Chapter 5

DISCUSSION
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5.1 Neural induction

Neural induction is an important event during early embryonic development. Embryonic induction is defined as an interaction between one inducing tissue and another responding tissue, as a result of which the responding tissue undergoes a change in its pathway of differentiation. Embryonic induction is a remarkable phenomena which initiates the differentiation of cells leading to the organisation of cells into tissues and organs. It is believed that during induction certain messages are transferred from the inducing to the responding tissue. This acts as the causative agent bringing about the differentiation of cells of the responding tissue.

The phenomenon of neural induction brings about the transformation of ectoderm lying over the developing notochord into neural plate during gastrulation. This was first demonstrated by Spemann (1918) in an amphibian embryo in the course of his investigation on the role of dorsal lip of blastopore as the Organizer. Through the dorsal lip of blastopore the cells from the surface of amphibian blastula start moving into the interior
of the amphibian embryo. By the time the blastopore is completely formed, the presumptive notochordal material is arranged lengthwise mid-dorsally on the roof of the archenteron and neural plate has been formed over the notochordal material in the mid-dorsal region. A number of workers (see for review Spemann, 1938; Nieuwkoop, 1952 among others) have demonstrated that the ectoderm above the presumptive notochord is transformed into neural plate under its inductive influence. Spemann and Mangold (1924) showed that when dorsal lip of blastopore is implanted into the blastocoel of another gastrula of the same age, it induces the formation of a secondary embryonic axis. The induction of neural plate by the underlying roof of the archenteron is an important event at this stage.

The phenomena of neural induction in chick embryo was first demonstrated by Waddington (1932, 1933). Waddington (1932) demonstrated that Hensen's node of the primitive streak of the chick embryo acts as an 'Organiser' similar to that in the amphibians. When implanted below the epiblast of another primitive streak stage embryo, the Hensen's node induced a secondary embryonic axis. In 1933, he published the result of his further experiments and argued that the underlying chordal mesoderm appeared
to have the capacity to induce the formation of neural plate in the host ectoderm. The mesoderm which caused the induction of neural plate, was actually composed of prechordal and chordal material has been conclusively confirmed by subsequent workers (see for review Hara, 1978).

Further, experimental works on amphibian embryos (see for review Nieuwkoop, 1973) and chick (see for review Hara, 1978; Khare and Choudhury, 1985) provide us with an insight that the first stimulus for neural induction emanates from embryonic endoderm. This is followed by the stimulus from the prechordal and chordal mesoderm. In the chick embryo many workers (such as Waddington, 1932, 1933; Spratt and Haas, 1960 a,b; Vakaet, 1964, 1965; Eyal-Giladi and Wolk, 1979, 1981) have shown that the embryonic endoderm had a definite role in the induction of the neural plate. Gallera (1971) writes that "The stimulus for the neural induction, then, would originate in the presumptive embryonic endoblast, and it is reinforced later by the inductive stimulus from the chorda mesoblast. In any event, once invaginated, the embryonic endoblast lose their inducing capacity" (Gallera and Nicolet, 1969).
Competence of the responding system

Competence is a term defined as the physiological state of the tissue which permits it to react in the morphogenetically specific way to determinative stimuli, or, more in keeping with the molecular biological outlook, "a term which sums the ability of the enzyme complement of the embryonic cell to adapt to a particular ratio of metabolites." Whatever it may be, competence is always related to particular stimuli and particular corresponding response. With regard to primary induction, therefore we may speak of neural differentiation as a primary competence of the ectoderm.

In chick embryos several workers such as Woodside (1937) attempted to investigate the part played by the responding system and also the developmental age of the host on the responding tissues in the presence of inducing tissues. He used Fell and Robinson (1929) watch glass technique to culture the grafts. He performed experiments on embryos ranging from the first appearance of short broad primitive streak to the 5 somite stages. He performed three sets of experiments according to the age of the host from 16 - 19 hours of incubation. He demonstrated that competence of ectoderm to react to inducing action of the graft is best at middle
to full primitive streak stage. It declines from full primitive streak stage to head process stage. In early experiments of the present investigation, the author also noted that the ectoderm of the host responded best to the grafts of the primitive streak slightly before full primitive stage, and as such hosts of this stage only were used for the experiments in the present investigation.

5.2 **Inducing capacity of different parts of the primitive streak at stages 3, 4, 5 and 6**

Waddington and his collaborators during (1930-1940) notably, Abercrombie, Taylor and Schmidt already discussed and demonstrated that the anterior part of the primitive was capable of inducing neural structure in the competent ectoderm from both pellucid and opaque areas. The inducing capacity of the definitive primitive streak has also been systematically analysed by some other notable workers such as Mulherkar (1958) and later reinvestigated by Gallera (1964). All these workers concentrated mainly on the definitive streak stage except Vakaet (1964) and Gallera and Nicollet (1969), who also work on pre-streak stage (stage 3). As these workers have described the change in the inducing capacity of the Hensen's node from stage to stage,
the experiments in the present investigation were conducted to re-examine the neural inducing capacities of different parts of the primitive streak grafts A, B, C and D (Figs. 3.5a, 3.5b) at four developmental stages namely stage 3, stage 4, stage 5 and stage 6. The grafts were prepared with (1) all germ layers at stages 3, 4, 5 and 6 (2) without endoderm at stages 3, 4 and 5 (3) without endoderm and mesoderm at stages 3, 4 and 5.

These three experiments were designed to test the inductive behaviour of the three germ layers when they were present together as well as when they were separated. The experiments have been provided following dynamic picture of the inducing capacity of the different parts of the primitive streak.

5.2.1 By the grafts having all germ layers

In the present investigation, the neural inducing capacity of the Hensen's node as well as posterior parts of the primitive streak has been examined at pre-streak stage (H and H stage 3); definitive primitive streak (H and H stage 4); head process stage (H and H stage 5) and head fold stage (H and H stage 6). In the first series of experiments of the 183 grafts implanted, 49 died
(Table 4.1.1) at stages 3, 4, 5 and 6. The study of the sections revealed that the structures differentiated were 'Complete' embryonic axis which had all the three components namely, neural tube or neural plate, notochord and somites. 'Incomplete' embryonic axis had either the somites or notochord in addition to the neural plate. In some instances neural plate is the only induced axial structure.

Complete embryonic axis was induced by only A and B grafts (Figs. 4.1A - ECMEn) while C and D grafts did not show any induction (Figs. 4.1C - ECMEn, 4.1D - ECMEn). Graft A of stage 4 (Figs. 4.1A - ECMEn) shows that it can induce the complete embryonic axis and the inducing capacity is greater while reaching stage 5 and again shows a decline at stage 6.

Induction of incomplete embryonic axis was observed by A, B, C grafts only D grafts did not show any induction of incomplete embryonic axis except at stage 4 (Figs. 4.1A - ECMEn, 4.1B - ECMEn, 4.1C - ECMEn and 4.1D - ECMEn). The grafts A, B, C at stages 3, 4, 5 and 6 showed that induction starts at stage 3, increases at stage 4 and stage 5 and showed a decline at stage 6. Except graft C at stage 6 (Fig. 4.1C - ECMEn) did not show any induction of
incomplete embryonic axis, this may be due to high mortality rate (60%) of the grafts implanted (Table 4.1.1).

Induction of neural plate alone was achieved by all the grafts A, B, C and D and as in other instances observed as early as stage 3 and decline at stage 6 (Figs. 4.1A - EcMEn, 4.1B - EcMEn, 4.1C - EcMEn and 4.1D - EcMEn). The figures showed that all the grafts have the capacity to induce the neural plate and the frequency of neural induction declined at stage 6.

The above finding shows a dynamic picture in the inducing capacity of the primitive streak with all germ layers present. The primitive streak of donors at stage 3, 4, 5 and 6 have a comparable capacity to induce neural plate formation. But induction of a complete embryonic axis or incomplete embryonic axis was induced only by A and B grafts. Incomplete embryonic axis was induced only by A, B, C grafts while graft D did not have the capacity to form axial structures other than the neural plate. The anterior portion have greater inducing capacity than the posterior region. This works also supports the earlier findings that anterior half of the primitive streak has clear neural inducing capacity.
Washington, Abercrombie, Taylor and Schmidt (1930-1940) have demonstrated that anterior part of the primitive streak is capable of inducing neural structure in the competent ectoderm. Mulherkar (1958) Gallera (1964) and Vakaet (1964) confirmed these findings and demonstrated that these capacities were restricted to middle half of the primitive streak. While the present author supports the finding that anterior half has clear neural inducing capacity this investigation indicates that the posterior half is not devoid of such capacity.

While reviewing the earlier contributions on primary induction, Gallera (1971) pointed out that neural inducing capacity of the anterior part of the primitive streak is much reduced at stage 6. In the present investigation, graft A comprising Hensen's node from the anterior part of the primitive streak with all germ layers (Fig. 4.1A - ECMeN) showed that percentage frequency of neural plate induction showed a comparatively high inducing capacity which was 87.5% at stage 3 and decline at stage 6 to 66.6%.

5.2.2 By the grafts without endoderm

In the second set of experiments the donors were taken at stage 3, 4 and 5. In the present
investigation it shows that complete embryonic axis was not induced by any grafts after removal of endoderm only. Incomplete embryonic axis was induced only by A and B grafts of stage 3; A, B and C grafts of stage 4; and A graft of stage 5 (Figs. 4.1A - EcM, 4.1B - EcM, 4.1C - EcM). Graft A at stage 3 and 4 showed the same level of induction and the capacity which increase 42.7% at stage 5 (Fig. 4.1A - EcM). This shows that the ability to induce other axial structures beside neural plate starts as early as stage 3 and increases at stage 5. Graft B showed same level of induction 14.3% at stage 3 and stage 4 but no induction at stage 5, this may be due to high mortality rate (Fig. 4.1B - EcM and Table 4.1.2). While C graft showed induction only at stage 4 (Fig.4.1C - EcM). From the above picture it shows that induction of other axial structure apart from neural plate is restricted largely to grafts having all the three germ layers.

Neural plate was induced by A, B and C grafts only (Figs. 4.1A - EcM, 4.1B - EcM and 4.1C - EcM). In this investigation it shows that the induction of neural plate occurs even as early as stage 3 and then there is a gradual decline as development proceeds further. The percentage frequency of neural plate induction showed a similar
trend as in the first set of experiments where greater induction was observed at early stages and again there is a decline in inductive capacity. After removal of endoderm, induction of other axial structures besides the neural plate is reduced. This suggests that the endoderm might enhance the induction of these structures in the competent ectoderm. Several authors have studied the inductive action of the early hypoblast on the epiblast in the chick blastoderm (Waddington, 1932, 1933; Spratt and Haas, 1960 a,b; Eyal-Giladi and Wolk, 1970; Azar and Eyal-Giladi, 1970, 1981). But all these experiments revealed mainly on the role of the hypoblast in the morphogenesis of the epiblast. However, they suggest strong inducing action of hypoblast.

Other workers, Azar and Eyal-Giladi (1981) have studied the interaction of epiblast and hypoblast to induce the formation of primitive streak and embryonic axis. This present investigation also shows that in the absence of endoderm, the formation of a complete embryonic axis does not occur. Graft D showed a very high mortality and it was not possible to have enough number of successful cases in order to draw meaningful conclusions.
5.2.3 By the grafts without endoderm and mesoderm

In the third set of experiments when both endoderm and mesoderm was removed from donor embryos at stages 3, 4 and 5, the results were similar to those obtained in the second set of experiments described above. No induction of complete embryonic axis occurred in any of the grafts.

Incomplete embryonic axis was induced only by A graft of stages 3, 4 and 5 (Fig. 4.1A - Ec) while B, C and D graft did not show any induction. This shows that only anterior part of primitive streak has the greater inducing capacity even when these two other layers are removed. Again, the inducing capacity is observed even as early as stage 3 and 4 increases at stage 5. Similarly, the grafts without the endoderm (Fig. 4.1A - EcM) also shows an increase at stage 5. Here it is suggested that the mesoderm might have the capacity to induce axial structures in the competent ectoderm; B, C and D grafts did not show induction of any axial structure other than neural plate.

Neural plate is induced by grafts A, B and C (Figs. 4.1A - Ec, 4.1B - Ec, 4.1C - Ec). High mortality occurred in D graft experiments and therefore no meaningful conclusions could be drawn.
Induction of neural plate is observed as early as stage 3 and continues at increased frequency but declines as development proceeds.

In this investigation it is observed that after the removal of two germ layers the ectoderm is capable of inducing only neural plate, it is therefore suggested that the information necessary to bring about neural induction is already present in the donor ectoderm. In the third set of experiments like the second set of experiments it was found no formation of complete embryonic axis; incomplete embryonic axis was induced only by graft A at stages 3, 4 and 5. Only neural plate was induced by A, B and C grafts and induction of other axial structures are greatly reduced which suggest that the presence of endoderm enhance to bring about induction of other axial structures in the competent endoderm.

5.3 Histological changes in the neurectoderm induced by the grafts of Hensen's node of stages 4, 5 and 6; stage wise analysis

Instructive investigations in this field have been carried out by Gallera (1964, 1965, 1966). His experiments consisted of explanting oriented grafts of a standard size taken from the anterior part of primitive streak and then implanting in area
pellucida and another in area opaca. To obtain this information Gallera (1965) implanted grafts of Hensen's node on the competent ectoblast in the area opaca and assured a direct contact between them. At various intervals, grafts were detached from the ectoblast and host blastoderms were allowed to grow in order to assess the type of inductive response given by the ectoblast. Under these conditions 6 hours of contact between the inductor and the ectoblast was found necessary to obtain the induction of a well thickened neuroidal plate. Longer contact was required for the differentiation of cerebral structures. Typical neural induction was obtained after a contact of $8\frac{1}{2}$ hours or longer.

Leikola and Maccallion (1967) used alcohol killed chick liver as the inductor. They found a neuroid response after 4 hours of contact and a typical neural induction after 6 hours.

In the present investigation the host embryo together with the grafts were removed from the incubator at different time intervals, fixed and processed for histological analysis. Neural induction by the grafts of Hensen's node at stages 4, 5 and 6 has been investigated at different
time intervals with two types of grafts:

(1) Grafts of Hensen's node with all the germ layers of stage 4, stage 5 and stage 6.
(2) Grafts of Hensen's node without the endoderm of stage 4, stage 5 and stage 6.

5.3.1 By the grafts of Hensen's node having all germ layers

In the first set of experiments, the grafts of Hensen's node at stage 4 caused distinct and gradual changes in the histomorphology of the cells at time interval of 5, 10, 15, 20, 25 and 30 minutes. The cells become slightly elongated with intercellular spaces between them at 10 minutes of contact and at 15 minutes of contact they were prominently elongated and stratified with very little intercellular spaces in between them. At 30 minutes of contact the reacting ectoderm showed formation of neural plate (Plates 4.2.1c, 4.2.1d). In short time interval of contact appearance of intercellular spaces between the cells were observed but with increase time interval of contact the ectodermal layer appeared to become thickened, stratified, cells were tightly closed together without any intercellular spaces (Figs. 4.2.1(i), 4.2.1(ii)).
At stage 5, these grafts induced comparatively sharp and more prominent changes in the reacting ectoderm at different time intervals of 5, 10, 15, 20, 25 and 30 minutes. At 10 minutes of contact there was distinct change resulting into the formation of neural plate as well as neural groove (Plates 4.2.1d and 4.2.1e) with few intercellular spaces in between the neuralised cells.

At stage 6, these grafts showed no change in the reacting ectoderm even for a contact of 50 minutes. Definitive neural response was seen only at 2 hours of contact.

Histologically, the neural plate induced by grafts of stage 4 as well as stage 5 and stage 6 show similar changes, the cells show a tendency to become elongated, bottle-shaped cells show an increase in length with increase time interval of contact (Fig. 4.2.1) and slowly the intercellular spaces present between the cells disappear and the whole ectodermal layer appeared to be stratified and resembling a normal neural plate.

The experiments though designed, to examine the histological changes in the reacting ectoderm at different time intervals of contact with the grafts of Hensen's node with all germ layers intact,
reveal that the inducing effect has been manifested as early as at 10 minutes of contact. The fully formed neural plate was seen only at 30 minutes of contact.

It is also not possible here to comment on the difference between present observations and observations made by Gallera (1965, 1970), because he also put the graft in contact and then removed it and allowed the reacted host embryo to develop.

5.3.2 By the grafts of Hensen's node without endoderm

In the second set of experiments the grafts of Hensen's node stripped free of its endoderm at stages 4, 5 and 6 and kept in contact at different time intervals of 10, 15, 20, 25 and 30 minutes and at stage 6 'for 2 hours and 2 hours 30 minutes revealed the following picture.

The grafts at stage 4 caused a slow and weak response. Even at 15 minutes of contact the cells show a tendency to elongate and presence of some intercellular spaces in between them. But only at 30 minutes of contact the reacting ectodermal layer was somewhat thickened and closely packed without intercellular spaces and it appeared as a thickened neural plate.
The grafts at stage 5 showed similar weak response. A response was seen at 15 minutes of contact, when ectodermal layer cells become elongated and densely packed. But at 20 minutes the whole ectodermal layer appeared to be thickened. It was more prominent at 30 minutes of contact (Plates 4.2.2c, 4.2.2d).

The grafts at stage 6 showed a weak response at 2 hours of contact, only at 2 hours 30 minutes of contact with the graft, the study of the sections revealed that the reacting ectoderm formed a thickened neural plate. The cells show a tendency to elongate and they were closely packed without any intercellular spaces where it showed formation of a thickened neural plate.

In our experiments when endoderm was removed from the graft of Hensen's node, it showed a slow and weak response in the reacting ectoderm. It may however be noted that the grafts of Hensen's node without the endoderm caused induction of thickening of reacting ectoderm at 15 minutes of contact similar to the grafts of stage 4 which had all germ layers. The grafts of Hensen's node at stage 5 blastoderm bring about transformation of reacting ectoderm into neural plate at 20 minutes of contact.
almost double the time than those grafts of stage 5 which had all germ layers intact which caused changes in the reacting ectoderm only at 10 minutes of contact and those of stage 6 grafts without endoderm bring about changes at 2 hours 30 minutes of contact while stage 6 grafts with all germ layers initiated this process at 2 hours of contact. The result indicate that presence of endoderm in the first series of experiments did have some influence on the phenomena of induction.

In the present investigation a comparative analysis has been made whether there is any difference in the timing of induction under experimental condition by grafts of the Hensen's node at stage 4, stage 5 and stage 6. We did note a distinct difference when the graft was isolated at stage 4, the clear histological change in the responding ectoderm was seen at 15 minutes of stage 5, contact but when the graft was isolated at it brought about a clear histological change in the cells of the responding ectoderm at 10 minutes of contact. By stage 6 the inducing power of the Hensen's node was highly reduced as it did not cause any induction up to 50 minutes of contact. The result is indicated that at stage 5 the Hensen's node had cells which had better inducing capacity.
than at stage 4 or stage 6. The cells which are migrating through the Hensen's node downwards are mainly the cells of chorda-mesoderm and endoderm.

5.4 Histological changes in the normal neurectoderm at stages 3, 4, 5 and 6

In the present investigation, sections of the neurectoderm of the chick embryo at stages 3, 4, 5 and 6 have been studied. At stage 3 the sections of the neurectodermal layer reveal that it is a thin strip of cells composed of different types of cells with large intercellular spaces between them. The cells are mainly of the cuboidal type some bottle-shaped, tall columnar, attached and forming a thin epithelial sheet of cells, where both the prospective neural plate and ectodermal cells could not be distinguished from each other.

The study of sections of the neurectodermal layer of stage 4 reveals that it is similar to stage 3 except that the ectodermal layer is slightly stratified. It is composed of different types of cells with large intercellular spaces between them as in stage 3. Cells were bottle-shaped, tall columnar and cuboidal cells and irregular-shaped cells.

At stage 5, the entire neurectodermal
layer appears as a thickened strip of cells which are closely packed with little inter-cellular spaces in between them. Cells were mostly bottle-shaped and tall columnar.

At stage 6, the neural plate appears as a thickened strip of cells that closely packed with little intercellular spaces between them. However, the neurectoderm at the neural fold region is stratified with distinct intercellular spaces between cells. Cells were mostly bottle-shaped, tall columnar and cuboidal. The cells of the neural plate become elongated preparatory to migration while cells in the middle region remain cuboidal shape. As the cells become elongated or bottle-shaped changes occur at their cell surfaces. The cells lose their epithelial arrangement and the sections of this layer reveal it to have become stratified.

As could be inferred from the foregoing observations it is clear that the changes observed in the normal neurectoderm of stages 3, 4, 5 and 6 revealed conspicuous difference with increasing development. The presumptive neural plate appear as a thin strip of epithelial layer even as early as stage 3. An epithelium is usually defined as a sheet of cells that lines a body cavity or covers the surface of the body (Hay, 1968; Trinkaus, 1976)
and it also possesses a basal lamina at one side and a free apical edge at the other. There is high mitotic rate in the presumptive neural plate region at stage 4 than in the more laterally situated ectoderm (Emanuelsson, 1961). This may be the reason that the presumptive neural plate at stage 4 appear most stratified than at stage 3. Similarly, at stage 5 and stage 6, cells are densely packed with less inter-cellular spaces this may be also due to the high rate of cell division during elongation of the head process.

5.5 Comparative histological changes of normal and induced neurectoderm

A comparative analysis of the histological changes in the normal neurectoderm at stages 3, 4, 5 and 6 with those of the neurectoderm induced by grafts of Hensen's node of stages 4, 5 and 6 reveals a following picture.

In the normal neurectoderm the prominent characteristics are:

1. The sections of neurectoderm at stage 3 is a thin strip of cells with large intercellular spaces between them. Cells are mainly cuboidal in appearance.
2. At stage 4, the cells begin to appear stratified and distinct intercellular spaces occur between cells. Tall columnar and irregular shaped cells are present.
and 6, at stage 5, cells are closely packed with very little intercellular space between them. Most of the cells are now bottle-shaped and tall columnar. At stage 6 in the neural fold region the cells are stratified, as in stage 4, with some intercellular spaces.

A comparative analysis of the induction of neurectoderm at stages 4, 5, and 6 revealed that the grafts of Hensen's node at stage 4 with all germ layers intact caused neuralisation of host ectoderm evidenced by distinct and gradual changes in its histology.

In the neurectoderm induced by the grafts of Hensen's node with all germ layer intact the following changes were observed:

After 15 minutes of contact with the graft of Hensen's node at stage 4 the cells of host tissue become prominently elongated and stratified with little intercellular spaces between them. After 30 minutes of contact a prominently thickened neural plate with a neural groove is formed in the host tissue. At stage 5, the changes were distinct and prominent in the reacting ectoderm. Even at 10 minutes contact with the graft, there is a distinct change in the reacting ectoderm which appeared
like a thickened neural plate. After 30 minutes of contact with the graft, a thickened neural plate is formed and the cells are tightly packed without any intercellular spaces between them. Mostly bottle-shaped and tall columnar cells are present and they have an elongated nucleus. At stage 6, very little change is observed in the competent reacting ectoderm. Even such small changes are slow in occurring. At stage 6, a longer time is taken for a neuroid response to occur in the reacting ectoderm. No change is observed at 50 minutes contact. A thickened neur ectodermal layer appears only after 2 hours of contact. The cells in the induced ectoderm are of a variety of shapes some are cuboidal, rounded or irregular in shape but most cells are elongated and bottle-shaped. These cells are closely packed with very little intercellular spaces in between them.

In the neur ectoderm induced by the grafts of Hensen's node without the endoderm, the following changes were observed:

Grafts of Hensen's node without the endoderm, at stage 4 evoked a slow and weak response in the host tissue. Even after 15 minutes of contact the host ectoderm appeared merely as a thickened ectoderm, where cells tend to be elongated and with
large intercellular spaces. It is only after 30 minutes of contact that the entire host ectodermal layer is induced to form a neural plate. The cells tend to elongate and densely packed. Graft taken at stage 5, induced only a weak response even at 20 minutes of contact with the host ectoderm. Cells were densely packed and elongated. The induced ectoderm appeared to consist of different types of cells. The host ectoderm layer appeared to form a thickened neural plate only at a 30 minutes contact with the graft. At stage 6, a longer time of contact was required to bring about neuroid response in the host tissue. Only at 2 hours 30 minutes of contact did the host cells become elongated to form neural tissue. After 2 hours of contact only a feeble neuroid response was elicited and the host ectoderm appeared as thickened ectoderm with less intercellular spaces between the cells.

After removal of endoderm from Hensen's node at the above mentioned stages it is seen that it takes a longer time of contact with the inductor to bring about a neuroid response in the reacting ectoderm.

As could be inferred from the foregoing observations it is clear that the changes observed
in the induced neurectoderm are similar to those seen in the normal neurectoderm.

Studies by Rosilo and Leikola (1976) on neural induction by previously induced in avian embryos in vitro established that neutralised cells were usually easily recognised by their elongated nuclei. In the present study of the cells composing the induced neural plate, bottle-shaped and columnar, cells had elongated nuclei. The cells appear to elongate and columnar in shaped in the reacting ectoderm. Similarly, (Spratt, 1946) revealed that the cells of the neural plate even are more columnar than the surrounding prospective epidermal cells.

5.6 Ultrastructural changes in the normal neurectoderm

In the present investigation, ultrathin sections of the normal neurectoderm of the chick embryo at stages 4, 5 and 6 were studied in order to follow the changes in the arrangement and presence of different types of cells.

At stage 4, the neurectoderm is composed of two types of cells, namely, elongated and amoeboid cells, whereas at stage 5, the cells are densely packed throughout the thickness of the neurectoderm and with very few lightly staining mesenchymal cells in the dorsal and ventral portions of the
neurectoderm. At stage 6 the competent cells appear to be very much elongated and mesenchymal cells are absent. The elongated cells have large prominent nuclei and the cytoplasm is dense with ribosomes, rough and smooth endoplasmic reticulum, lipid droplets and yolk granules. Neighbouring ectodermal cells are attached to each other by demosomes and gap junctions. In particular, gap junctions have been demonstrated from stage 4 by Revel et al (1973), who carried out a freeze etch study. It is generally accepted that gap junctions are the sites of electrical activity and might therefore serve to keep the cells in communication.

At stage 4, in certain cells lysosomal vesicles are seen surrounding lipid droplets, indicating some sort of ongoing metabolic processes in the cells. As there is higher mitotic rate in the presumptive neural plate region than in the more laterally situated ectoderm at stage 4 (Emanuelsson, 1961) and there is also larger amount of debris in the neural plate region than laterally (Banerofft and Bellairs, 1974), this is also likely to indicate a region in which many cells have recently undergone mitosis. At stage 4, stage 5 and stage 6 yolk granules along with mitochondria are abundantly present in the elongated cells. The
reason seems to be that in elongating cells more energy is required for their elongation and morphogenesis but once their shape is established the function of these organelles is also reduced to some extent. Large amount or high density of yolk granules seem to have stored raw material for providing energy during the elongation and morphogenesis of these neur ectodermal cells.

5.7 Embryonic Endoderm—Origin and Formation

Earlier workers (Kionka, 1894; Wetzel, 1939; Merbach, 1935. Hunt, 1937, Pasteels, 1937; Peter, 1938; Jaco, 1938) believed that the hypoblast arises either by separation or by polyinvagination of the epiblast and later gives rise to the endoderm of the embryo. But Spratt and Haas 1960 a, b, 1965 and Vakaet, 1962, 1967 and Vakaet and Mares, 1964, Modak, 1963, 1965, 1966, Rosenguist, 1966, Nicolet, 1965, 1967 have conclusively demonstrated that the embryonic endoderm arises from the base of Henson's node at stage 3, 4 and 5. The original hypoblast, also called primary hypoblast, is pushed anteriorly in the germinal crescent area. The new secondary hypoblast arising from the base of Henson's node gives rise to endoderm of the embryo. Vakaet (1970) called the primary hypoblast,
endophyll and the secondary hypoblast, as sickle hypoblast. Wolk and Eyal - Giladi (1977) by immunofluorescence technique and Wakely and England (1978) by SEM have further confirmed and elaborated the manner in which this layer arises.

Comparative analysis of the inducing capacity of the two types of grafts of the Hensen's node with all germ layers and without endoderm at different time intervals in the present investigation reveals that there was not much difference in the timing of neural induction by grafts of stage 4 blastoderms there was distinct difference in the time of induction caused by stage 5 and stage 6 grafts. The grafts at stage 5 from which endoderm had been removed took almost double the time 20 minutes to induce the formation of a neural plate in the competent responding ectoderm than those which had all germ layers intact. The stage 6 grafts with all germ layers intact took 2 hours to elicit neural induction in the host tissue whereas stage 6 grafts without endoderm do not cause any induction in less than 2 hours of contact with the graft.

The explanation for these results seem to be the following :-

(1) At stage 4, the Hensen's node still has
prospective embryonic endoderm and chordal mesoderm cells in it. Thus the two types of grafts with all germ layers and grafts without endoderm did not show much difference in the induction time. 

(2) At stage 5, the chordal mesoderm has emigrated out anteriorly from the Hensen's node to give rise to the head processes and many endoderm cells have also emigrated at the base of the Hensen's node to give rise to embryonic endoderm, this may be the reason why the grafts with all germ layers intact caused induction at a faster rate as soon after 10 minutes of contact then those which did not have endoderm seen after 20 minutes of contact.

At stage 6, all endoderm cells are known to have emigrated at the base of the Hensen's node this seem to be the reason for delayed action where no induction was seen even at 50 minutes of contact by the grafts with all germ layers intact. When endoderm was removed from such grafts their inducing capacity was observed to be greatly diminished with induction occurring at 2 hours 30 minutes of contact.

The role of chordal mesoderm is already well understood the role of embryonic endoderm in neural induction has also been explained by some workers (Waddington, 1932, 1933; Spratt and Haas 1960 a, b; Vakaet 1964, 1965, Eyal-Giladi and Wolk 1979, 1981)
who have established that embryonic endoderm has a definite role in the induction of the neural plate.

In chick embryo (see for review Hara, 1978; Khare and Choudhury, 1985) provided us with the insight that the stimulus for neural induction emanates from embryonic endoderm. The stimulus from the prechordal and chordal mesoderm is given out at second stage. Galler (1971) writes that "the first stimulus for the neural induction, then, would originate in the presumptive embryonic endoderm, and it is re-inforced later by the inductive stimulus from the chorda-mesoblast. In any event, once invaginated, the embryonic endoblast lose their inducing capacity (Galler and Nicolet, 1969)."

5.8 Role of endoderm in neural induction

In the present investigations it was observed that after removal of endoderm from the inducing graft at stage 3, 4 and 5 the inducing capacity to form other axial structure is reduced except the neural plate. This suggest that the endoderm might enhance the induction of those axial structures in the competent ectoderm. The role of the primary hypoblast in prosencephalic induction before the laying down of pre-chordal mesoderm has also been reported (Vakaet, 1964, 1965; Galler, 1971 and Eyal-Giladi, 1971) and morphogenetic changes during
neural induction have been better investigated with the help of SEM, TEM and transfilter techniques (for reference see Gallera, 1968; England, 1973; Eyal-Giladi, 1975; Rasilo and Leikola, 1976; England and Cowper, 1976).

The inducing role of hypoblast was discovered by Waddington as early as 1933. He showed that if it is excised and rotated by $180^\circ$, the orientation of the developing primitive streak is also changed. Similarly, the primary hypoblast was found to be capable of inducing a primitive streak in the competent epiblast when separated from it by a millicore filter (Eyal-Giladi and Wolk, 1970). The hypoblast was also found to support and stabilise the primitive streak during the initial steps of its formation (Eyal-Giladi, 1970; Azar and Eyal-Giladi, 1979). Recently, Azar and Eyal-Giladi (1981) investigated the interaction of epiblast and hypoblast in the formation of the primitive streak and embryonic axis and explained the dynamics of the inductiveness of the hypoblast and the competence of the epiblast.