cannot be assimilated in the induction-system and that is why their presence amount to fragmentation of the inducing centre. With the removal of the organizer cells, the remaining cells of the mesoderm presumably acquire a better inductive-state because some non-inductive material have taken the place of the original high gradient. In terms of Yamada's (1940) 'high' and 'low' gradients, it could be explained by certain theoretical postulations. Mookerjee (1953a) has already put forward the latero-dorsal topographic relations in inductive process. His idea of 'position-effects' can be brought in the present cases of double axis. The other type of lowly induced neural structures can be explained better if one thinks that the grafts possibly could not be assimilated and invaginated properly during gastrulation. It is likely that the organizer centre, represented here only by the remaining cells of the mesoderm (ineffective inductor, as shown by Waddington, 1936 and by Mookerjee, 1953a) could not induce a better type of neural structures.

The mode of differentiation of the ventral blastopore in these grafts does not give the impression that they can be easily differentiated into notochord structures. Yamada (1950) has got them to differentiate into notochord by a cytolytic process of alkalization. The series of experiments that we have conducted do not make much room for the idea that under the experimental conditions in our materials, they become chorda-wise. The process of dorsalization as Yamada has been able to produce, seems to be resulted from a particular type of reaction, perhaps provided by the action of the alkali upon his cells. Mookerjee (1953b) has entertained such an idea of specific 'type' of reaction and the mode of differentiation produced. Thus the negative results will not minimise but in fact strengthen the idea of Mookerjee that sublethal cytotoxicity, not all cytotoxicity, can bring about a directive

change in tissue differentiation. The other possibility which should not be ignored is the specific difference of the material studied. Yamada's *T. pyrrogaster* may have a special susceptibility to dorsalization than the cells of *T. alpestris* studied by us.

SUMMARY.

(1) The ventral blastopore of a gastrulae when replaces the dorsal lip of a gastrulae, cannot bring about neuralization by itself.

(2) The doubling of the neural axis is the outcome of the implantation of the non-inductive ventral blastopore.

(3) The doubling phenomenon has been explained by supposing that the two lateral halves of the mesoderm become inductive in the presence of a non-inductive material in between them.

(4) Theoretical implications about the position effects in the process of induction have been discussed.

(5) The differentiation of the ventral blastopore cells are not 'dorsalized' either by implantation or by physiological treatments of some category.

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