ANALYTICAL INVESTIGATIONS ON THE NEURAL INDUCING CAPACITY OF THE PRIMITIVE STREAK OF THE CHICK *(Gallus domesticus)* EMBRYO WITH AND WITHOUT ENDODERM

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I certify that the thesis entitled, " Analytical investigations on the neural inducing capacity of the primitive streak of the chick (Gallus domesticus) embryo with and without endoderm ", submitted by Mrs Rupa Nylla Hooroo for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under the supervision of (Late) Prof. M.K.Khare. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph. D. degree. This work has not been submitted for any degree of any other University.

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Dedicated to the Memory

of

(Late) Professor Mahendra Kumar Khare

C O N T E N T S

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RUPA NYLLA HOOROO

Chapter 1

INTRODUCTION

1

I N T R O D U C T I O N

The chick embryo has been one of the favoured experimental materials among Developmental Biologists world over since ancient times. As seen by the naked eye its development was first described by Aristotle as early as in 342 B.C. but detailed embryological and histological investigations were carried out with the help of microscope only in late 19th. century (see for reference Lillie,1908; revised by Hamilton,1952; Romanoff,1960; Patten,1971 among others). Analytical apporach to morphogenetic study seems to have been started by Wilhelm His (1874) who pointed out that the chick blastoderm is an aggregate of organ forming areas. The morphogenetic nature of the primitive streak of the early chick embryo that it resembles amphibian blastopore was first described by Rauber in 1876. Experimental investigations to examine the casual relationships to development were started with the work of Waddington (1932) who discovered that the anterior part of the primitive streak of the chick embryo known as the Hensen's node acts as organizer, corresponding to the dorsal lip of blastopore in amphibia as described by Spemann in 1918.

The most important role of the primitive streak is one that may be essential in the control of the development in body patterning. The steady, forward growth of the primitive streak brings cells from the posterior end of the area pellucida to the middle of the blastoderm, whilst its gradual retreat spreads them out again along the axis. The formation of the primitive streak in the chick embryo coincides with the start of gastrulation. During gastrulation, the cells of the epiblast proceed to move from the anterior and pile up at its posterior end. The heap of cells so formed stretch anteriorly and gives rise to the primitive streak. The cells of this primitive streak rudiment subsequently pass downwards through the primitive streak and in the opposite directions to form the mesodermal and endodermal layers. When the primitive streak is fully formed at Hamburger and Hamilton (1951) stage 4, it has a primitive groove along its entire length bounded laterally by primitive folds. The anterior part of the primitive streak is slightly enlarged and is known as Hensen's node. The primitive streak is not a fixed structure, rather a morphogenetic event. It retreats and disappears as soon as all prospective organ forming areas of the epiblast destined to pass through it have done so.

There is strong evidence that cell movement in the epiblast leading to the formation of the primitive streak is induced by hypoblast (see for review Bellairs,1986). Jaffe and Stern (1982) have attempted to explain the morphogenetic movements in the chick blastoderm via electric coupling. They have explained that extra-cellular electric current pathways operate at this stage. Mitrani and Farverov (1982) however feel that as fibro-nectin appear between hypoblast and epiblast, it may have some role in bringing about the formation of the primitive streak. Once formed the primitive streak exhibits two major morphogenetic roles :
(1) Migration of cells through it to their respective destinations and (2) Embryonic induction. So far as embryonic induction is concerned Bellairs (1986) reviews that there are two major aspects of induction in relation to the primitive streak. The first is concerned with the induction of the streak itself and second, the induction of embryonic structures especially the neural plate. Several decades ago this aspect was considered of prime importance but it received relatively little attention. The phenomenon of embryonic induction was first observed in amphibians and most of the information now available on this phenomenon has

been acquired from this material. This work was stimulated by classical experiments on the amphibian organizer by Spemann (1918).

A direct analogy between the processes of primary induction in amphibians and birds was established during the thirties. Owing to technical difficulties definite proof of embryonic induction in the chick embryo was not obtained until 1932 when Waddington demonstrated that Hensen's node does indeed act as organizer in the chick embryo. He showed that if Hensen's node was extirpated, stripped free of adherent endoderm and implanted beneath the ectoderm at the side of the area pellucida of another embryo it could induce there a secondary embryonic axis. However, it soon became apparent that this ability was not restricted to Hensen's node but was present in throughout the anterior third of the primitive streak. It was therefore considered by most workers at this time that the anterior part of the primitive streak, possibly its entire length, correspond with the amphibian organizer. However, the most well established finding about the node is perhaps that it has greater power of bringing about an induction of the neural tissue than any other part of the primitive streak or indeed any other region of the blastoderm.

The dynamic picture of the process of neural induction and establishment of neural differentiation pattern in the chick embryo has been provided by Hara,1961; Rao,1968; Choudhury and Khare,1978 and Khare and Choudhury,1985.

Other workers, Abercrombie (1950) extirpated lengths of the primitive streak and grafted them back into the blastoderm in reversed orientation, so that the original anterior and Hensen's node now lay towards the posterior end of the area pellucida, care being taken to maintain the original dorso-ventral polarity of the graft. It might expected that if successful healing takes place the embryo would develop with its head directed towards the posterior end of the area pellucida as a whole, thus the fate and development of the whole primitive streak including the node be strongly influenced by the surrounding tissue. The influence of area pellucida upon the orientation of the graft is however restricted in its action in the antero-posterior organisation.

Mulherkar (1958) and Gallera (1964) showed that the neural inductions have been obtained also by grafts taken from behind the node or from

regions immediately lateral or anterior to it. Gallera (1964), Gallera and Nicolet (1969), Vakaet (1964) have shown also that the middle of primitive streak also causes neural induction. However, the middle of the primitive streak does not induce neural tissue directly, but it first induces an additional primitive streak which then gives rise to an embryonic axis (Gallera 1968). The grafts of the posterior third of the primitive streak have not been reported to cause neural induction in the host ectoderm except in one case reported by Waddington and Schmidt (1933).

The middle region of the primitive streak apparently has presumptive head mesenchyme and the last posterior third of the primitive streak lateral plate mesoderm only.

In review on primary induction in birds, Gallera (1971), on the basis of work done by Mulherkar (1958) and his own work summarised that the inducing capacity of the fragments derived from the streak diminishes in the antero-posterior direction and disappears completely posterior to the mid-point of the definitive streak. He referred to the work of Vakaet (1964, 1965) who demonstrated that the inducing capacity of the Hensen's node

changes during its development. At stage 3 and 4 the node induces brain structure while from stage 5 onwards it induces medullary structures.

Based on these and the work of Gallera and Nicolet (1969) and Nicolet (1970), Gallera (1971) felt that the induced brain structure in its own turn may influence the embryonic endoblast so that it differentiates into a typical foregut. Gallera and Nicolet (1969) wrote "the first stimulus for the neural induction, then, would originate in the presumptive embryonic endoblast, and it is later reinforced by inductive stimulus from chorda-mesoblast. In any event, once invaginated, the embryonic endoblast cells loose their inducing capacity." The decline in the neural inducing capacity of Hensen's node by stage 6 has been attributed to complete disappearance of endodermal cells from this region.

In the present work the following aspects have been investigated :

(1) Neural induction by the grafts of different parts of the primitive streak of stage 3, 4, 5 and 6 with all germ layers intact, without endoderm and without endoderm and mesoderm.

(2) Histological changes in the neurectoderm induced by the grafts of the Hensen's node at different time intervals of stage 4, 5 and 6 by the grafts with all germ layers intact and without endoderm.

(3) Histological changes in the normal neurectoderm at stages 3, 4, 5 and 6 and ultra-structural changes at stages 4, 5 and 6.

Though the process of induction has been well investigated and the dynamics of neural plate formation well established, very little attention has been paid to the quality of induced neural tissue. This work is a study on the cellular composition of neural tissue. The investigations described here would establish the quality of induced tissue in relation to the quality of the inductor tissue. The histological changes in the induced neural tissue, as development progresses, are studied in comparison with similar events during normal development. The results obtained in these investigations would provide new insights on the role of endoderm and endomesoderm not only in neural induction but also in neural differentiation.

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Neural induction and Spemann's Organizer in Birds

The phenomenon of embryonic induction was discovered in amphibians by Spemann (1918). He grafted a piece of the dorsal lip of blastopore of an early amphibian gastrula into the flank of another similar embryo at the same stage of development, and demonstrated formation of a secondary embryonic axis. Subsequently, Spemann and Mangold (1924) modified the experiment so that they were able to decide which of the tissues of this axis were formed from the host and which from the graft. This was done by taking the graft and the host from two separate amphibian species which differed from one another in their intracellular pigmentation. It was then possible to see which were host tissues and which were of the graft. They found that most of the neural tissues were formed from the host and that in general, there was a collaboration of host and graft tissues to form a unified axis. It became apparent that the graft had influenced the host tissues around it to form an embryo. Grafts taken from other parts of the donor embryo could not produce this result. Thus, Spemann concluded that the dorsal lip of blastopore possessed special

properties which enabled it to 'organize' the tissues around it to form an embryonic axis. As a result of these Classical experiments a great deal of interest was stimulated and a number of research workers started similar research on other animals.

Waddington (1932) demonstrated that chick blastoderm could be dissected away from the yolk and grown in vitro, in a watch glass with the technique of Fell and Robinson (1929). His experiments revealed that if the anterior end of the primitive streak (Hensen's node), stripped free of any adherent endoderm, implanted beneath the ectoderm at one side of the area pellucida of another embryo it induced a secondary embryonic axis. The results were comparable with that of a primary organizer of amphibians.

Waddington and Schmidt (1933) reported that an embryonic axis of chick could also be induced using grafts of the head process as well as of the sinus rhomboidalis (that is, the most posterior part of the neural plate), although the inducing capacity of these regions was not so great as that of Hensen's node. How neural inductive stimulus emanates from the pre-chordal and chordal mesoderm and how neural differentiation pattern is established in the neural plate has been

investigated by Hara (1961), Rao (1968), Choudhury & Khare (1978), Khare & Choudhury (1985).

Neural inductions have been obtained by grafts taken from behind the node or from regions immediately lateral or anterior to it (See Mulherkar, 1958; Gallera, 1966 among others). According to the prospective fate map of Pasteels (1937) these regions were probably presumptive somite and presumptive head mesenchyme. Similarly, the middle part of the primitive streak (Gallera, 1964; Vakaet, 1964; Gallera and Nicolet, 1969) and the head process mesoderm of embryos possessing not more than four pairs of somites (Gallera, 1965) were also found capable of bringing about neural induction when transplanted to the area opaca of a primitive streak stage host. Grafts taken from the middle part of the primitive streak, however, do not induce neural tissue directly; instead, they first induce an additional primitive streak in the host and this then give rise to an embryonic axis (Gallera, 1968). If some embryonic endoderm is formed by this new primitive streak it may play a role in inducing neural tissue (Gallera and Nicolet, 1969).

Attempts to obtain inductions by grafts taken from the posterior third of the primitive streak were less successful in early experiments (Waddington, 1932)

but one successful case was recorded by Waddington and Schmidt (1933). This region consists largely of presumptive lateral plate mesoderm overlain by ectoderm and is not normally capable of differentiating when isolated (Spratt, 1952; Butros, 1960). It was found that in birds, as in amphibians, simple neural placodes could be induced by a variety of substances, living, dead or inert (Waddington, 1938a; Abercrombie, 1939). Pasternak and McCallion (1962) have shown that neural inductions can be obtained in chick by implants of liver and kidney; Viswanath et al (1968) demonstrated that alcohol killed Hensen's node is able to induce various tissues from competent ectoderm.

England (1981) showed that the scanning electron microscopy (SEM) is a valuable tool for the analysis of morphogenetic events. The role of extracellular materials in primary neural induction in the early stage 5 chick embryo may be analysed by SEM as well as by histochemical techniques. During primary neural induction, extracellular materials in the early stage 5 chick embryo form a fan-shaped region on the ectoderm anterior to Hensen's node. Fibronectin and sulphated glycosaminoglycans are present anterior to Hensen's node on the ventral ectodermal layer. It is proposed that the fan-shape

of extracellular materials has a dual function; as a chemical substrate to form close contacts between the inducing cells and the target ectodermal cells, and also to serve as a contact guidance system for the pre-notochordal cells.

Smith, Dale and Slack (1985) studied cell lineage labels and region - specific markers in the analysis of inductive interactions. The work reviews cell lineage labels and cell type - specific markers in the analysis of inductive interactions in early amphibian development. The results provide clear evidence for the existence of three such interactions. Mesodermal induction occurs in the early blastula and results from the action of vegetal pole cells on the animal hemisphere. At least two mesodermal rudiments are formed, one dorsal and one ventral. During the next interaction, which we call dorsalization, the ventral mesodermal rudiment becomes subdivided into several territories under the influence of the dorsal marginal zone, or organizer. Finally, during gastrulation, the involuting organizer induces neural tissue from the overlying ectoderm. This interaction is called neural induction.

Although these phenomena can readily be demonstrated under experimental conditions, direct evidence that they occur in normal development awaits

an understanding of the molecular basis of induction.

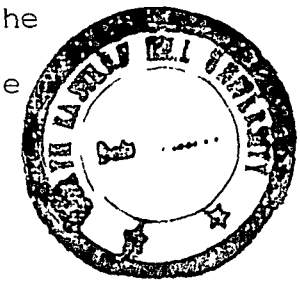
In the following review we shall deal mainly with contributions on the role of primitive streak in this connection.

2.2 Inducing capacity of the primitive streak and its derivatives

Following the work of Waddington and his collaborators, notably Abercrombie, Taylor and Schmidt during 1930-40, which established the capacity of the anterior part of the primitive streak to induce a neural structure in the ectoblast of both pellucid and opaque areas, the inducing capacity of the definitive streak was systematically analysed by Mulherkar (1958) and later reinvestigated by Gallera (1964). According to these authors, grafts of fragments derived from the anterior half of the definitive streak alone have the capacity to induce neural structure. Inducing capacity of the fragments derived from the streak diminishes in the antero-posterior direction and disappears completely posterior to the mid-point of the definitive streak.

Vakaet (1964) reported that the middle part of a medium primitive streak induced the formation of a secondary primitive streak if grafted to a long streak blastoderm, while the anterior part of such a streak induced secondary head formation.

According to Vakaet, the anterior third of the streak (stage 3) induced brain, and the middle third region induced a new primitive streak in the host ectoblast. The differences in the inducing capacity of these two regions were found to be of a quantitative nature only. Thus both of these fragments induce either a neural structure or a new primitive streak, but the frequency of neural induction is indeed higher for the anterior third fragment. Moreover, the frequency of primitive streak inductions decreases as function of the age of the host ectoblast; beyond stage 4, the host ectoblast is no longer competent for the induction of a primitive streak (Gallera and Nicolet, 1969). They also found that streak inducing capacity is present not only in the middle third but also in the anterior third of the streak more often gave rise to axial and paraxial mesoderm than the middle third.



There are a number of contributions which have revealed the importance of certain components of blastoderm such as hypoblast and embryonic endoderm in the inductive processes. It would be proper now to examine them.

Wakely and England (1977) studied the primitive streak by SEM of the chick embryo. The structure of the cells forming the primitive streak was examined by SEM in a series of embryos at Hamburger and Hamilton's stages 2-5. Specimens were prepared by stripping the

endoderm from fresh embryos in New Culture and by fracturing whole fixed embryos along and at right angles to the primitive streak. At all stages of examination the SEM appearance of cells within the primitive streak was quite different from that of ectodermal, endodermal or mesodermal cells away from the streak. Streak cells were closely packed, lay with their long axes directed from ectoderm to endoderm and possessed many flat leaf-like processes. By contrast the ectoderm formed a columnar epithelium, the endoderm a flat epithelium and the mesoderm was a layer of loosely arranged cells with long, thin processes.

Within the streak SEM did not show any differences between cells that could identify them specifically as future endoderm or mesoderm cells. It was concluded that during gastrulation all the cells migrating through the primitive streak have the same appearance regardless of their eventual destination in the embryo. This structure may be attributed to the type of movement made by cells during invagination.

Wakely and England (1979) studied the chick embryo late primitive streak and head process by Scanning electron microscopy. They observed that

changes in cell shape during the formation of the head process and regression of Hensen's node from stage 5 to stage 8. The endoderm was removed and the embryo mounted for New Culture such that the mesoderm could be viewed from ventral surface. As the primitive streak shortens its cells were seen to flatten and lose the flap-like processes characteristic of earlier stages. The notochord differentiates , and its cells become transversely orientated, and between stages 6 and 8 then re-align along the embryonic axis. Simultaneously, at the same time a split or cleft appears between notochord and head mesoderm, and extracellular fibrils are formed which are the first sign of the formation of a notochordal sheath.

Bellairs (1986) attempted to show the critical role played by the primitive streak in the early development of the embryonic axis. Its appearance follows the establishment of antero-posterior polarity and leads to the formation of bilateral symmetry. It is the centre of a range of morphogenetic cell migrations including convergence, ingression, regression and the elongation of the areapellucida. Ingression through the primitive streak is associated with a range of corelated changes within the cells, at the surfaces of the cells and in the extracellular materials. The cells

lose their epithelial arrangement whilst passing through the primitive streak but regain it when they become associated into tissues at the end of the process (e.g., as endoderm or somites). Grafts of the primitive streak specially its anterior end can induce neural plate from ectoderm.

The most important role of the primitive streak in the development is one that may be essential in the control of the development in body patterning. The steady forward growth of the primitive streak brings cells from the posterior end of the area pellucida to the middle of the blastoderm, whilst its gradual retreat spreads them out again along the axis.

2.3 Hypoblast

Several authors have studied the inductive action of the early hypoblast on the epiblast in the chick blastoderm (Waddington, 1932, 1933; Spratt and Haas, 1960a,b; Eyal-Giladi and Wolk, 1970; Azar and Eyal-Giladi, 1979,1981). These experiments were concerned with the study of the role of the hypoblast in the morphogenesis of the epiblast.

The chick blastoderm has been described to be an assymmetrical system by Rudnick (1932) and Rawles (1936, 1943). They found that at the head process stage grafts from the left side of the blastoderm

possessed a superior developmental capacity compared to those from the right side when transplanted onto the chorio allantois.

Waddington (1930, 1932, 1933) noticed long ago that the hypoblast has an epigenetic influence on the epiblast. Summarising his earlier work in The Epigenetics of birds (1952), he proposes that there is an inherent polarity in the hypoblast as a whole in the form of a gradient field, which gives it an inductive effect. This is responsible for the formation of embryos in conformity with the polarity of the hypoblast, following rotation of the hypoblast in relation to the epiblast.

Eyal-Giladi and Wolk (1970) studied the inducing capacities of the primary hypoblast as revealed by transfilter induction studies. The inducing powers of the primary hypoblast of the chick embryo were studied by the insertion of a TH millipore filter between the hypoblast and the reacting epiblast. Two successive inducing capacities were discovered in the hypoblast. The first to appear and disappear is the inductor of the primitive streak, while the second is a prosencephalic inductor. The formation of a mature primitive streak which contains a Hensen's node is dependent on direct cellular contact between the epiblast and

hypoblast, and most probably on the inclusion of hypoblastic cells in the forming node and notochord. The formation of spinal cord-like sturctures and undifferentiated neural plate can be related to the presence of nodeless primitive streaks in the blastoderms. These neural structures are probably induced by the mesoderm produced by the defective primitive streaks, which were unable to form notochord or somites. The primary hypoblast, by virtue of its prosencephalic inducing power, can be compared with the presumptive pharyngeal endoderm of the amphibian embryo.

Eyal-Giladi (1970) in a study of the differentiation potencies of the young chick blastoderm as revealed by different manipulations namely localised damage and hypoblast removal concluded that removal of hypoblast from a primitive streak stage blastoderm reduces its developmental potencies to the level of those of the unincubated stage. These experiments which were designed to test the embryo-forming potency of the blastoderm whose hypoblast has been removed, showed that the naked epiblast is at least as labile as an unincubated blastoderm. It was concluded that during the period of development of a blastoderm from the unincubated stage till the formation of a full length primitive

streak, the epiblast and its surrounding marginal zone neither undergo irreversible differentiation on the one hand and nor lose, even slightly, the potency for the induction of heterotopic embryo-forming centres on the other.

It is the influence of the polarised hypoblast which creates the environmental conditions promoting the formation of an embryo-forming centre at the posterior marginal zone. This effect is not limited to the period of the fountain - like movement of the hypoblast, but is exercised as a long term continuous influence. It is probably firstly concerned with the formation of the primitive streak, which again needs further influencing by the hypoblast for its own normal development.

Litke (1978) investigated the development and hypoblast formation in early chick embryo, (Stages 1-13) in eggs incubated from 0-52 hours by light microscopy, SEM and TEM, and supported the theory of delamination as a means of forming the hypoblast formation and prior to it, appendages (microvilli, blebs and ruffles) were numerous.

Sanders, Bellairs and Portch (1978) conducted in vivo and in vitro experiments on the hypoblast and definitive endoblast of avian embryos. They reported

that this was an unusual example of invasion of one tissue by another during gastrulation in the chick embryo when the definitive endoblast becomes inserted into the hypoblast.They also examined the two tissues morphologically by SEM and TEM. They resemble each other in being of an epithelial type, though neither posseses a basal lamina. The definitive endoblast cells are flatter than the hypoblast cells and more closely attached to one another. When they were explanted in hanging drop cultures, the two tissues were found to exhibit differences in their behaviour. In comparison with the definitive endoblast, the hypoblast cells attached more readily to the glass, produced larger ruffle membranes, moved more rapidly, showed poorer contact-inhibition of locomotion and showed a greater tendency to break away from the main explant.

When a hypoblast explant was confronted with a definitive endoblast explant, the hypoblast cells became displaced by the definitive endoblast. The hypoblast explant tended to fragment into smaller groups of cells, many of which migrated around the definitive endoblast, thus mimicking the situation in vivo. Control experiments comprised confronting hypoblast with hypoblast, hypoblast with somites, definitive endoblast with definitive

endoblast, and definitive endoblast with somites. The hypoblast explants behaved in a consistent manner, always fragmenting when coming into contact with cells from a confronting explant. The definitive endoblast explants showed more contact inhibition of locomotion when confronted with definitive endoblast or with somites than when confronted with hypoblast. It is suggested therefore that the ability of the hypoblast cells to separate from one another may play an important role in the penetration of the hypoblast by the definitive endoblast both *in vitro* and *in vivo*.

Azar and Eyal-Giladi (1979) showed that the removal of both area opaca and the marginal zone of the area pellucida from a blastoderm stripped free of its hypoblast prevents the regeneration of a normally functioning hypoblast. The cellular contribution of the marginal zone to the primary hypoblast is instrumental for the latter's capacity to induce a primitive streak.

By hypoblast rotation experiments, Azar and Eyal-Giladi (1981) studied the interaction of epiblast and hypoblast in the formation of the primitive streak and the embryonic axis. Three types of experiments were performed to determine the interaction between the epiblast and hypoblast for

primitive streak formation : (1) Hypoblast of blastoderms from stages XIII E.G & K to 3 H & H were separated from the epiblast and rotated by 90° counter clockwise. (2) hypoblasts from stages XIII E.G. & K to 3 H & H blastoderms were rotated by 180°; (3) hypoblasts were exchanged between blastoderms of different developmental stages and placed at 90° counter clock-wise to the axis to the recipient epiblast. In all blastoderm studied only a single PS developed. After rotation of the hypoblast by 90°, the direction of the PS was according to the orientation of the hypoblast at stage XIII, whereas at older stages it gradually shifted towards the axis of the epiblast. At stage 3 H & H the PS is already imprinted in the epiblast and cannot be shifted. After rotation of the hypoblast by 180° the PS originated at the point near the marginal zone at which the inductive part of the hypoblast interacted with a competent epiblast. Conclusions were drawn about the dynamics of the inductiveness of the hypoblast and the competence of the epiblast in the formation of the primitive streak and its orientation.

Mitrani and Eyal-Giladi (1981) reported that the hypoblastic cells can form a disk inducing an

embryonic axis in chick epiblast. The primitive streak of the chick embryo develops from one of the two layers of cells of stage XIII blastoderm, the epiblast. The other layer of cells, the hypoblast, seems to be necessary for the induction of the primitive streak and also determines its orientation. Rotation of the hypoblast by 90° is followed by a similar rotation of the embryonic axis. Cells of stage XIII hypoblast, showed that two functions, induction and orientation are independent and that, with reconstituted hypoblast, the orientation of the primitive streak is determined by the epiblast.

With such understanding on the role of hypoblast, increasing attention has been paid on its morphology, development and in inductive action.

Weinberger, and Brick (1982) studied on the development of primary hypoblast in chick by SEM indicated that the primary hypoblast forms beneath the area pellucida during the first 8 hours of incubation mainly by establishment of contact among cells which move downward from the epiblast. This movement or polyingression, begins posteriorly and continues antero-laterally. Polyingression produced many pits and possibly a crescentic fold in the embryo upper surface. Fixation in situ helps

to prevent formation of artifactual folds and wrinkles facilitating interpretation of the SEM images. Formation of intercellular adhesions lead to development of primary hypoblast from posterior to anterior direction. This epithelialization begins with the elaboration of numerous filamentous processes by cells as they arrive from the epiblast and continue with ongoing input of cells, merging of cells into cell clusters and cell flattening. Proliferation of ingressing cells provides additional cells for hypoblast development.

Shimoni
Mitrani, and Eyal-Giladi (1983) studied the nature of the influence of the hypoblast on the early chick embryo epiblast. The chick blastoderm at stage XIII when deprived of the marginal zone, the area opaca and the posterior half of the hypoblast, and incubated further, developed axes whose orientation in 50% of the cases, was according to the original blastoderms orientation, whereas in the other 50% cases, the embryonic axis developed at 90° to the posterior side. These results illustrate the quantitative differences in inductivity between the anterior and the posterior halves of the hypoblast. The posterior region has the greatest effect but other regions can also bring about the development of an embryonic axis if allowed to act upon the

epiblast for a sufficiently long time.

Mitrani and Eyal-Giladi (1984) studied the differentiation of dissociated and reconstituted chick epiblast under the influence of a normal hypoblast. Cell suspensions of chick epiblast cells cultured under defined conditions form a flat disk which differentiates and generates axial embryonic structures, when covered with a primary hypoblast. Macroscopically identifiable axes developed in 25 to 33 cases. In all cases axes developed in a direction consistent with the postero-anterior polarity of the normal hypoblast. Almost invariably, the epiblast cells differentiate into ectoderm, neural plate or tubes and endoderm. In some cases typical primitive streaks were found sometimes accompanied by signs of axial mesoderm, in other cases the primitive streak seemed to regress. In the absence of a hypoblast no differentiation of neural tissue or any signs of axial development were observed.

Similarly, Mitrani (1984) studied the mitosis in the formation and function of the primary hypoblast of the chick. A normal stage XIII hypoblast ~~is~~ submitted to an X-ray dose of 6000 rad (sufficient to stop cell division), appears to retain its

capacity to induce axis formation in the epiblast. Examination of the process of hypoblast formation indicates an agreement with previous findings, that a hypoblastic layer can form in the absence of cell division and apparently can induce the development of an embryonic axis when combined with a normal stage XIII epiblast.

Azar and Eyal-Giladi (1983) observed that the retention of primary hypoblastic cells underneath the developing primitive streak allows for their prolonged inductive influence. An experimental study was made of the distribution of the primary hypoblastic cells in the lower layer of the avian blastoderm throughout primitive streak formation and until stage 10 (Hamburger and Hamilton, 1951). The primary hypoblast of stage XIII (Eyal-Giladi and Kochav, 1976) chick blastoderm was exchange for either an [H^3] thymidine-labelled similar chick hypoblast, or a quail primary hypoblast. During the entire period of primitive streak formation, the lower proved to be a mosaic of labelled hypoblastic and non-labelled entodermal cells (Chick cells of epiblastic origin). The persistence of hypoblastic cells underneath the developing primitive streak is regarded as a possible way to prolong the inductive influence of the hypoblast upon the forming streak.

2.4. Embryonic endoderm

Gallera and Nicolet (1969) while working on the inducing capacity of the primitive streak observed that the inducing capacity is present not only in the anterior third but also in the middle third of the medium - primitive streak. Their experiments with autoradiographic techniques revealed that the anterior third of the streak more often gave rise to axial and para-axial mesoderm than its middle third. However, regardless of the origin of the graft, if the graft contributed exclusively to endoderm formation either a primitive streak or neural structures were formed, whereas only neural structures were induced if marked graft cells were found both in the endoderm and the mesoderm. Since isolated pieces of the endodermal layer of the medium to long streak blastoderm do not show any inductive effect on host ectoderm, the authors concluded that the neural or streak inductive capacity of the embryonic endoderm is retained only during invagination, and that the secondary neural structures observed in the former case must have been formed by inductive influence emanating directly from the prospective embryonic endoderm in the graft.

The authors feel that induction occurs possibly in two steps. First, the grafted streak induces the formation of a secondary streak, as a result of which new endo-mesodermal cells of host origin invaginate (in some cases intermingled with graft cells); and subsequently the invaginated host mesoderm induces neural structure.

The autoradiographic study of Nicolet (1970), using [^3H] thymidine as a marker, revealed the changes in prospective significance of Hensen's node and other parts of the primitive streak during the short streak to head process stages. He discovered that the anterior end of the streak of the short streak blastoderm consists almost exclusively of prospective endodermal cells. As the stage advances the percentage of prospective endodermal cells decreases while conversely that of the prospective mesodermal cells increases. At the definitive primitive streak stage, Hensen's node contains prospective cells for endoderm (60%), mesoderm (35%) and ectoderm (5%). The mesodermal component of the primitive streak at this stage comprises (roughly from front to back) prospective cells for prechordal plate, notochord, head mesenchyme, somite, heart, lateral plate, and extra-embryonic mesoderm. At the head process stage, the

anterior streak (exclusive of the node) contains prospective somite cells and the more posterior streak prospective lateral plate cells.

Similarly, based on cinematographic study in combination with carbon marking Vakaet (1970) visualised the dynamics of the centrifugal spreading of the embryonic endoderm (tertiary hypoblast) with Hensen's node at its centre during the latter half of the primitive streak formation, and supported the idea of the epiblastic origin of the embryonic endoderm. It also showed that the initial layer (the primary hypoblast) is pushed forward by the secondary hypoblast spreading from 'Koller's Sickle,' and subsequently by the centrifugally expanding embryonic endoderm (the tertiary hypoblast), until it finally comes to lie in the extra-embryonic germinal cresent area. It was observed that the spreading of the embryonic endoderm with Hensen's node as its centre starts at the medium streak stage, when groove formation in the streak begins.

Rosenquist (1972) studied endoderm movements in the chick embryo between the early short streak and head process stages. Endoderm movements in the chick embryo between the early short streak and head process stages were studied by tracing the migration of tritiated thymidine-labeled grafts excised from

explanted donor embryos and transplanted to identical positions in explanted recipient embryos of the same stage.

During the primitive streak stages, the endoderm layer migrates in an orderly fashion away from a centre of the primitive streak which is located about one fourth to one third of the distance from its anterior to its posterior end; all endoderm grafts placed anterior, lateral or posterior to this centre migrated respectively anteriorly, laterally and posteriorly toward the outer margin of the area pellucida. During this migration, the grafts became narrowed and stretched out to conform with the rounded circumference of the area pellucida. Endoderm cells located in the endoderm centre itself never completely left the streak, but spread out in all directions until they occupied a central circular area destined to form the gut.

Veini and Hara (1975) using the intracoelomic technique, demonstrated changes in the differentiation tendencies of the hypoblast - free Hensen's node during the stages from medium streak to head fold. Endodermal differentiation tendencies (various gut and gland structures) gradually decreased from the medium streak to the pre-head process stage and

completely disappeared at the head process stage, whereas controls (hypoblast not removed) gave rise to endodermal structures throughout all stages. There was a constant high incidence of notochord, muscle and cartilage formation. The incidence of mesonephric structures, sometimes accompanied by adrenal gland, rose steadily throughout all stages both in experimental and controls. Neural differentiation tendencies (rhombencephalon and/or spinal cord) were always present in the nodes isolated, with or without hypoblast, from the definitive primitive streak stage onwards, but in nodes from earlier stages the incidence of neural differentiation was significantly lower.

England and Wakely (1977) using SEM followed the development of the mesoderm layer in chick embryos from stage 3 to stage 5. It was revealed that the secretion of basement membrane by the ectoderm preceeded the arrival of mesodermal cells. Ectodermal cells alone can synthesize basement membrane without mesodermal contribution.

England and Wakely (1978) investigated by SEM that a new endoderm was formed in situ. The area of regenerated endoderm coincides with the mesoderm area at the time of endoderm removal, confirming the mesodermal origin of the new layer. Remnants of

the original endoderm did not contribute to the regenerated layer and contact inhibition was observed at the boundary between original and regenerated endoderm.

Solursh and Revel (1978) showed by SEM that epiblast cells become progressively elongated in the primitive streak region until flask cells predominate medially. The flask cells have a broad basal end directed toward the endoderm. In addition to fine filopodia, broad lamellipodia are found anchoring the flask cells to subjacent cells. The primary mesenchyme cells are at first round in shape and closely packed, but laterally are flattened and more dispersed. The mesenchyme cells are associated with each other by filopodia and lamellipodia and with the epiblast and endoderm by filopodia. It is suggested that cell movement through the primitive streak occurs by cell extension, attachment by basal lamellipodia and cell shortening that results in the movement of individual cells in a cell stream.

Stern and Ireland (1981) conducted an integrated experimental study of endoderm formation in avian embryo by microsurgery, time lapse filming, use of chick- quails chimeras, tritiated thymidine autoradiography and a novel technique for identifying the morphology of the cells after small pieces of

tissue from known areas have been maintained in the culture for 24 hours. These techniques confirmed that the ventral layer of the early chick embryo receives contributions from both marginal and the central region of the area pellucida.

Vanroelen, Verplanken and Vakaet (1982) studied the effects of partial hypoblast removal on the cell morphology of the epiblast in the chick blastoderm. The experiment was performed at the early primitive streak stage chick blastoderm where the hypoblast was partially removed. This provokes a reaction in the epiblast which curls up and becomes even at its ventral surface. The basal lamina underlying the epiblast is also dependent upon the presence of hypoblast. During culture after partial hypoblast removal, active hypoblast wound healing is observed. Where the hypoblast underlines the epiblast again, the effects of the removal disappear and normal development proceeds. The results suggests that the normal epiblast morphology is dependent upon the presence of hypoblast. This influence of hypoblast on epiblast is thought to be concerned with the morphology of the epiblast and not directly with its morphogenesis.

Ishiziya - Oka Atsuko (1983) investigated the self-differentiation potency of the endoderm of the chick embryo mainly by TEM. The self-differentiation potencies of the upper and lower layers of blastoderm of Hamburger and Hamilton (H & H) stage 1-5 were investigated. Both stomach type and small intestinal type epithelial cells developed only when fragments of the lower layer were isolated from the blastoderms. When embryos older than stage 3 were cultured the results suggest that cells possessing the potency to differentiate into the stomach and small intestine type epithelia exist in the definitive endoderm at the beginning of its formation.

While inducing a new primitive streak, the invaginated cells mix with those of the grafted material and make the recognition of the fate of the graft impossible. In order to overcome this difficulty, grafts labelled with thymidine - ^{3}H were employed (Gallera and Nicolet, 1969). The autoradiographic analysis provided results falling into two distinct groups. In the first, the graft material gave rise to embryonic endoblast, axial and para-axial mesoblast, and it induced a neural structure in the host ectoblast. In the second group, labelled cells were found only in the embryonic

endoblast, and induction of either a neural structure of a new primitive streak was observed in the host ectoblast. It seems necessary to indicate that the induced brain structure, in its own turn, may influence the invaginated embryonic endoblast so that it differentiates into a typical fore-gut. The results suggest that the presumptive embryonic endoblast cells exercise a neural inductive influence while they are still in the streak. This suggestion is more relevant when one considers the fact that a major portion of cells in Hensen's node or in a corresponding region between stage 2 to 4 are presumptive embryonic endoblast cells (Nicolet, 1970). The first 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, Gallera and Nicolet (1969) felt that once invaginated the embryonic endoblast cells lose their inducing capacity.

Grunz and Tacke (1986) studied the inducing capacity of the vegetal hemisphere of early amphibian blastulae by placing a nuclear pore filter (pore size 0.4 μm) between isolated presumptive endoderm and animal (ectodermal) caps. The inducing effect was known to traverse the nucleopore

membrane. The reacting ectoderm differentiated into mainly ventral mesodermal derivatives. Explants consisting of five animal caps also formed dorsal mesodermal and neural structures. Those results together with data published elsewhere indicate that in addition to a vegetalizing factor, different mesodermal factors must be taken into consideration for the induction of either the ventral or the dorsal mesodermal derivatives. The neural structures are thought to be induced by the primarily induced dorsal mesodermal tissue. Electron microscopic (TEM) examination did not reveal any cell processes in the pores of the filter, the results indicate that factors other rather than signals via cytoplasmic or gap junctions are responsible for the mesodermal induction of ectodermal cells. The data support the view that mesoderm is determined by the transfer of inducing factors from vegetal blastomeres cells of the marginal zone (presumptive mesodermal cells).

2.5 Changes in the induced ectoderm

England (1974) as a result of her study of Cytoplasmic changes in primary neural induction, suggested that the primary neural induction in the chick embryo was associated with cellular communication between ectoderm and mesoderm cells.

Furthermore, the cytoplasmic changes in the mesoderm emphasize the close interaction between ectoderm and mesoderm during induction.

Rasilo and Laikola (1976) studied the neural induction by previously induced epiblast in avian embryo *in vitro* and showed that pieces of previously neurally induced and competent epiblast respectively of chick and Quail primitive streak blastoderms were cultured in close contact with each other for 48 hours. In several cases, both pieces differentiated in the neural direction, indicating the occurrence of a homoiogenetic induction. There was considerable mixing of cells of different origin, especially in the undifferentiated controls. In general, the dorsoventral orientation of the previously induced epiblast was retained, but the orientation of the competent epiblast cells was more flexible and could be influenced by the neighbouring neuralised cells.

Toivonen and Wartiovaara (1976) studied the mechanism of cells interaction during primary embryonic induction by transfilter experiments. The Transmission mechanisms operative at different stages of neuralisation during primary embryonic induction of the newt *Triturus vulgaris* were studied in experiments employing nucleopore filters placed between interactive tissue explants. The transmission

time of the neuralising effect was determined with 0.2 µm nucleopore filter. In another series of experiments the transformation of neuralised ectoderm by archenteron roof mesoderm into other parts of the CNS was studied. Although sufficiently long induction times was used no transformation into hindbrain structures could be induced across filters with pore sizes from 0.1 µm to 1.0 µm. However, electron microscope demonstrated cytoplasmic penetration into 0.6 µm filters at 15 hours of induction. The result speaks against free long range diffusion of inductive material at the stage of transformation of the neuralised ectoderm to more caudal parts of CSN and warrant a more detailed structural study of the transmission phenomenon in question.

England and Cowper (1976) studied primary neural induction using transmission and scanning electron microscopy. A single mesoderm cell is usually in contact with several ectoderm cells. The mesoderm cells are also contacting other mesodermal cells. It is suggested that ectodermal cells are induced in groups and that induction is synchronised by these contacts. At the points of contact between mesoderm and ectoderm cells cytoplasmic changes are present in the induced tissue.

It has been often suggested that changes in the relative adhesiveness of cells may play an important role in embryonic differentiation (e.g. Townes and Holtfrater, 1955; Curtis, 1967 Ede and Agerbak, 1968 Steinberg, 1970; Johnson, 1970). There are however curiously few attempts to investigate whether or not changes in adhesiveness take place in the cells of a particular tissue as it differentiates. Several authors have proposed that changes of this type are a major factor in the segmentation of somites (Waddington, 1956; Zeeman, 1971) and also during the formation of the neural plate (Brown, Hamburger and Schmidt 1941). Bellairs, Curtis and Sanders (1978) studied cell adhesiveness and embryonic differentiation. The investigation was to decide whether cell to cell adhesiveness took place during embryonic differentiation. The technique of Curtis (1969) was used to measure the adhesive behaviour of several types of ectodermal, neural and mesodermal cells of the chick embryo at stages 7 and 12 of differentiation. Cells dissected from segmented mesoderm were found to be more adhesive than cells from unsegmented mesoderm. Cells from both ectoderm and neural tissue became more adhesive between stages 7 and 12. It is concluded that an increase in adhesiveness may play a role in somite segmentation but not neural tube formation.

Bellairs, Sanders and Portch (1978) carried out *in vitro* studies on the development of neural and ectodermal cells from young chick embryos. A brief account is given of morphological and fine structural changes in these tissues at Hamburger and Hamilton stages 4, 7 and 12. The behavioural differences have been studied by taking neural and ectodermal tissues from the same three stages of embryos and growing them *in vitro*. They were explanted either as sheets or as semi-dissociated cells and were grown as hanging drops on collagen coated glass or as sitting drops on plastic. The behaviour of the cells was studied by time - lapse cinematography. It was found that the proportion of cells that settled on the substrate was higher with the ectodermal than with the neural explants. During the process of settling on the substrate they underwent vigorous blebbing. When they were well attached they became spindle - shaped in appearance. Finally, if they made contact with other cells they usually spread out on the substrate and lost their spindle - shaped appearance. It was found that these cells possessed many of the characteristics which are typical of other types of epithelial cells *in vitro*, though they also exhibited certain characteristics of their own. The neural cells from the stage 12

embryos differed from those of the other tissues in that they seldom form a sheet but remained fibroblast-like in appearance. They had difficulty in making firm adhesions to other cells and tended to migrate over or under one another with few signs of contact inhibition of locomotion. The relevance of these results is discussed in relation to their behaviour and morphology in their normal environment in the embryo.

2.6 The chemical nature of inductors

Work on the chemical basis of induction has been reviewed by Toivonen (1967), Teilemann (1967 a,b) and Yamada (1967). Formerly, it was assumed that a single chemical was involved in induction but this now appears unlikely, for different results are brought about by different types of chemical inductor.

Another current idea is that some component of yolk may also not act as an inducing agent, at least in amphibia (Flickinger, 1961; Brachet, 1967; Yamada, 1967). This concept is based largely on the finding of nucleic acids in yolk.

Experiments by Sherbet (1963) and Sherbet and Mulherkar (1963, 1965) reminds us that follicle stimulating hormone is capable of calling forth inductive capacity in chick blastoderms. Earlier Abercrombie (1937) demonstrated, that the chick

resembles the amphibian embryo in responding to a wide variety of inductive agents.

Nicolet (1965 b) who treated young chick embryo *in vitro* with lithium chloride concluded that the main effects were brought by changes affecting the morphogenetic movements and the mitotic rhythm.

According to Sherbet and Mulherkar (1965) isolated fragments taken from the posterior part of the primitive streak when treated with FSH acquire a certain capacity to induce neural structures.

Lakshmi (1962) found that under the influence of increasing doses of chloroacetophenone, Hensen's node progressively loses its inducing capacity.

Diwan (1966) observed that a similar effect is exercised by colchicine which however does not affect the neural competence of the host ectoblast. Inhibition of the inducing capacity of these drugs is probably due to a blockage of - SH (- thiol) groups on sulphydryl proteins (Lakshmi, 1962; Diwan, 1966) which play an important role in the primary induction (Brachet, 1960, 1964).

Lakshmi (1962) and Diwan (1966) could show that the action of these inhibitors could be reversed by the addition of cysteine to the culture

medium at an appropriate moment. They also remarked that an inhibition of the graft differentiation is not the factor responsible for the loss of its inducing capacity.

According to Waheed and Mulherkar (1967), isolated fragments taken from the posterior part of the primitive streak, when treated with cysteine, acquire a certain capacity to induce neural structures.

Gallera (1970) incubated chick blastoderm in physiological saline solution containing actinomycin D. He found that although the development of the host axis was delayed the reacting tissue was still able to respond (by producing a neural plate) if the Hensen's node from a normal, untreated donor embryo was grafted into it.

Zagris and Eyal-Giladi (1982) studied the effect of 5 - bromodexyuridine (Bud-R) inhibition of the epiblast competence for primitive streak formation in chick blastoderm stage XIII. Bud-R treated epiblast form a typical primitive streak and no axial mesoderm. A non-organised mesenchymal layer is formed between the epiblast and the hypoblast and a typical neural tissue in the epiblast. Bud-R interferes neither with hypoblast formation nor with its inductivity even when blastoderms are treated as early as uterine Stage VIII and later.

Penner and Brick (1984) studied the effect of acetylcholinesterase on polyingression in the epiblast of the primitive streak chick embryo. Cholinesterase histochemistry SEM were performed on whole chick blastoderms, stage 4, Hamburger-Hamilton, to study the relationship between acetylcholinesterase (ACHE) and cell movement in the epiblast.

Grunz (1985) studied in information transfer during embryonic induction in amphibians. Neural induction and differentiation has been studied using Concanavalin A, Cylic AMP, tunicamycin and calcium innophone A 23187. Competent ectoderm of Xenopus leavis treated with Concanavalin A differentiates into neural (archencephalic) structures. Binding studies with gold-labelled Con A indicate that the superficial ectodermal layer contains fewer Con A - sensitive sites (α - D-mannoside and α - D-glucoside residues of glycoproteins) than the inner ectodermal layer. The small number of Con-A sensitive sites can be correlated with the fact that the isolated superficial ectoderm layer, in contrast to the inner layer does not differentiate into neural structures. The gold Con-A particles bound to inner ectoderm are quickly (within 30 minutes) internalized presumably by

receptor-mediated endocytosis. However, endocytosis is not a pre-requisite for neural induction. On the contrary, Con A apparently must be bound to the plasma membrane for a certain period to initiate neural induction. The rapid internalization of Con A could explain why neural inductions are evoked only if ectoderm is incubated in Con A containing medium for longer than 30 minutes.

On the other hand cyclic AMP or calcium ionophone A 23187 does not elicit neural inductions. Calcium ionophone A 23187 apparently inhibits neural and mesodermal differentiation. These effects could be correlated with an increase of intracellular calcium level of the ectodermal target cells, which could influence the permeability of gap junctions resulting in a loss of cell communication followed by a change of differentiation and pattern formation.

Duprat, Kan, Gualandris, Foulquier, Marty (1935) observed that during neural induction embryonic determination elicits full expression of specific neuronal traits. In Pleurodeles waltl the early neuronal differentiation of precursor cells from late gastrula stage has been studied by culture in vitro from either isolated neural plate (NP) or isolated neural fold (NF). The aim of this study

was to delineate the information acquired by ectodermal target cells during neural induction. By culturing these cells *in vitro* either with or without the underlying chordamesoderm, they showed that in the absence of chordamesodermal influence such NP or NF cells exhibited a high degree of biochemical and morphological differentiation as revealed by the synthesis and the storage of neurotransmitters, the activity of specific enzymes, as well as by the expression of neuronal markers; specific changes in cell surface carbohydrates, tetanus toxin binding sites and neurofilament polypeptides. Remarkable changes in the cell adhesive properties were the first events observed in the different central (NP) and peripheral (NF) types.

In cocultures the chordamesodermal cells exert a beneficial influence on this differentiation, specially increasing acetylcholine synthesis. There are some differences between central (NP) or peripheral (NF) neuroblast response to this further notochord or mesodermal influence.

Chapter 3

MATERIAL AND METHODS

MATERIAL AND METHODS

3.1 Incubation of eggs and sterilization measures

Mixed breed (white and red leghorn) hatchable hens' eggs were obtained from the All-weather Government Poultry farm at Nayabungalow near Shillong. They were stored below 14°C and incubated in forced air drift type NSW incubator for a desired period at 37.5°C (±1°C) and 60% - 70% humidity. The operation table was sterilised with 70% ethanol before starting the operations. The sterilized solutions, Corning glasswares and steel instruments were exposed to a UV germicidal lamp as an additional sterilizing measure before starting each operation. The operations were carried out near a table lamp fitted with a 60 watt bulb so that a temperature of around 30°C (±1°C) was achieved around the lamp on the operation table.

3.2 Preparation of solutions and agar bases

For solution, BDH (Analar grade) and E Merck (GR grade) chemicals were used. The following three solutions were used for operation work.

1. Normal Locke Solution

Nacl	M/6.2	-	94.5 ml
Kcl	M/6.2	-	3.5 ml
$CaCl_2$	M/9.3	-	2.0 ml
Glucose		-	0.2 g
$NaHCo_3$		-	0.02g(for buffering to maintain pH ± 7.4)

2) Ca - Free Locke Solution

Nacl	M/6.2	- 96.4 ml
Kcl	M/6.2	- 3.6 ml
Glucose		- 0.20 g

(non-buffered ± 6,0)

3) Normal Saline - 0.9%

The solutions were boiled and after boiling $NaHCO_3$ was added to normal Locke Solution to maintain pH ± 7.4 and the containers were covered with aluminium foils to keep them in sterilized condition.

2% solutions of agar in normal saline was sterilized in a pressure cooker for 10 minutes and poured into Petridishes. When cooled and set, the agar was cut into rectangular pieces and stored in normal saline in the refrigerator.When required, the desired number of pieces were melted and poured into flame sterilised watch glasses and allowed to set for 24 hours.

3.3 Separation of germ layers of the donor embryos

The germ layers of the donor blastoderms were separated and grafts were prepared on the agar bases in the watch glasses using tungsten needles (Tindall, 1960) and under a Leitz Stereoscopic Binocular Microscope with reflected beam of light in a dark field (Figs. 3.3a and 3.3b). The

Fig. 3.3a Diagramatic section through the operation dish. The main light beam is indicated by arrows.

Fig. 3.3b Separation of the Endomesodermal layer from the ectoderm. The figure shows a sagittal section at the level of primitive streak.

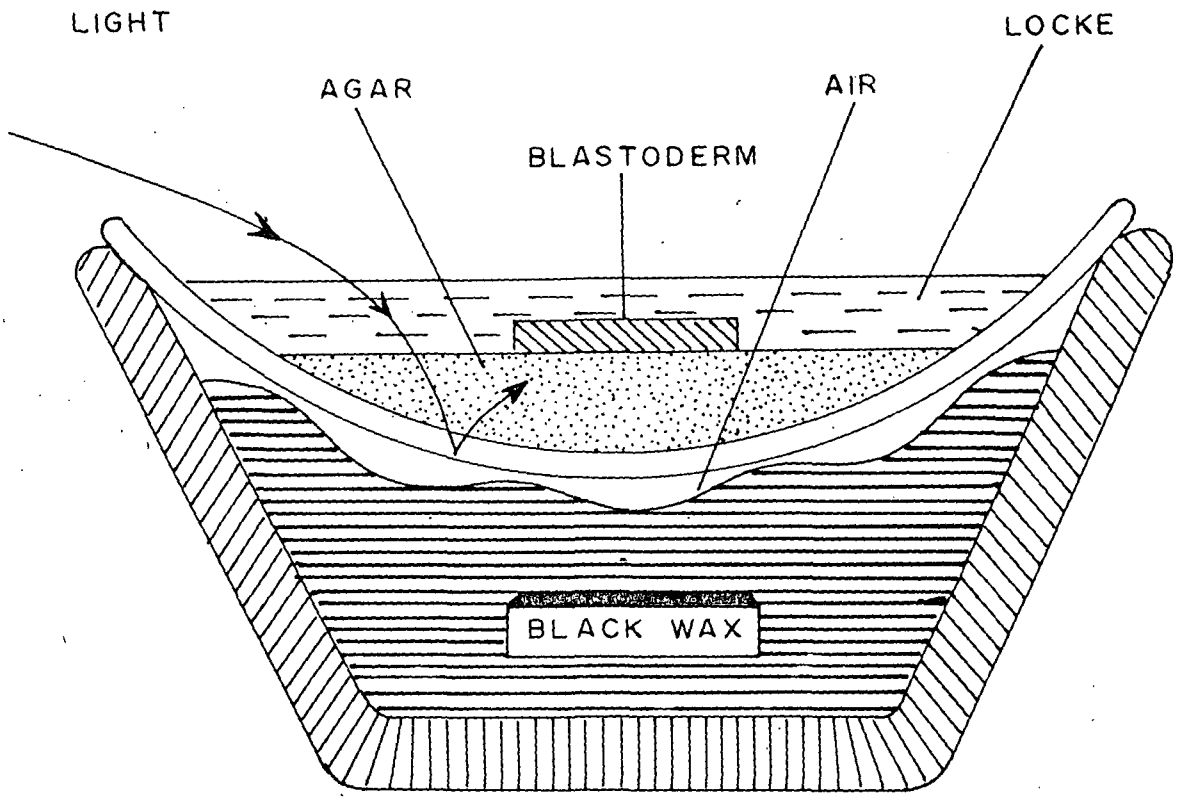


FIG. 3·3a

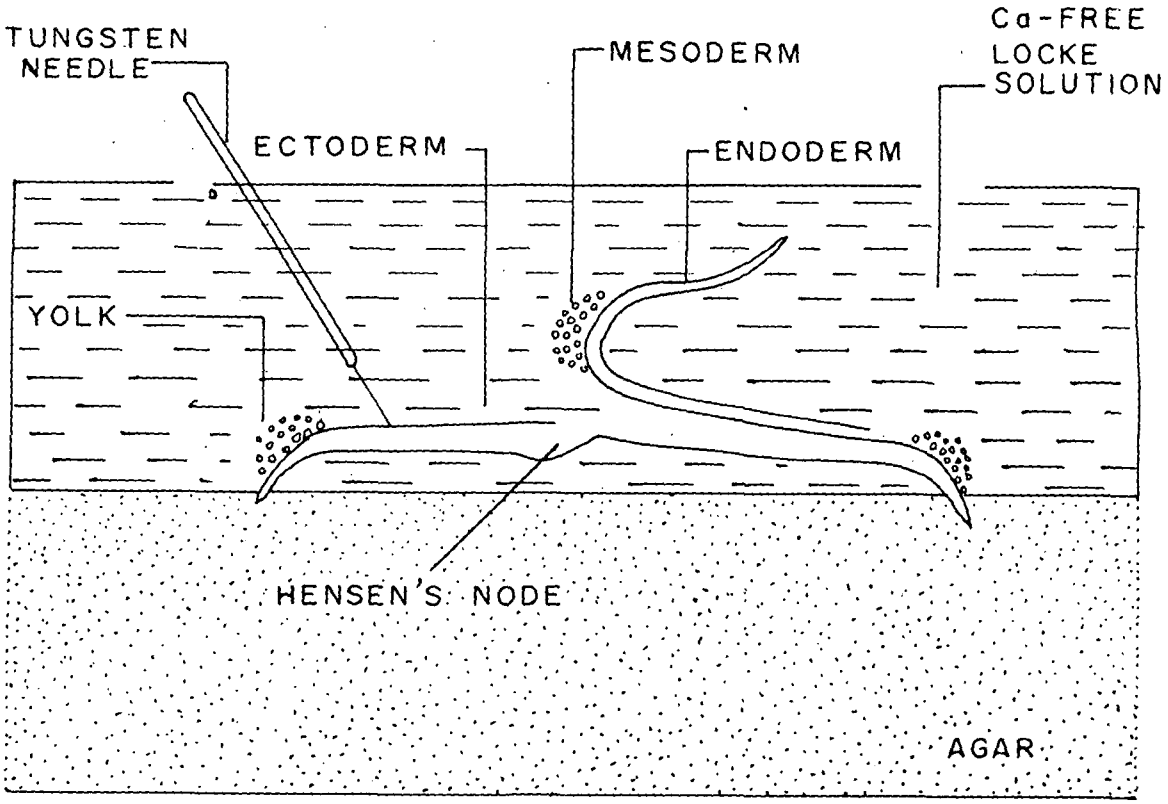


FIG. 3·3b

instruments were sterilised after each use by passing them through 70% ethanol, boiling water and cold sterile water.

3.4 Technique for culturing the grafts in the host embryos

The grafts were cultured according to New's technique as described below :

In the method of New (1955) the explanted ectoderm is placed face downwards on a piece of vitelline membrane stretched across a glass ring in a watch glass. The watch glasses were placed in Petridishes on a Wad of cotton wool soaked in sterile water to maintain humidity (Figs. 3.4a, 3.4b, 3.4c and 3.4d).

3.5 Preparation and implantation of grafts

The graft pieces A, B, C and D were isolated from the primitive streak region of the chick embryos at Hamburger and Hamilton (1951) stages 3, 4, 5 and 6.

Three types of grafts were prepared and implanted :

(1) Graft pieces with all the germ layers intact at stages 3,4, 5 and 6. They have been designated as EcMEn grafts.

(2) Graft pieces at stages 3, 4 and 5 without endoderm designated as EcM grafts.

(3) Graft pieces at stages 3, 4 and 5 without endoderm as well as mesoderm designated as Ec grafts.

The separation of germ layers and isolation of grafts were carried out with tungsten needles in Ca^{++} free Locke's solution in dark field background. The grafts were implanted below the ectoderm of the host embryo nearing stage 4 in the antero-lateral margin of the area pellucida as shown in Fig.3.5a and 3.5b . The host blastoderm with implants were incubated for a maximum of 24 hours.

3.6 Analysis of induction

After 24 hours of incubation the host embryos (with the grafts) were fixed in Bouin's fluid. The induced structures were examined morphologically and then histologically.For morphological analysis the embryos were stained with borax carmine. For histological analysis the embryos were passed through graded series of ethanol and butanol, embedded in paraffin wax, sectioned at 8 µm and stained by the Azan method.

Histological structures differentiated under the inductive influence were neural tube, neural plate, notochord and somite. Based on the presence, absence and extent of differentiation of

Fig. 3.4a Explanting the chick blastoderm for culturing on vitelline membrane.

Fig. 3.4b Contents of watch glass after removal from dish saline.

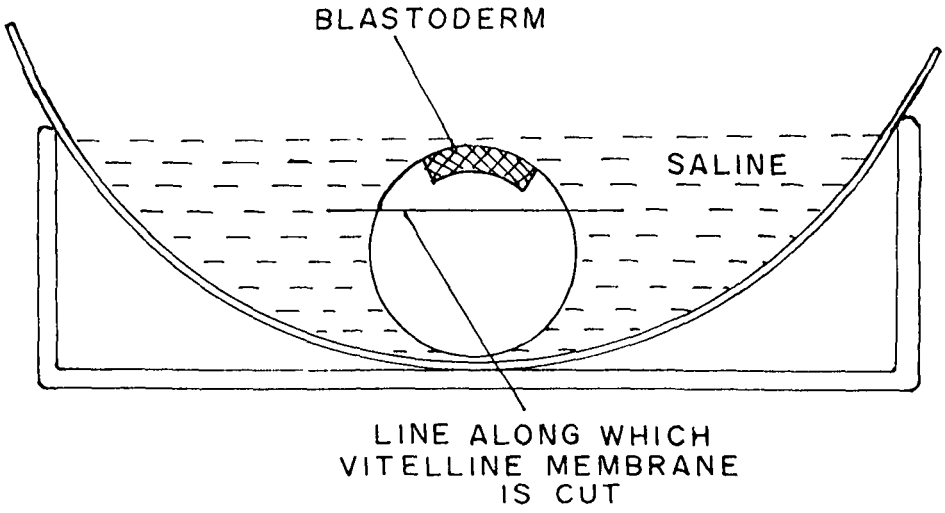


FIG. 3·4a

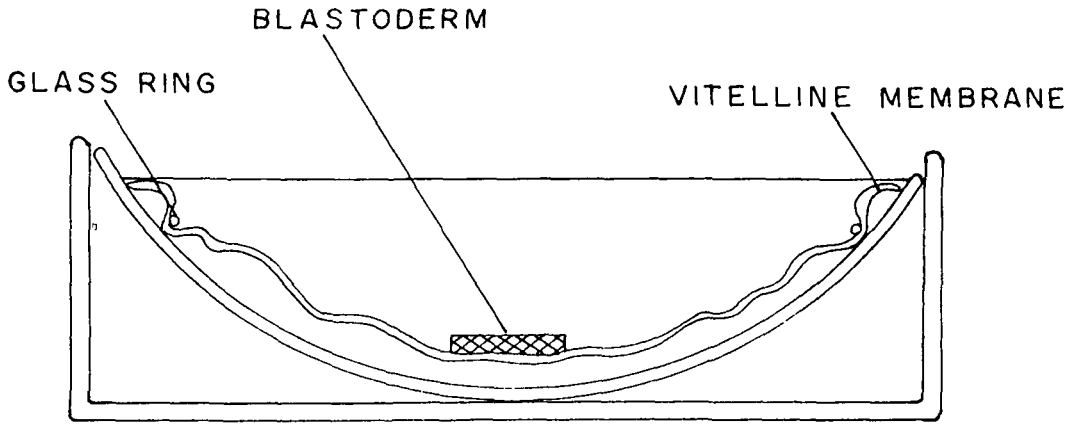


FIG. 3·4b

Fig. 3.4c Completed preparation. Contents of watch glass housed in a covered petri dish Side view.

Fig. 3.4d Completed preparation from above. Dorsal view.

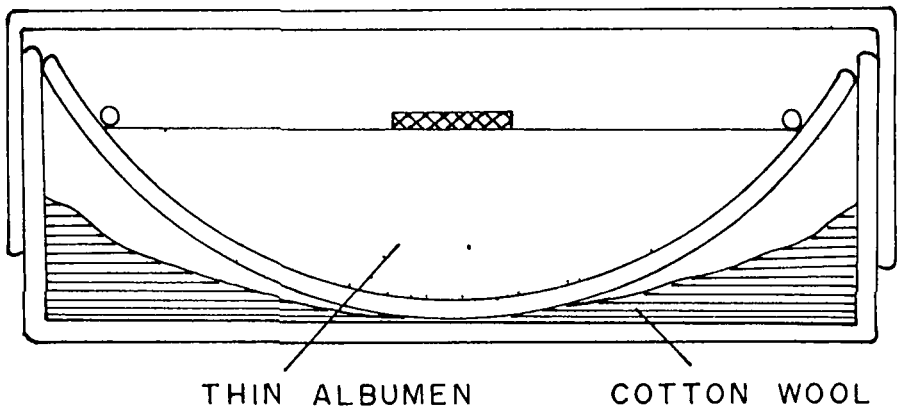


FIG. 3·4c

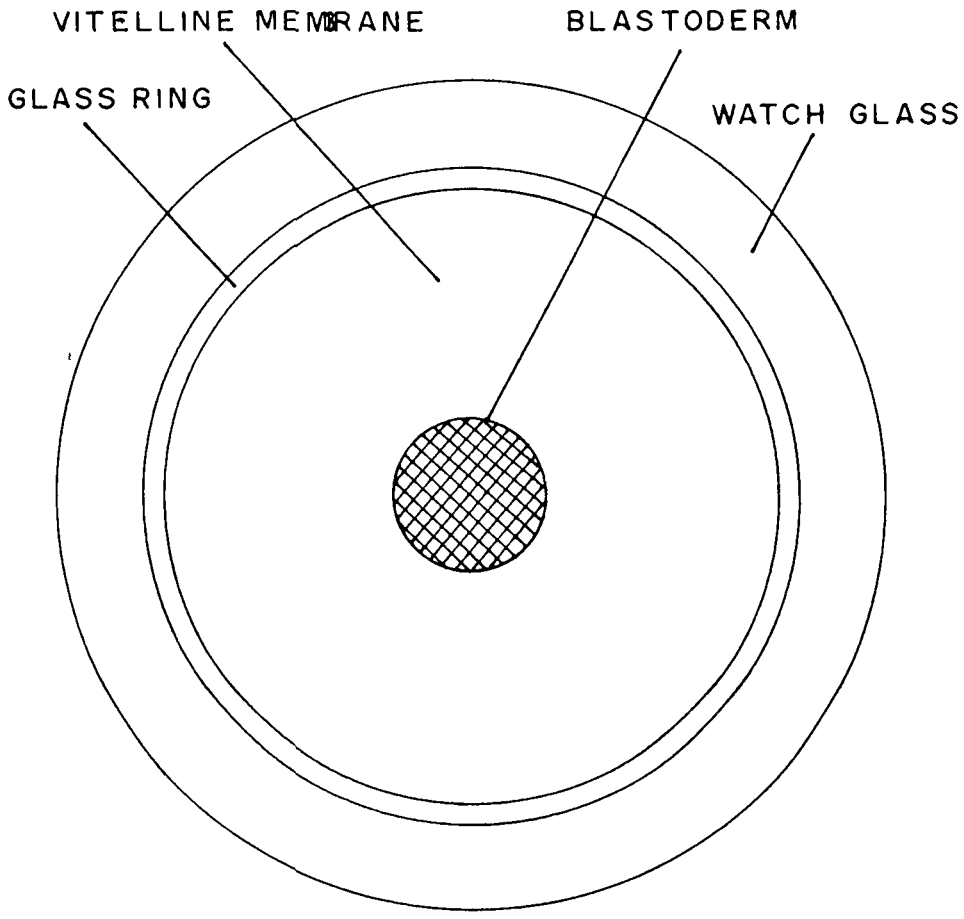


FIG. 3·4d

Fig. 3.5a Operation on Host Embryo : G indicates site of implantation. Letters A, B, C and D indicate the parts of the primitive streak from where grafts were isolated from donor embryos stage 3 and stage 4.

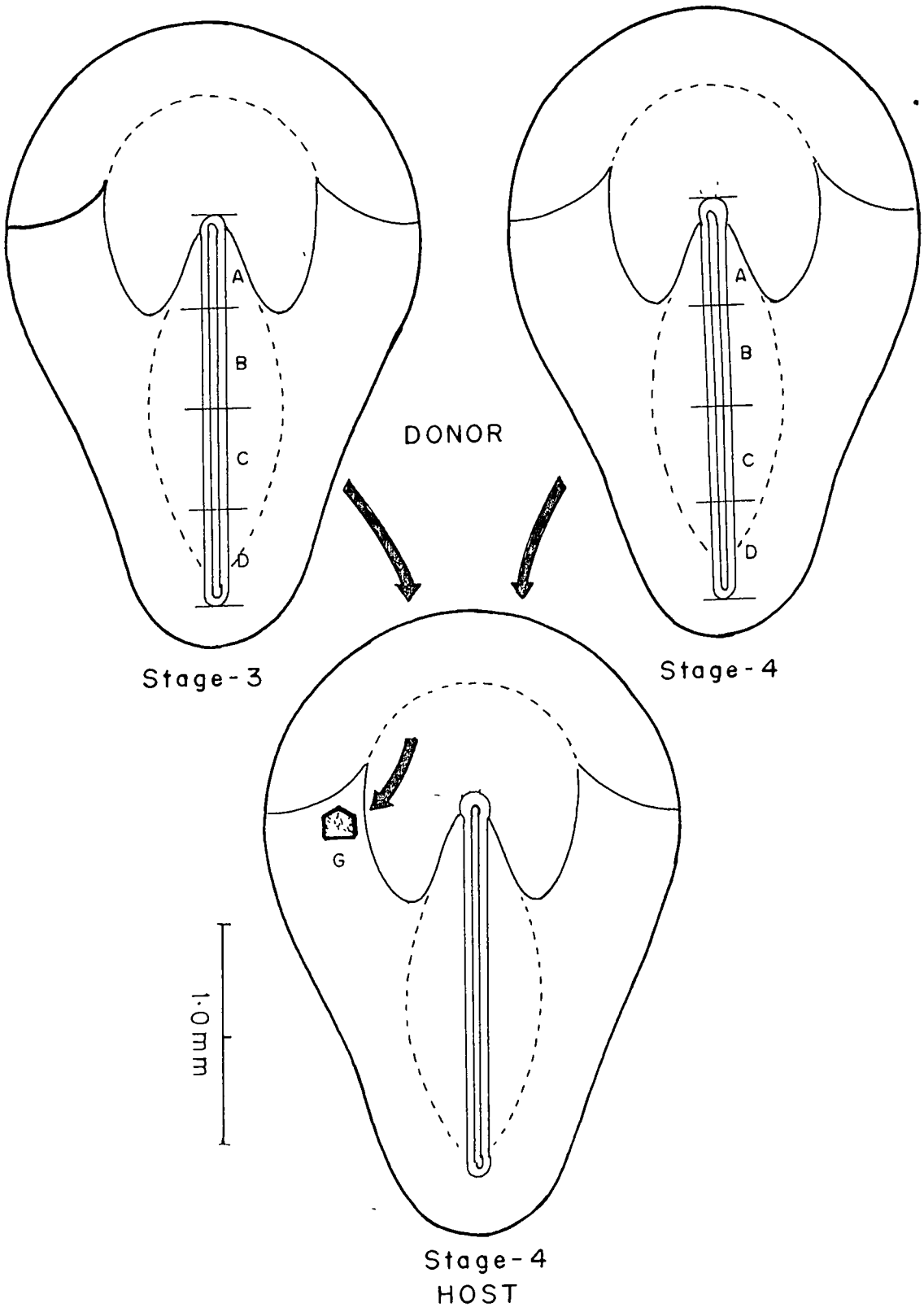


FIG. 3.5a

Fig. 3.5b Operation on Host Embryo : G indicates site of implantation. Letters A, B, C and D indicate the parts of the primitive streak from where grafts were isolated from donor embryos stage 5 and stage 6.

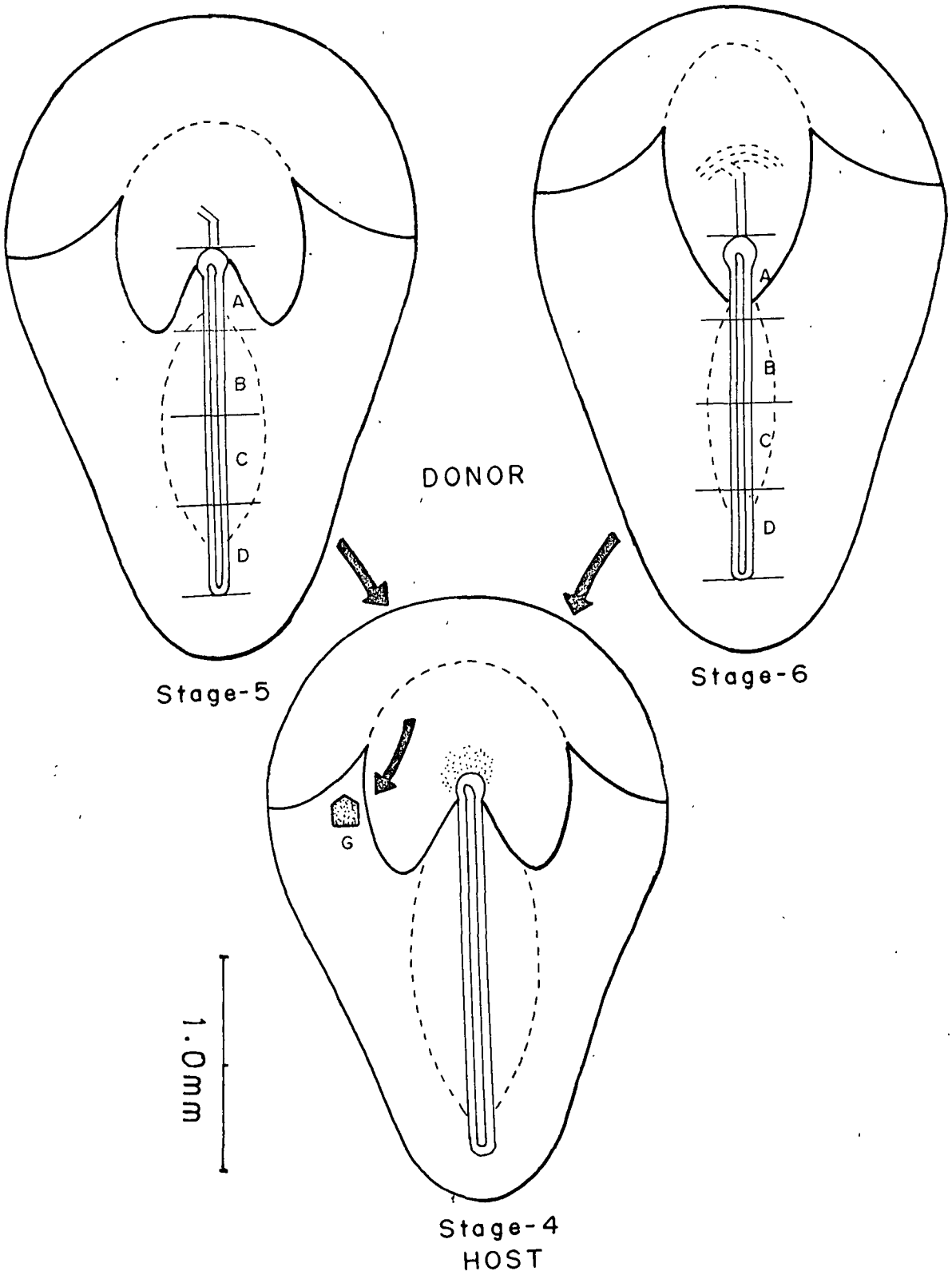


FIG. 3·5b

neural tube, neural plate, notochord and somite, a quantitative analysis of induction was made. According to this, the differentiation of 'complete' embryonic axis is indicated by the presence of neural tube or neural plate, notochord and somites and embryonic is termed 'incomplete' if neural plate and either somites or notochord is induced. The third category is when the neural plate is the only axial structure formed.

3.7 Analysis of histological changes in the neurectoderm induced by the grafts of Hensen's node at different time intervals

Two types of grafts were prepared for each of stage 4, 5 and 6.

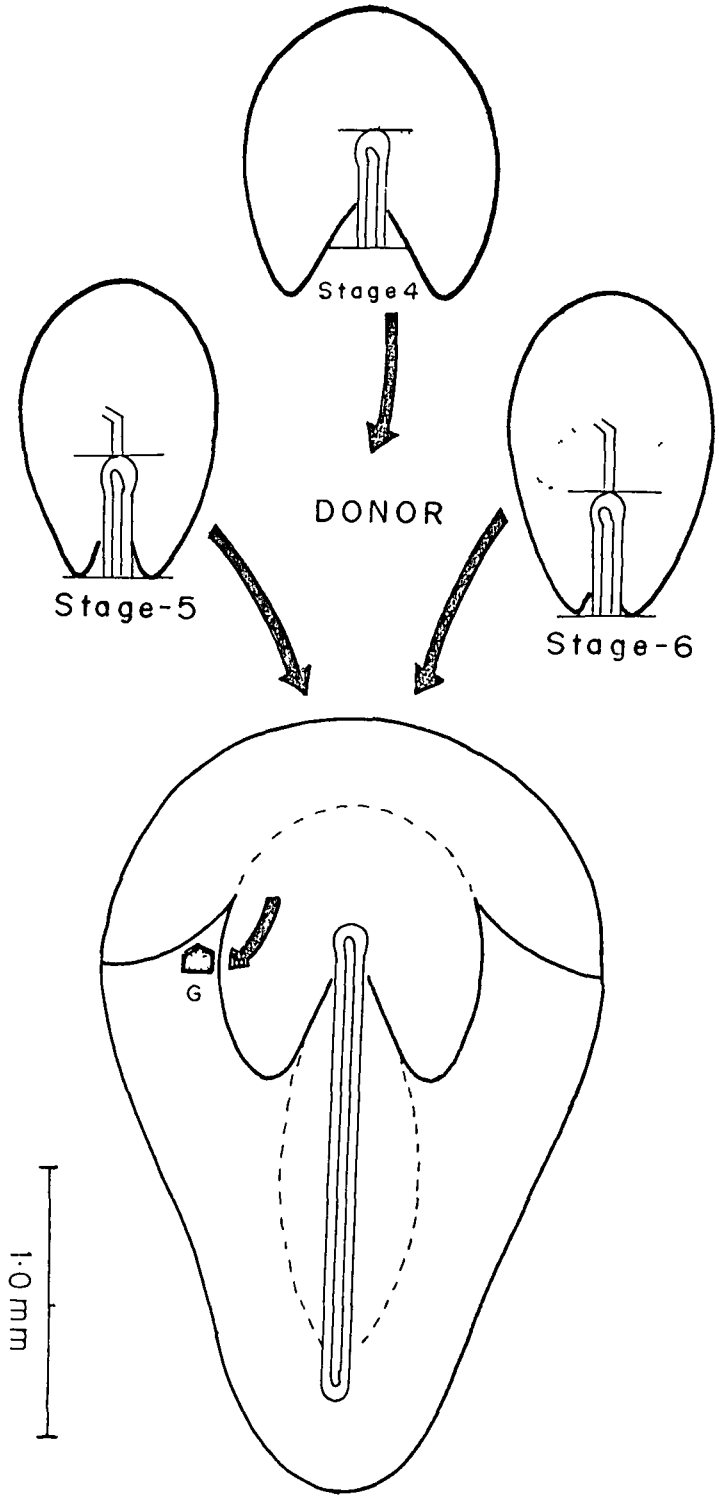
1. Grafts of Hensen's node with all the germ layers. The grafts have been designated as 4 EcMEn, 5 EcMEn and 6 EcMEn with the numbers indicating the stage of the donor embryo.
2. Grafts of Hensen's node without endoderm designated as 4 EcM, 5 EcM and 6 EcM with the numbers indicating the stage of the donor embryo as above.

The grafts were implanted below the ectoderm (Fig. 3.7a) at the border of the area pellucida almost at the level of the Hensen's node of the host embryo nearing Hamburger and Hamilton (1951) stage 4 and the cultures were placed back in the incubator. The cultures having gfafts 4 EcMEn, 5 EcMEn, 4 EcM and 5 EcM were taken out at time intervals of every 5 minutes upto 30 minutes and those having grafts 6 EcMEn and 6 EcM at 50 minutes, 2 hours and 2 hours 30 minutes as neural induction was not observed in pilot experiments before 50 minutes. Histological analysis has been carried out in semithin plastic sections.

The host embryos were fixed in ice cold 2.5% glutaraldehyde in 0.1M sodium Cacodylate 1 hour to 4 hours. They were washed three times in 0.1M sodium Cacodylate containing 0.333 g $CaCl_2$ for 1 hour 30 minutes. They were post-fixed with 1% osmium tetroxide in phosphate buffer and washed twice in the same buffer. They were dehydrated in graded ethanol followed by two changes of propylene oxide, embedded in epoxy resins, sectioned at a thickness of 1 µm with ultramicrotome, model LKB 2088 and stained with toluidene blue.

Some experimental embryos were fixed in Bouin's fluid, embedded in paraffin, sectioned at 10 µm and stained by the Azan method.

Fig. 3.7a Implantation of the grafts of Henson's node at different time intervals from donor embryos stage 4, stage 5 and stage 6. G indicates site of implantation.



Stage-4
HOST
FIG. 3 7a

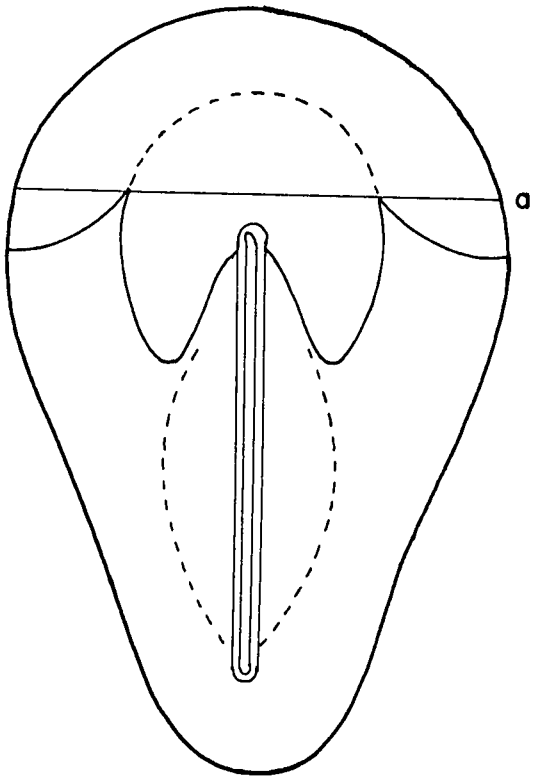
The sections were studied under the Olympus Research Microscope. The presence of different types of cells were noted and their dimensions, length (L), width (W) and nuclear diameter (ND) were measured with a graduated ocular micrometer. Presence or absence of neuroid response in the host ectoderm was recorded and indicated by a positive (+) or a negative sign (-) respectively.

3.8 Analysis of histological changes in the normal neurectoderm

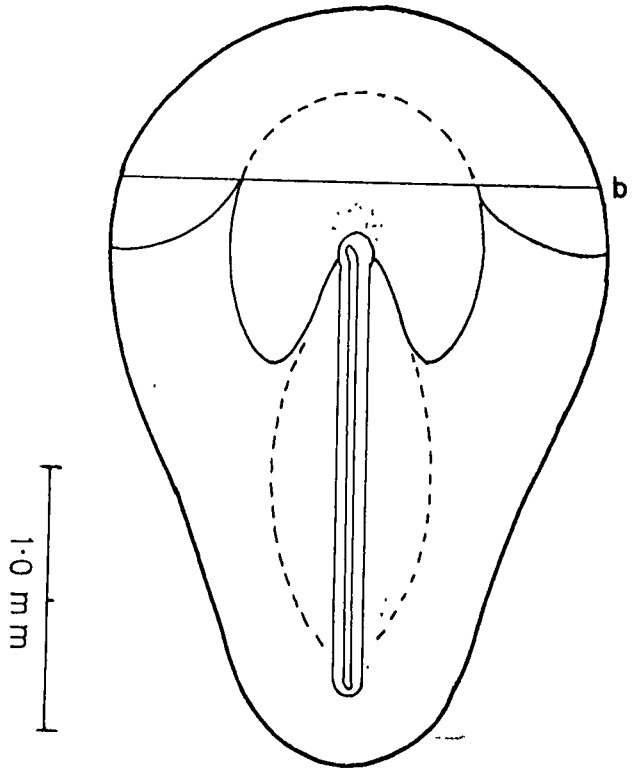
Embryos at Hamburger and Hamilton (1951) stages 3, 4 5 and 6 were excised from incubated eggs in a bowl of Tyrode solution and the vitelline membrane was separated. The excised embryos were then transferred to a Petridish, fixed and processed for embedding in plastic as described in section 3.7. They were then sectioned in the normal neural plate region (Fig. 3.8a, 3.8b) at a thickness of 0.5 or 1 µm in the LKB 2088 ultramicrotome and stained with toluidene blue for histological analysis.

The sections were studied under the Olympus Research Microscope. The presence of different types of cells was noted and their length (L) width (W) and nuclear diameter (ND) were recorded.

Fig. 3.8a Embryos at stage 3 and stage 4. The lines a and b indicate the plane of sections.



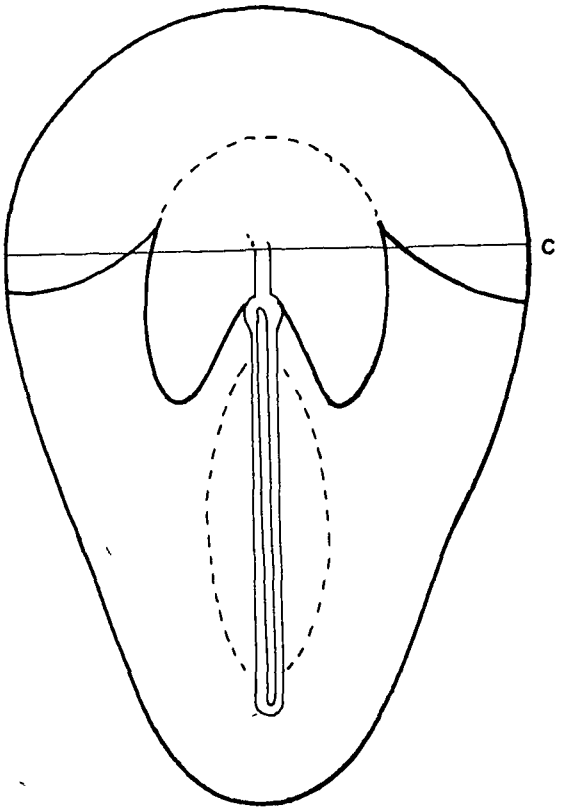
Stage-3



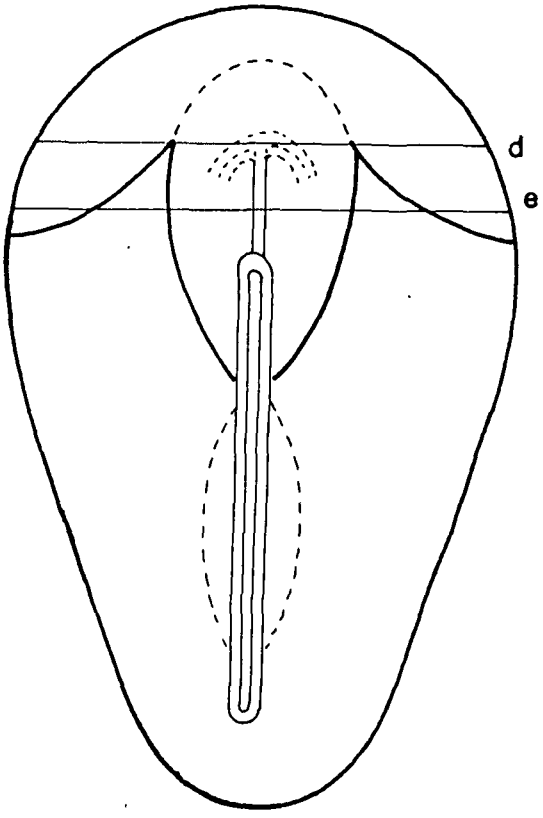
Stage-4

FIG. 3·8a

Fig. 3.8b Embryos at stage 5 and stage 6. The lines c, d and e indicate the plane of sections.



Stage-5



Stage-6

FIG. 3·8b

3.9 Analysis of the ultra-structural changes in the normal neuroectoderm

(A) Solutions for Transmission Electron Microscope:

(1) Tyrode Saline

NaCL	-	8.00 g (M/6.2)
KCL	-	0.20 g (M/6.2)
$CaCL_2$	-	2.00 g (M/9.3)
$MgCl_2.6H_2O$	-	0.10 g
$NaH_2PO_4.2H_2O$	-	0.05 g
$NaHCO_3$	-	1.00 g (for buffering to maintain pH ± 7.4)
Glucose	-	1.00 g
Distilled Water to make	-	1000 ml

(2) Fixatives

(a) Glutaraldehyde 2.5% in 0.1 M Sodium Cacodylate buffer.

(b) Osmium tetroxide 1% in 0.1 M buffer solution.

(3) Preparation of buffers

(a) Sodium Cacodylate 0.1 M + 0.33 g. $CaCl_2$

(b) Sodium phosphate 0.2 M buffer solution.

Solution A : Monobasic sodium phosphate

NaH_2PO_4 - 27.80 g

Distilled water - 1000 ml

Solution B : Dibasic sodium phosphate

$Na_2HPO . 7H_2O$ - 53.65 g

or

$Na_2HPO_4 . 12H_2O$ - 71.70 g

Distilled water - 1000 ml

The desired pH was obtained by mixing the two solutions in the ratio of 19 ml of Solution A and 81 ml of Solution B and diluting it to a total volume of 200 ml.

(4) Stains

(a) Aqueous uranyl acetate 2%.

(b) Lead Citrate :

Lead Citrate - 0.003 g

Distilled Water - 1 ml

10 N NaOH - 10 µl

The ingredients are mixed in a small test tube with the help of a cyclo mixer for 10 minutes and then this solution was centrifuged for 2 minutes at 1000 rpm. The supernatant is taken for staining.

(5) Embedding Medium :

The Dowex epoxy resin was prepared with the following recipe :-

DER 332	-	7.0 ml	} Resin
DER 732	-	3.0 ml	
DDSA	-	5.0 ml	- Hardner
DMP	-	0.3 ml	- Accelerator

The ingredients are mixed slowly but thoroughly with a cyclo mixer (Remi).

(B) Removal of embryo

The eggs were incubated at 37.5°C (±1°C) to get embryos at stages 4, 5 and 6. The embryos were removed in ice cold Tyrode solution in Petridishes, the vitelline membrane was separated and the desired portion of the embryo was cut out. The fixation, post fixation, washing and dehydration was also done in ice cold condition. Finally, dehydration in 100% ethyl alcohol and clearing was done at room temperature (18°C - 20°C). We obtained good results even if the processing of dehydration and clearing was done at room temperature after post-fixation and washing.

Fixation : The material was fixed in ice cold 2.5% glutaradehyde in 0.1 M sodium cacodylate (pH ± 7.4)

for 1 hour to 4 hours.

Washing : The material was washed in 0.1M sodium cacodylate buffer three times for 1 hour 30 minutes with three changes.

Post fixation : After washing the material was post fixed in 1% osmium tetroxide in phosphate buffer for 1 hour and washed in phosphate buffer with two changes of 15 minutes each.

Dehydration : After post fixation, the material was dehydrated with ethanol grades 30%, 50%, 60%, 70%, 80%, 90% and 100% for 15 minutes at each step. At absolute ethanol stage, the alcohol was changed two times.

Clearing : The dehydrated material was cleared in following mixture for about 10 minutes in each step.

Absolute ethanol	:	Propylene Oxide
3 parts	:	1 part
2 parts	:	1 part
1 part	:	3 parts

Pure propylene oxide (two changes)

Embedding : The material was passed through following mixtures for embedding in the Dowex epoxy resin

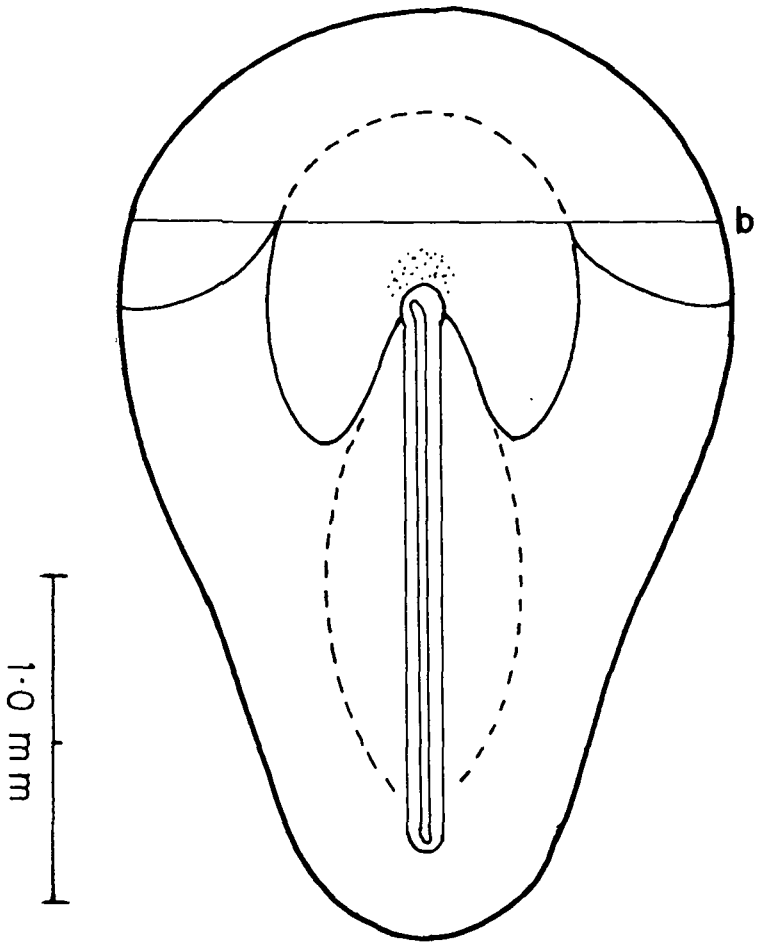
Propylene Oxide	:	Resin
3 parts	:	1 part (15 mins.)
2 parts	:	2 parts (30 mins.)
1 part	:	3 parts (60 mins.)
Pure Embedding medium (2 hrs.)		

The material was kept in pure embedding medium overnight for 24hours. The container was kept during infiltration on an automatic shaker with slow speed. After infiltration, the material was transferred to the pure embedding medium in the oven at 60°C for polymerization.

Sectioning : The polymerised blocks were trimmed and sectioned with LKB 2088 ultramicrotome at the desired position as shown in (Fig. 3.9a, 3.9b). At first, the semithin sections were cut with glass knife at a thickness of 0.5 or 1 µm and stained in toluidene blue. The desired portion of the sections was selected, the blocks were further trimmed and the ultrathin sections were cut with LKB diamond knife. The thickness of these sections was judged by interference colours. Gold colour sections (thickness ± 600Å to 900Å) were picked up on grids.

The sections were picked up on the 300 mesh grids. The grids were placed blotted on filter paper in a Petridish, dried and then stored in the grid box. The sections were stained in 2% in aqueous uranyl acetate for 5 to 10 minutes followed by lead citrate for 2 minutes. For staining, drops of stain were placed on dental wax and grids were floated on it with the sections in contact with the stain. The stained sections were then examined with the Transmission Electron Microscope JEM 100C X II.

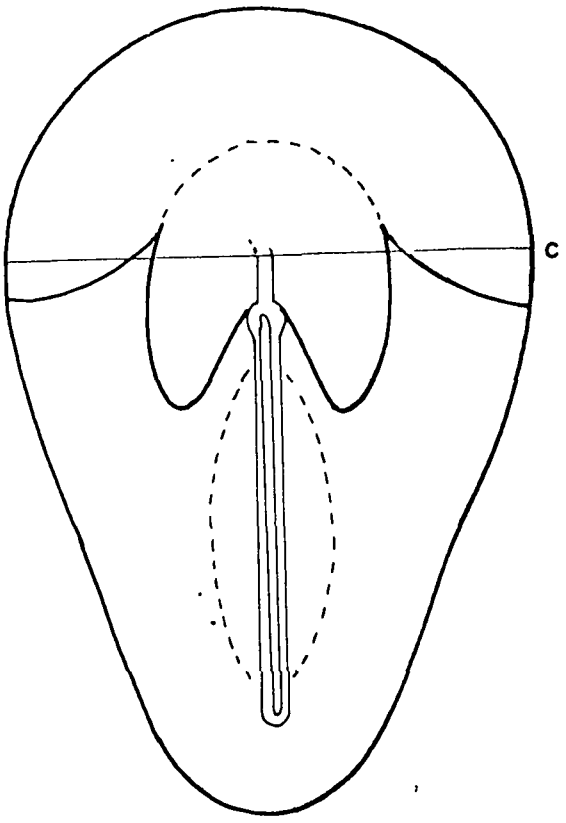
Fig. 3.9a Embryo at stage 4. The line b, indicates the plane of section.



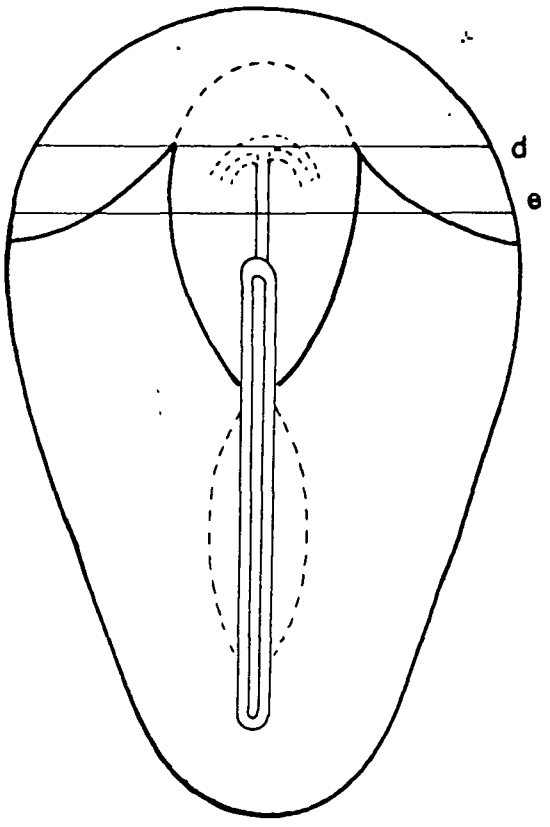
Stage-4

FIG. 3.9a

Fig. 3.9b Embryos at stage 5 and stage 6. The lines c, d and e indicate the plane of sections.



Stage-5



Stage-6

FIG. 3.9b

Chapter 4

EXPERIMENTS AND RESULTS

EXPERIMENTS AND RESULTS

4.1 NEURAL INDUCTION BY DIFFERENT PARTS OF THE PRIMITIVE STREAK OF STAGES 3, 4, 5 and 6

4.1.1 By the grafts having all germ layers (designated as EcMEn grafts)

The primitive streak of the chick embryo at H and H stages 3, 4, 5 and 6 was divided into four parts A, B, C and D (Figs. 3.5a and 3.5b) and each graft so prepared was implanted near the antero-lateral margin of the area pellucida below the ectoderm of the host embryo approaching stage 4. The culture dishes were placed in the incubator and the host embryos were removed and fixed after 24 hours. The changes in the induced ectoderm were examined morphologically, histologically and analysed at each stage.

Stage 3 :

A total of 47 EcMEn grafts were implanted into stage 4 hosts. This included 16A 15B, 11C and 5D grafts. The study of the sections of all host blastoderms revealed that complete embryonic axis was induced by only 1 EcMEn 'B' graft, incomplete embryonic axis (all parts of embryonic axis not properly formed or some parts missing) by 9 grafts. Only induced neural plate by 24 grafts, while

2 grafts did not show any induction and the 11 remaining grafts were dead (Table 4.1.1).

Stage 4 :

At stage 4, a total of 74 EcMEn grafts were implanted. This included 26A, 19B, 18C and 11D. The study of the sections of all host blastoderm revealed that complete embryonic axis was induced by 4 EcMEn grafts (Plate 4.1.1a) incomplete embryonic axis by 19 grafts and only induced neural plate by 32 grafts, 2 grafts did not show any induction while the 17 remaining grafts were dead (Table 4.1.1).

Stage 5 :

At stage 5, a total of 41 EcMEn grafts were implanted. This included 17A, 14B, 5C and 5D grafts. The study of all sections revealed that complete embryonic axis was induced by 4 anterior grafts (Plates 4.1.1b–4.1.1f) incomplete embryonic axis by 11 grafts, an induced neural plate by 15 grafts while 1 graft did not show any induction and the 10 remaining grafts were dead (Table 4.1.1).

Stage 6 :

At stage 6, a total of 21 EcMEn grafts were implanted. This included 6A, 5B, 5C and 5D grafts. The study of all sections revealed that complete embryonic axis was induced by only 1 EcMEn grafts, incomplete embryonic axis by 2 grafts and only neural plate by 7 grafts, (Plates 4.1.1g,4.1.1h) while 11 grafts were dead.

The analysis of the induced structures as seen in the sections were complete embryonic axis i.e. neural tube or neural plate, notochord and somite. Incomplete embryonic axis indicated presence of any two of the above structures and only neural plate was observed as the only induced structure. The percentage of induction of different types of grafts A, B, C and D at different stages are as follows :-

Complete embryonic axis :

Graft A at stage 3 did not show induction of complete embryonic axis but at stage 4 the percentage of induction is 11.5%; at stage 5 : 23.5%; at stage 6 : 16.6% (Fig. 4.1A - EcMEn). Graft B, at stage 3 : 6.6%; stage 4 : 5.2%; stage 5 : and at stage 6 no induction of complete embryonic axis (Fig. 4.1B - EcMEn). Graft C and D did not show any induction of complete embryonic axis from stages 3 to 6 (Figs. 4.1C - EcMEn, 4.1D - EcMEn).

Incomplete embryonic axis :

Graft A showed percentage of induction of incomplete embryonic axis at stage 3 : 37.5%; stage 4:27.0%; stage 5 : 29.5% and stage 6 : 16.6% (Fig.4.1A - EcMEn). Graft B at stage 3 : 13.3%; stage 4 : 31.5%; stage 5 : 28.5% and stage 6 : 20.0% (Fig. 4.1B-EcMEn). Graft C at stage 3 : 9.0%; stage 4:27.6%;stage 5 : 10.0% and stage 6 no induction (Fig.4.1C - EcMEn). Graft D did not show any induction

from stage 3 to stage 6 (Fig. 4.1D - EcMEn) except at stage 4 which is 9%.

Neural plate :

Graft A showed percentage of induction of neural plate alone at stage 3 : 50.0%; stage 4 : 30.7%; stage 5 : 29.5%; stage 6 : 33.3% (Fig. 4.1A - EcMEn). Graft B at stage 3 : 66.6%; stage 4 : 57.8%; stage 5 : 50.0%; stage 6 : 40.0% (Fig. 4.1B - EcMEn). Graft C at stage 3 : 54.5%; stage 4 : 44.4%; stage 5 : 40.0% and stage 6 : 40.0% (Fig. 4.1C - EcMEn). Graft D at stage 3 did not show any induction but stage 4 : 45.5%; stage 5 and stage 6 is 20.0% each.

The percentage frequency of neural plate induction of A, B, C and D grafts at stages 3, 4, 5 and 6 are as follows :-

Graft A at stage 3 : 87.5%; stage 4 : 69.2%; stage 5 : 82.5%; stage 6 : 66.5% (Fig. 4.1A - EcMEn). Graft B at stage 3 : 86.5%; stage 4 : 94.5%; stage 5 : 78.5% and stage 6 : 60.0% (Fig. 4.1B - EcMEn). Graft C at stage 3 : 63.5%; stage 4 : 72.0% stage 5 : 80.0% and stage 6 : 40.0% (Fig. 4.1C - EcMEn). Graft D showed no induction of neural plate at stage 3, at stage 4 : 54.5% stage 5 and stage 6 is 20.0% each.

TABLE 4.1.1

Structures induced by different types of grafts A, B, C and D of the primitive streak with all germ layers at stages 3, 4, 5 and 6 implanted into host embryos nearing stage 4

STAGE	TYPE OF GRAFTS	NO. OF GRAFTS IMPLANTED	MORTALITY		STRUCTURE INDUCED								% FREQUENCY OF NEURAL PLATE INDUCTION
			NO. DEAD	%	COMPLETE EMBRYONIC AXIS		INCOMPLETE EMBRYONIC AXIS		NEURAL PLATE ONLY		NO INDUCTION		
					NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	
3	A	16	2	12.5	0	0.0	6	37.5	8	50.0	0	0.0	87.5
	B	15	2	13.5	1	6.6	2	13.3	10	66.6	0	0.0	86.5
	C	11	3	27.2	0	0.0	1	9.0	6	54.5	1	9.0	63.5
	D	5	4	80.0	0	0.0	0	0.0	0	0.0	1	20.0	0.0
4	A	26	8	30.7	3	11.5	7	27.0	8	30.7	0	0.0	69.2
	B	19	1	5.2	1	5.2	6	31.5	11	57.8	0	0.0	94.5
	C	18	4	22.2	0	0.0	5	27.6	8	44.4	1	5.6	72.0
	D	11	4	36.3	0	0.0	1	9.0	5	45.5	1	9.0	54.5
5	A	17	3	17.6	4	23.5	5	29.5	5	29.5	0	0.0	82.5
	B	14	3	21.4	0	0.0	4	28.5	7	50.0	0	0.0	78.5
	C	5	1	20.0	0	0.0	2	40.0	2	40.0	0	0.0	80.0
	D	5	3	60.0	0	0.0	0	0.0	1	20.0	1	20.0	20.0
6	A	6	2	33.3	1	16.6	1	16.6	2	33.3	0	0.0	66.5
	B	5	2	40.0	0	0.0	1	20.0	2	40.0	0	0.0	60.0
	C	5	3	60.0	0	0.0	0	0.0	2	40.0	0	0.0	40.0
	D	5	4	80.0	0	0.0	0	0.0	1	20.0	0	0.0	20.0

Plate 4.1.1a Photomicrograph of chick embryo with complete embryonic axis indicated by arrow, induced by graft of donor embryo, Stage 4, having all germ layers (x25).

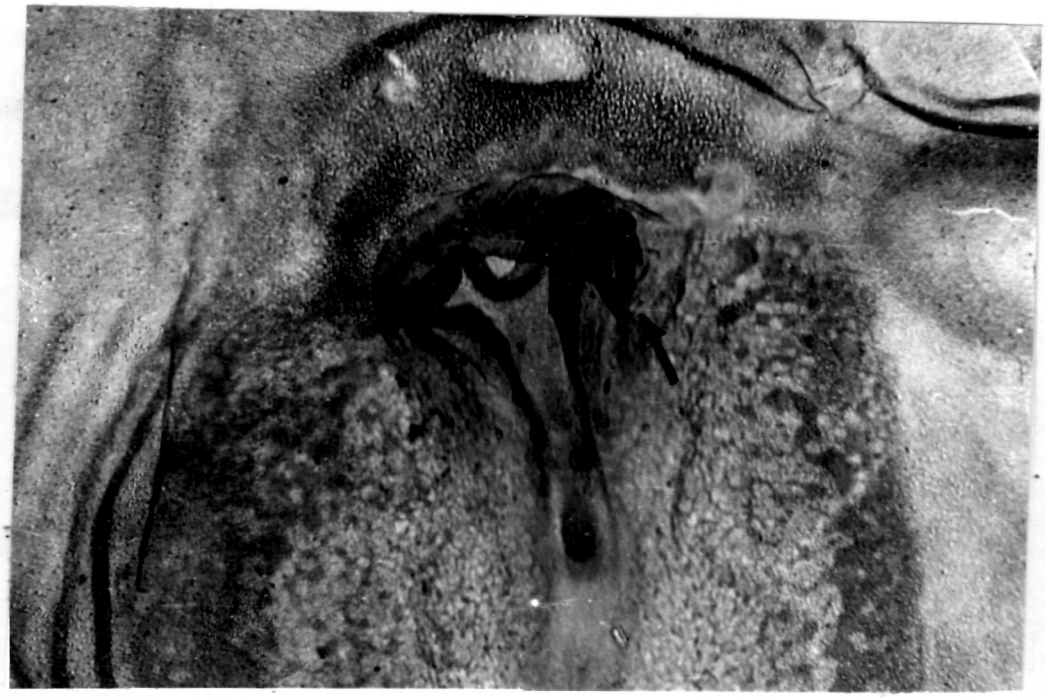


PLATE 4.1.1a

Plate 4.1.1b Photomicrograph of a section of chick embryo showing host neural tube and induced structures (x100).

Abbreviations :

Hnt - Host neural tube

I - Induced structures

nt - neural tube

s - somites

Plate 4.1.1c Photomicrograph of the above induced structures under higher magnification showing induced neural tube and somites (x400).

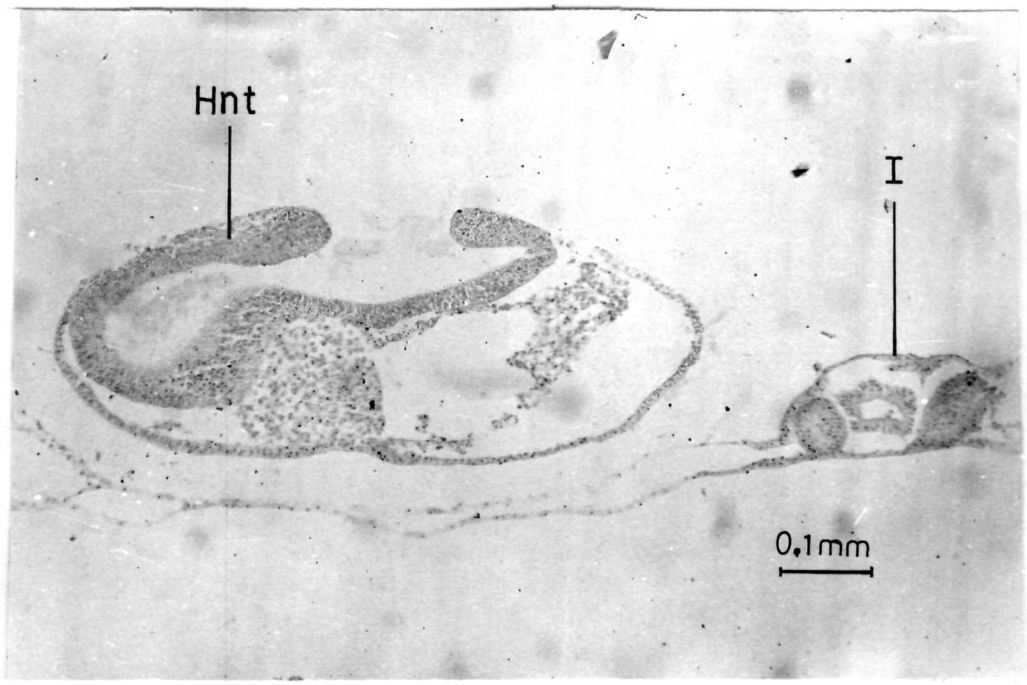


PLATE 4.1.1b

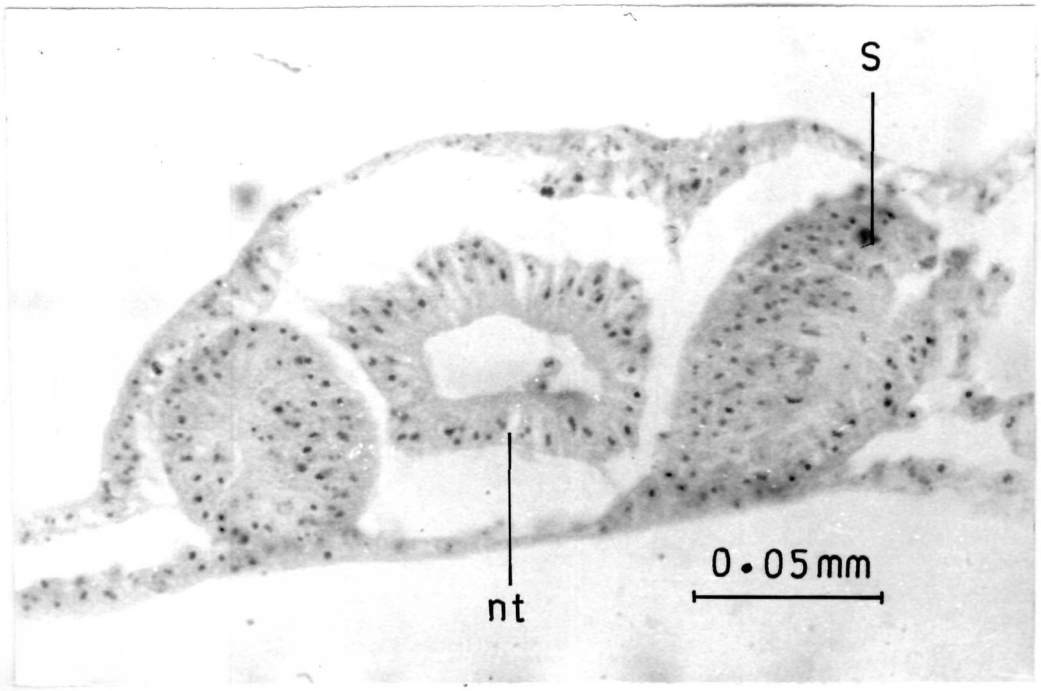


PLATE 4.1.1c

Plate 4.1.1d *Photomicrograph of chick embryo with complete embryonic axis indicated by arrow, induced by graft of donor embryo, Stage 5, having all germ layers (x300).*

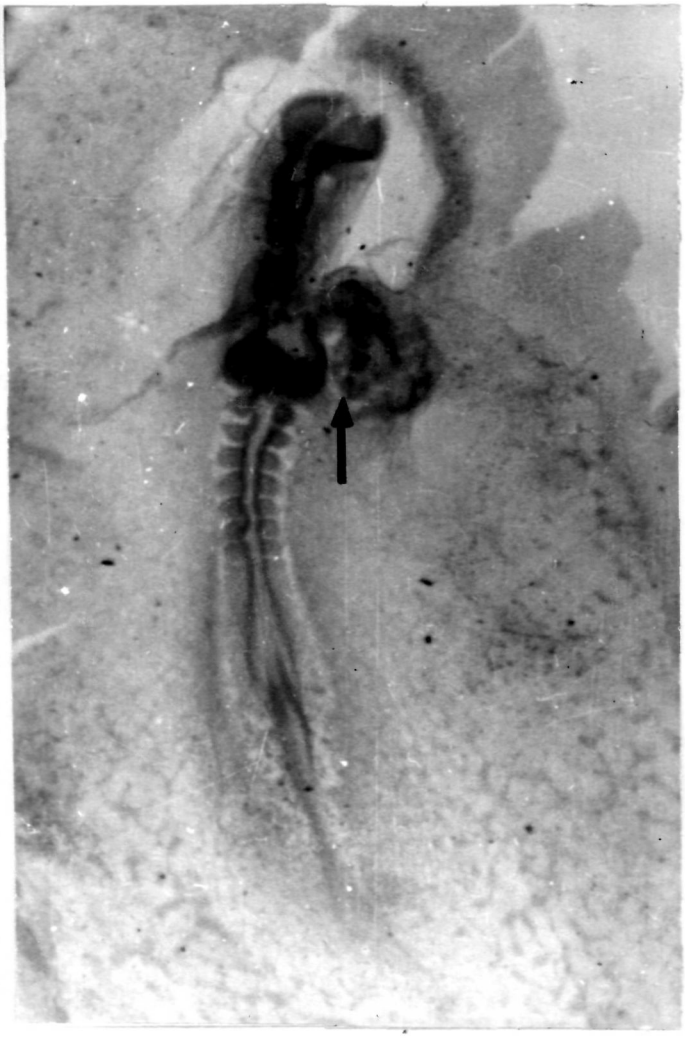


PLATE 4.1.1d

Plate 4.1.1e Photomicrograph of chick embryo with complete embryonic axis indicated by arrow, induced by graft of donor embryo, Stage 5, having all germ layers (x300).

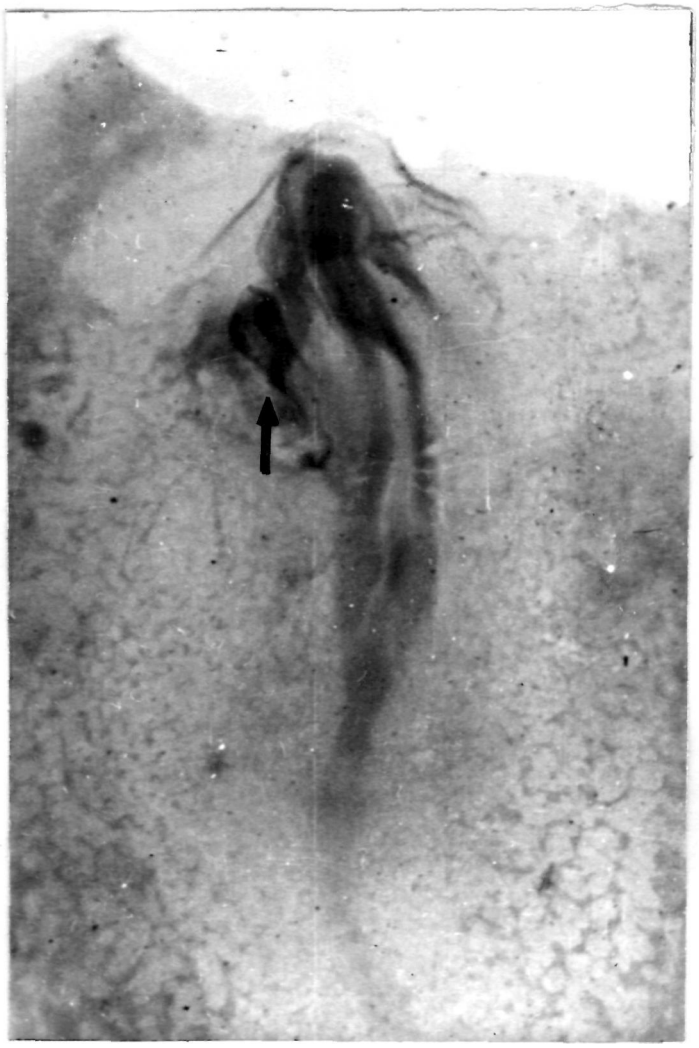


PLATE 4.1.1e

Plate 4.1.1f Photomicrograph of chick embryo with complete embryonic axis indicated by arrow, induced by graft of donor embryo, Stage 5, having all germ layers (x300).

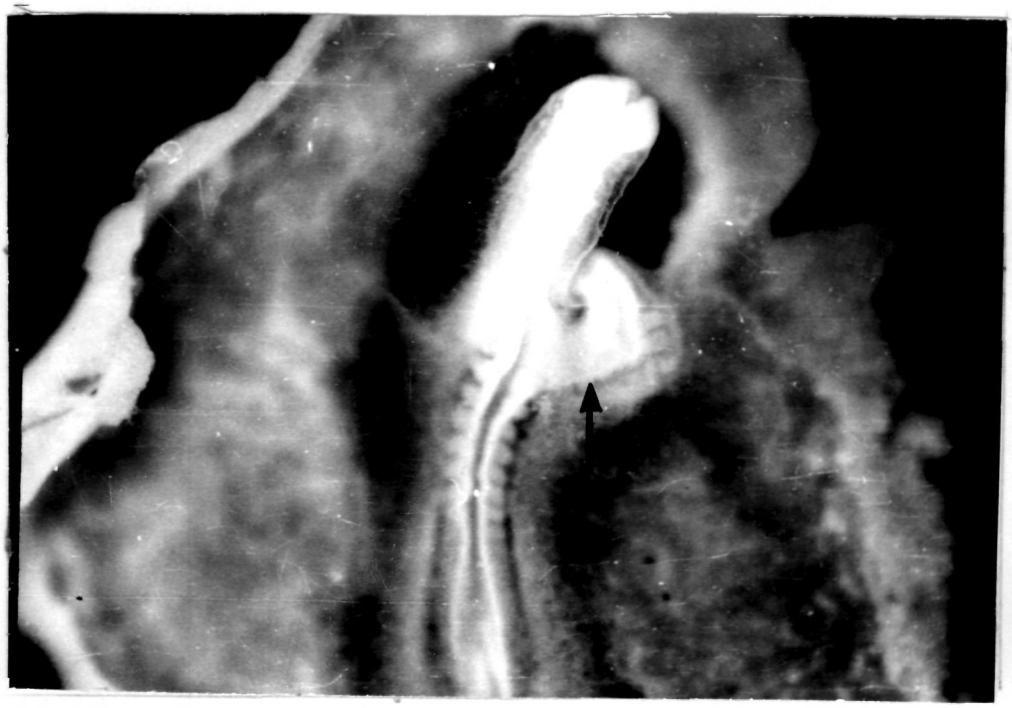


PLATE 4.1.1f

Plate 4.1.1g Photomicrograph of a section of chick embryo showing host neural tube and induced neural plate (x100).

Abbreviations :

Hnt - Host neural tube

Inp - Induced neural plate

Plate 4.1.1h Photomicrograph of the above induced neural plate under higher magnification (x400).

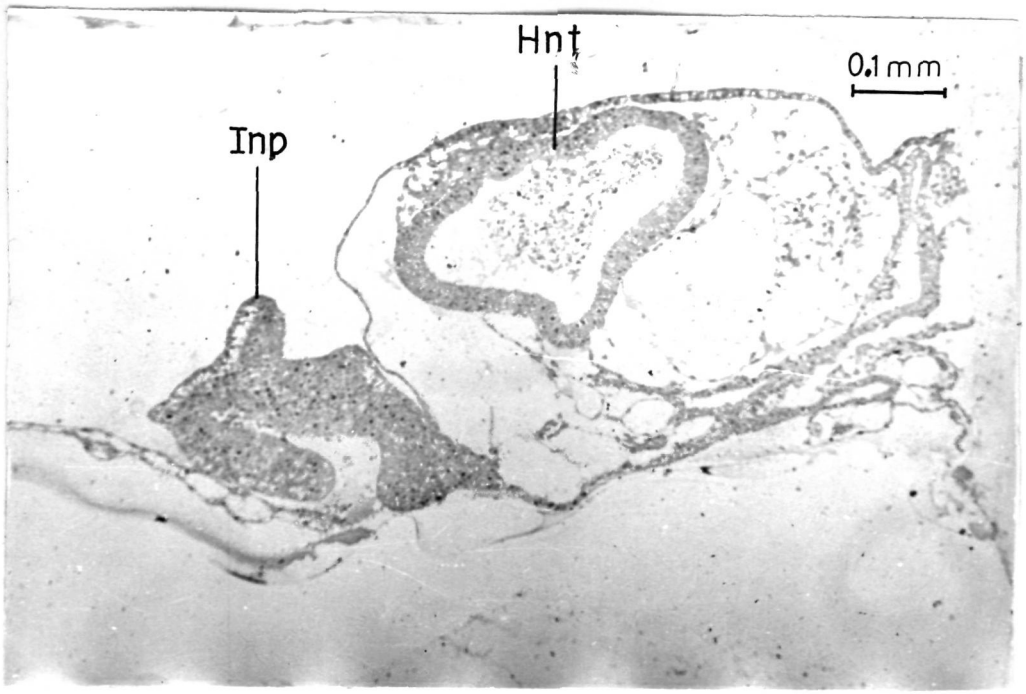


PLATE 4.1.1g

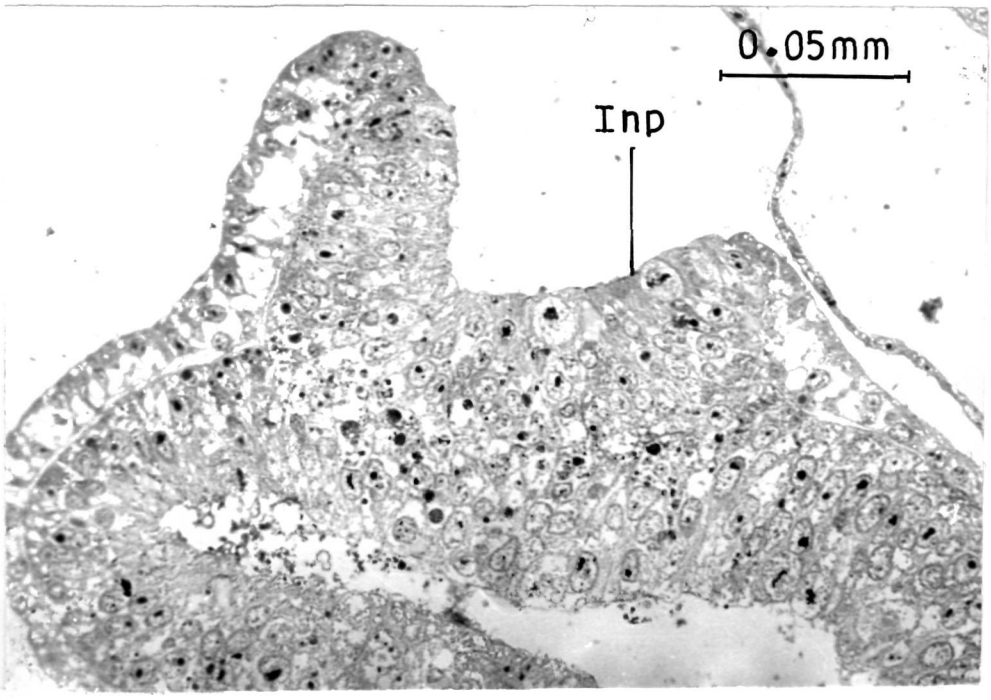


PLATE 4.1.1h

4.1.2 By the grafts without endoderm (designated as EcM grafts)

The primitive streak of the chick embryo at H and H stages 3, 4 and 5 was stripped free of endoderm and it was divided into four parts A, B, C and D (Figs. 3.5a, 3.5b). Separation of endoderm at stage 5 and 6 was dificult when compared to stage 4. The endoderm was separated in Ca^{++}- free Locke's solution with tungsten needles to remove the endoderm completely. Each graft so prepared was implanted near the antero - lateral margin of the area pellucida below the ectoderm of the host embryo approaching stage 4. The culture dishes were placed in the incubator and the host embryos were removed and fixed after 24 hours. The changes in the induced ectoderm were examined morphologically, histologically and analysed at each stage.

Stage 3 :

At stage 3, a total of 25 EcM grafts were implanted. This included 8A, 7B, 5C and 5D grafts. Complete embryonic axis was not induced by any graft; incomplete embryonic axis was induced by only 2 grafts and only neural plate by 10 grafts; while 2 grafts did not show any induction and all the remaining 11 grafts were dead (Table 4.1.2).

Stage 4 :

At stage 4, a total of 38 EcM grafts were implanted. This included 16A, 7B, 10C and 5D grafts. The study of all sections revealed that incomplete embryonic axis was induced by 4 grafts (Plate 4.1.2a) and neural plate by 17 grafts, while 1 graft did not show induction and the remaining 16 grafts were dead (Table 4.1.2).

Stage 5 :

At stage 5, a total of 22 EcM grafts were implanted. This included 7A, 5B, 5C and 5D grafts. The study of all sections revealed that complete embryonic axis was not induced by any graft at this stage. Incomplete embryonic axis was induced by 3 grafts and neural plate by 5 grafts only (Plates 4.1.2b and 4.1.2c), while 3 grafts did not show any induction. The remaining 11 grafts were dead.

In the second type of experiments when the endoderm was removed from donors at stage 3, 4 and 5, it was observed that complete embryonic axis was not induced by any EcM grafts. Only incomplete embryonic axis and neural plate was induced. The percentage of induction by the grafts A, B, C and D at different stages are as follows :-

Incomplete embryonic axis :

Graft A showed 12.5% of induction of the

incomplete embryonic axis at stage 3; stage 4 : 12.5% and at stage 5 : 42.7% (Fig. 4.1A - EcM). Graft B at stage 3 showed 14.3%; stage 4 : 14.3% and no induction at stage 5 (Fig. 4.1B - EcM). Graft C only stage 4 : 10% and no induction at stage 3 and 5, and graft D did not show any induction of incomplete embryonic axis at stages 3, 4 and 5 (Figs. 4.1C - EcM).

Neural plate :

Graft A showed percentage of induction of neural plate alone, at stage 3 : 62.5%; stage 4 : 50.0% and stage 5 : 14.3% (Fig. 4.1A - EcM). Graft B, stage 3 : 57.2%; stage 4 : 42.7% and stage 5 : 60.0% (Fig. 4.1B - EcM). Graft C, stage 3 : 20.0%; stage 4 : 60.0% and stage 5 : 20.0% (Fig. 4.1C - EcM).

The analysis of the percentage frequency of neural plate induction of grafts A, B, C and D at stages 3, 4 and 5 are as follows :-

Graft A, stage 3 : 75.0%; stage 4 : 62.5%; stage 5 : 57.0% (Fig. 4.1A - EcM). Graft B, stage 3 : 71.5%; stage 4 : 57.0% and stage 5 : 60.0% (Fig. 4.1B - EcM). Graft C, stage 3 : 20.0% stage 4 : 70.0%; stage 5 : 20.0% (Fig. 4.1C - EcM). Graft D did not show any induction at stages 3, 4 and 5

TABLE 4.1.2

Structures induced by different types of grafts A, B, C and D of the primitive streak without endoderm at stages 3, 4 and 5 implanted into host embryos nearing stage 4.

STAGE	TYPE OF GRAFTS	NO. OF GRAFTS IMPLANTED	MORTALITY		STRUCTURE INDUCED								% FREQUENCY OF NEURAL PLATE INDUCTION
			NO. DEAD	%	COMPLETE EMBRYONIC AXIS		INCOMPLETE EMBRYONIC AXIS		NEURAL PLATE ONLY		NO INDUCTION		
					NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	
3	A	8	2	25.0	0	0.0	1	12.5	5	62.5	0	0.0	75.0
	B	7	2	28.5	0	0.0	1	14.3	4	57.2	0	0.0	71.5
	C	5	3	60.0	0	0.0	0	0.0	1	20.0	1	20.0	20.0
	D	5	4	80.0	0	0.0	0	0.0	0	0.0	1	20.0	0.0
4	A	16	6	37.5	0	0.0	2	12.5	8	50.0	0	0.0	62.5
	B	7	3	42.8	0	0.0	1	14.3	3	42.7	0	0.0	57.0
	C	10	3	30.0	0	0.0	1	10.0	6	60.0	0	0.0	70.0
	D	5	4	80.0	0	0.0	0	0.0	0	0.0	1	20.0	0.0
5	A	7	2	28.7	0	0.0	3	42.7	1	14.3	1	14.3	57.0
	B	5	2	40.0	0	0.0	0	0.0	3	60.0	0.	0.0	60.0
	C	5	3	60.0	0	0.0	0	0.0	1	20.0	1	20.0	20.0
	D	5	4	80.0	0	0.0	0	0.0	0	0.0	1	20.0	0.0

Plate 4.1.2a Photomicrograph of chick embryo with incomplete embryonic axis indicated by arrow, induced by graft of donor embryo stage 4 without endoderm (x120).

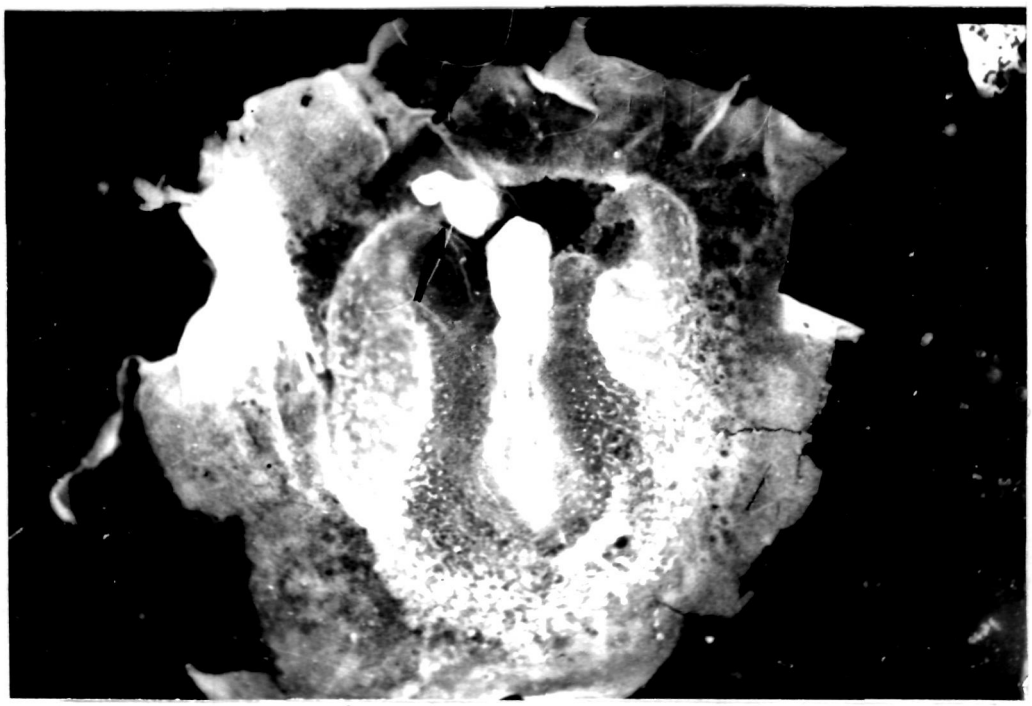


PLATE 4.1.2a

Plate 4.1.2b Photomicrograph of a section of chick embryo showing host neural tube and induced neural plate (x100).

Abbreviations :

Hnt - Host neural tube

Inp - Induced neural plate

Plate 4.1.2c Photomicrograph of the above induced neural plate under higher magnification (x250).

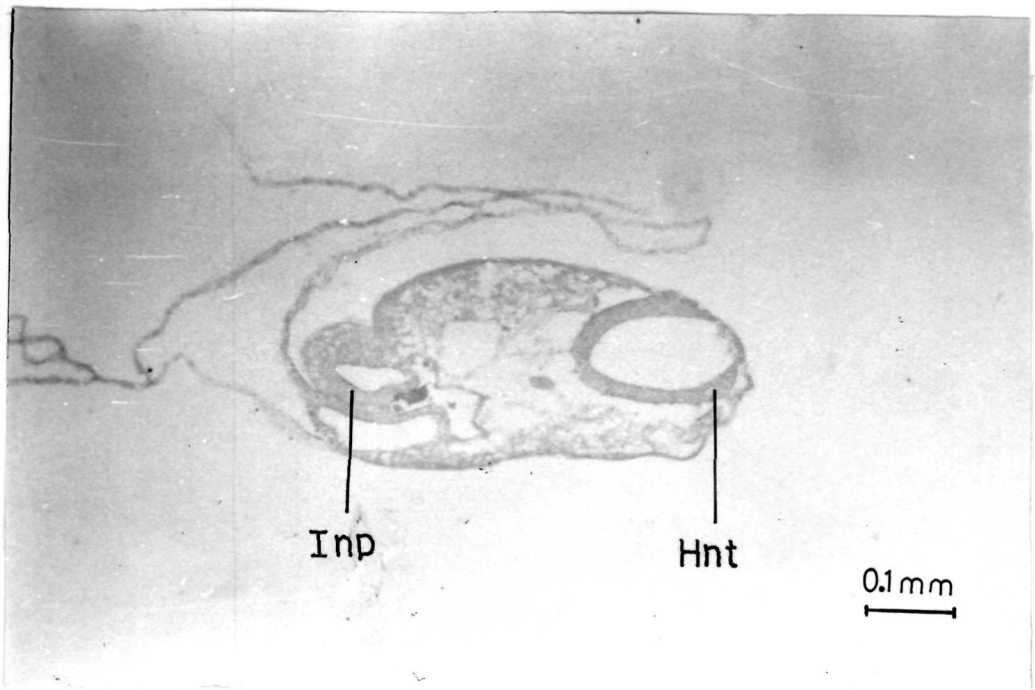


PLATE 4.1.2b

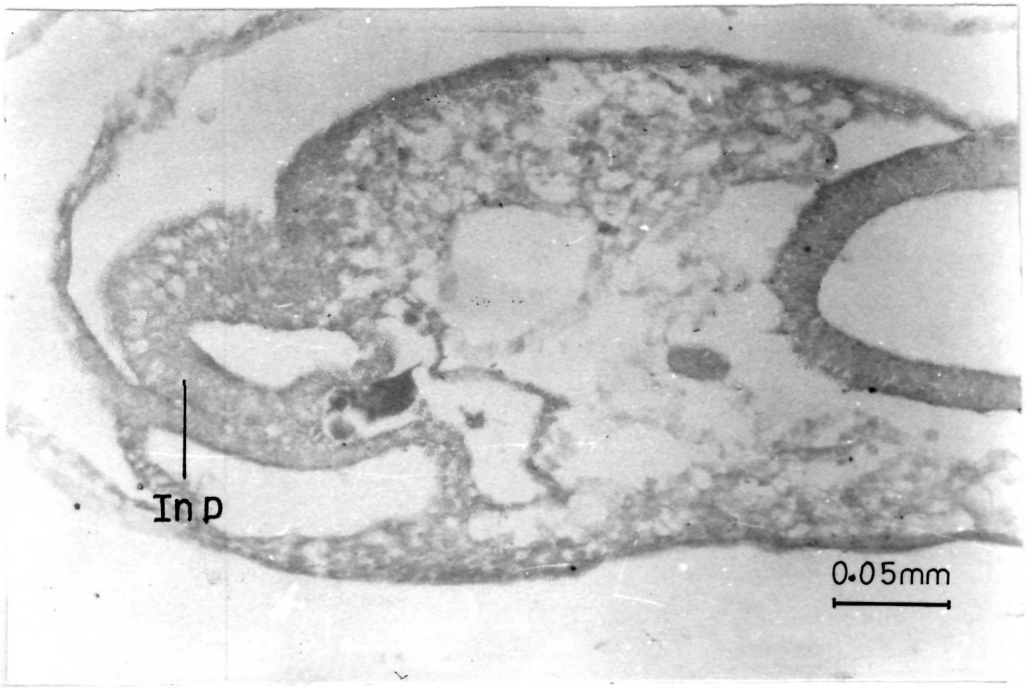


PLATE 4.1.2c

4.1.3 By the grafts without endoderm and mesoderm (designated as Ec grafts)

The primitive streak of the chick embryo at H and H stage 3, 4 and 5 stripped free of the endoderm and mesoderm was divided into four parts, A, B, C and D (Figs. 3.5a, 3.5b) and each graft so prepared was implanted near the antero - lateral margin of the area pellucida below the ectoderm of the host embryo approaching stage 4. Separation of germ layers of both endoderm and mesoderm at stage 5 was more difficult and it was performed with the help of Ca^{++} - free Locke's solution and tungsten needles to remove the layers completely. The culture dishes were placed in the incubator and the host embryos were removed and fixed after 24 hours. The changes in the induced ectoderm were examined morphologically, histologically and analysed at each stage.

Stage 3 :

At stage 3, a total of 31 Ec grafts were implanted. This included 12A, 9B, 5C and 5D grafts. The study of all sections revealed that complete emrbyonic axis was not induced induced by any graft, incomplete embryonic axis by 1 graft and neural plate by 16 grafts (Plate 4.1.3a), the other 2 grafts did not show any induction and 12 grafts were dead (Table 4.1.3).

Stage 4 :

At stage 4, a total of 29 Ec grafts were implanted. This included 12A, 7B, 5C and 5 D grafts. The study of the sections revealed that complete embryonic axis was not induced by any graft, incomplete embryonic axis was induced by 2 grafts and only neural plate formation by 8 grafts (Plates 4.1.3b, 4.1.3c); while 1 graft did not show any induction, the remaining 18 grafts were dead (Table 4.1.3).

Stage 5 :

At stage 5, a total of 20 Ec grafts were implanted. This included 5A, 5B, 5C and 5D. The study of all sections revealed that 2 grafts induced incomplete embryonic axis and 5 grafts induced only neural plate, while 13 grafts were dead.

In the third type of experiments when the endoderm and mesoderm was removed from donors at stage 3, stage 4 and stage 5, it was observed that complete embryonic axis was not induced by any Ec graft. Only incomplete embryonic axis and neural plate was induced. The percentage of induction by the grafts A, B, C and D at different stages are as follows :-

Incomplete embryonic axis :

Graft A, showed percentage of induction of incomplete embryonic axis at stage 3 : 8.3%;

stage 4 : 16.0% and stage 5 : 40.0% (Fig.4.1A - Ec). Graft B, C and D did not show any induction of incomplete embryonic axis at stage 3, stage 4 and stage 5 (Figs. 4.1B - Ec, 4.1C - Ec).

Neural plate :

Graft A, showed percentage of induction of neural plate at stage 3 : 50.0%; stage 4 : 25.0% and stage 5 : 20.0% (Fig. 4.1A - Ec). Graft B, at stage 3 : 66.5%; stage 4 : 42.5% and stage 5 : 40.0% (Fig. 4.1B - Ec). Graft C, stage 3 : 80.0%; stage 4: 20.0% and stage 5 : 40.0% (Fig. 4.1C - Ec). Graft D, at stage 3 and stage 5 did not show any induction but at stage 4, the induction of neural plate is 20.0%.

The analysis of the percentage frequency of neural plate induction of A, B, C and D grafts at stage 3, stage 4 and stage 5 are as follows :-

Graft A, at stage 3 : 58.3%; stage 4 : 41.0% and stage 5 : 60.0% (Fig. 4.1A - Ec). Graft B, stage 3 : 66.5%; stage 4 : 42.5%; stage 5 : 40.0% (Fig. 4.1B - Ec). Graft C, stage 3 : 80.0%; stage 4 : 20.0% and stage 5 : 40.0% (Fig.4.1C - Ec). Graft D, at stage 3 and stage 5 did not show any induction, only at stage 4, induction of neural plate was observed which is 20.0% (Table 4.1.3).

TABLE 4.1.3

Structures induced by different types of grafts A, B, C and D of the primitive streak without endoderm and mesoderm at stages 3, 4 and 5 implanted into the host embryos nearing stage 4.

STAGE	TYPE OF GRAFTS	NO. OF GRAFTS IMPLANTED	MORTALITY		STRUCTURE INDUCED								% FREQUENCY OF NEURAL PLATE INDUCTION
			NO. DEAD	%	COMPLETE EMBRYONIC AXIS		INCOMPLETE EMBRYONIC AXIS		NEURAL PLATE ONLY		NO INDUCTION		
					NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	
3	A	12	4	33.3	0	0.0	1	8.3	6	50.0	1	8.3	58.3
	B	9	3	33.3	0	0.0	0	0.0	6	66.5	0	0.0	66.5
	C	5	1	20.0	0	0.0	0	0.0	4	80.0	0	0.0	80.0
	D	5	4	80.0	0	0.0	0	0.0	0	0.0	1	20.0	0.0
4	A	12	6	50.0	0	0.0	2	16.0	3	25.0	1	8.3	41.0
	B	7	4	57.1	0	0.0	0	0.0	3	42.5	0	0.0	42.5
	C	5	4	80.0	0	0.0	0	0.0	1	20.0	0	0.0	20.0
	D	5	4	80.0	0	0.0	0	0.0	1	20.0	0	0.0	20.0
5	A	5	2	40.0	0	0.0	2	40.0	1	20.0	0	0.0	60.0
	B	5	3	60.0	0	0.0	0	0.0	2	40.0	0	0.0	40.0
	C	5	3	60.0	0	0.0	0	0.0	2	40.0	0	0.0	40.0
	D	5	5	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0.0

Plate 4.1.3a Photomicrograph of chick embryo with the graft without endoderm and mesoderm of donor embryo stage 3 causing induction of neural plate only. Graft indicated by an arrow (x120).

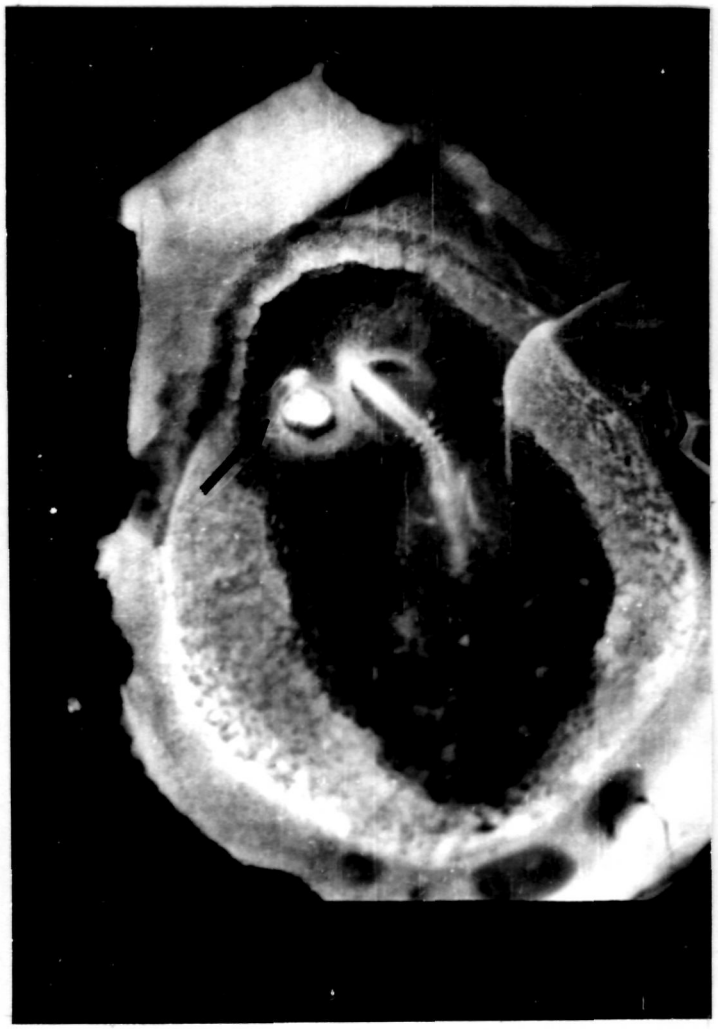


PLATE 4.1.3a

Plate 4.1.3b Photomicrograph of a section of chick embryo showing host neural tube and induced neural plate (x100).

Abbreviations :

Hnt - Host neural tube

Inp - Induced neural plate

Plate 4.1.3c Photomicrograph of the above induced neural plate under higher magnification (x250).

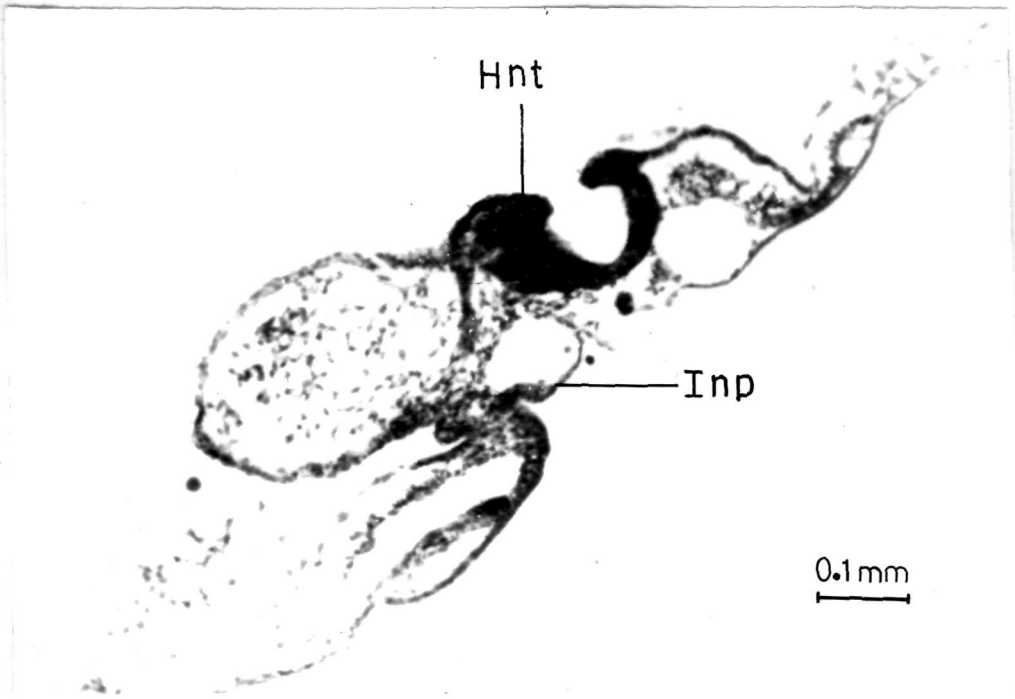


PLATE 4.1.3b

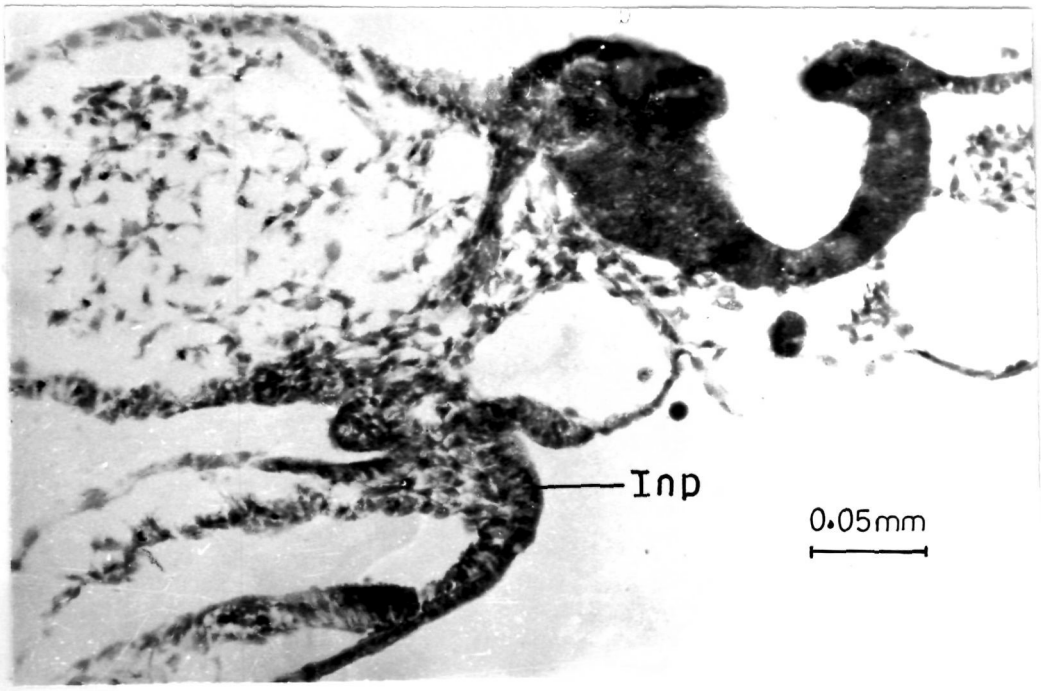


PLATE 4.1.3c

Fig. 4.1A Histograms representing percentage of inductive capacity of various structures induced by Graft A at stages 3, 4, 5 and 6 with all germs layers, without endoderm, and without endoderm and mesoderm.

Abbreviations :

EcMEn - Grafts with all germ layers

EcM - Grafts without endoderm

EC - Grafts without endoderm and mesoderm.

Structures induced :

- Complete embryonic axis
- Incomplete embryonic axis
- Induction of neural plate only

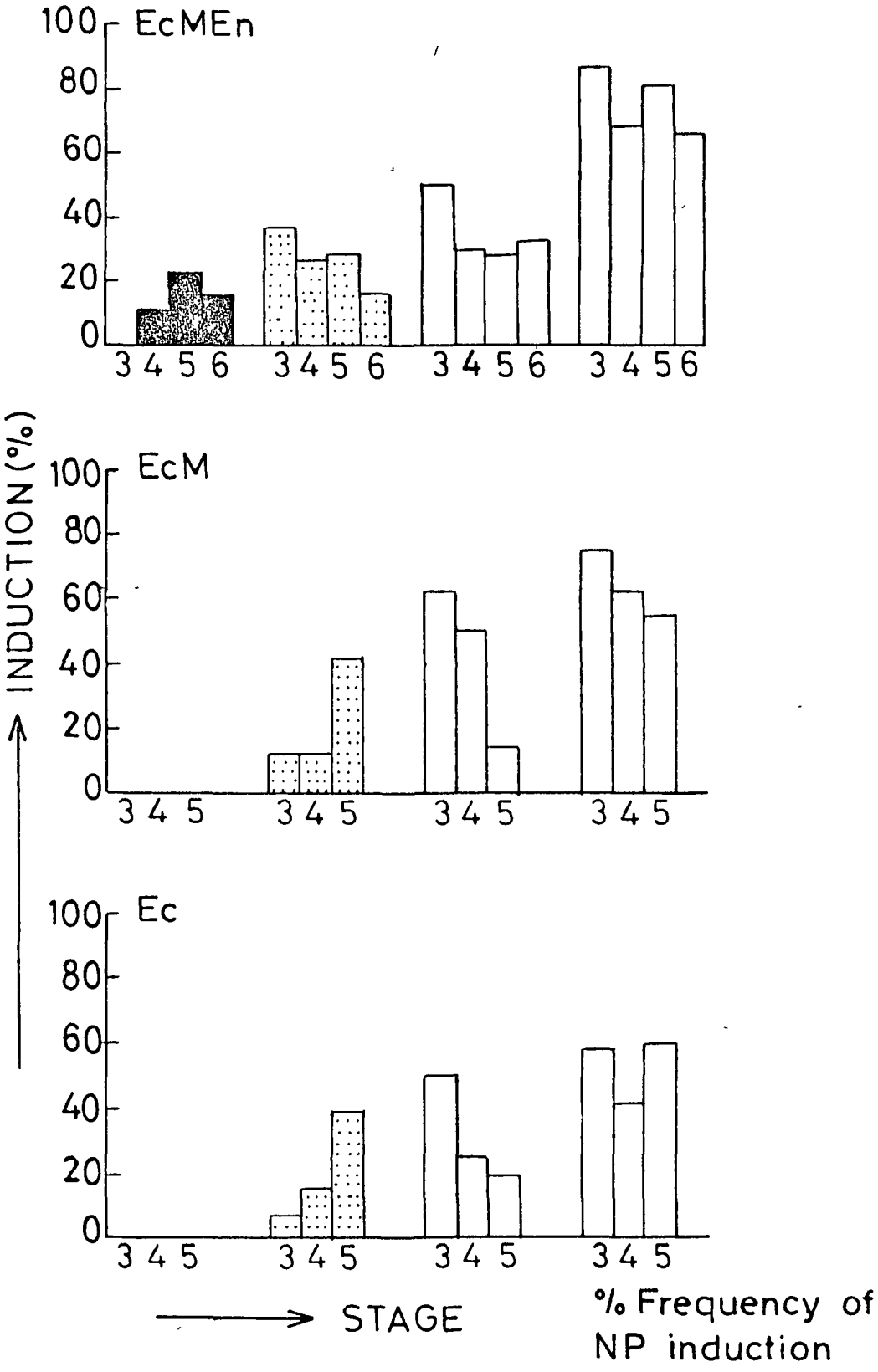

EcMEn
EcM
Ec
100
80
60
40
20
0
3 4 5 6
3 4 5
INDUCTION (%)
STAGE
% Frequency of NP induction

FIG. 4.1A

Fig. 4.1B Histograms representing percentage of inductive capacity of various structures induced by Graft B at stages 3, 4, 5 and 6 with all germs layers, without endoderm, and without endoderm and mesoderm.

Abbreviations :

EcMEn - Grafts with all germ layers

EcM - Grafts without endoderm

EC - Grafts without endoderm and mesoderm.

Structures induced :

- Complete embryonic axis
- Incomplete embryonic axis
- Induction of neural plate only

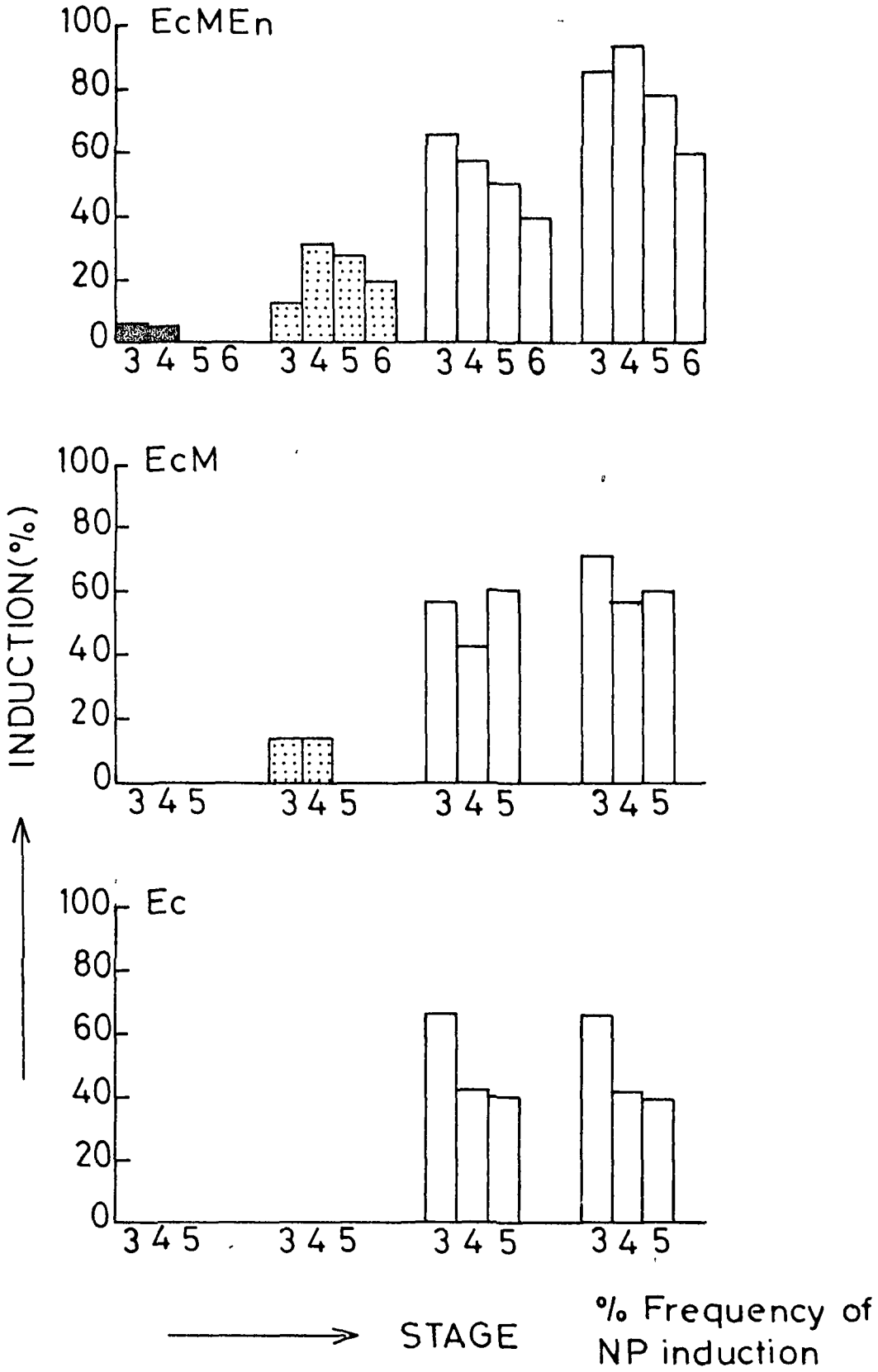

EcMEn
EcM
Ec
100
80
60
40
20
0
3 4 5 6
3 4 5
INDUCTION(%)
STAGE
% Frequency of NP induction

FIG. 4.1B

Fig. 4.1C Histograms representing percentage of inductive capacity of various structures induced by Graft C at stages 3, 4, 5 and 6 with all germs layers, without endoderm, and without endoderm and mesoderm.

Abbreviations :

EcMEn - Grafts with all germ layers

EcM - Grafts without endoderm

EC - Grafts without endoderm and mesoderm.

Structures induced :

- Incomplete embryonic axis

- Induction of neural plate only

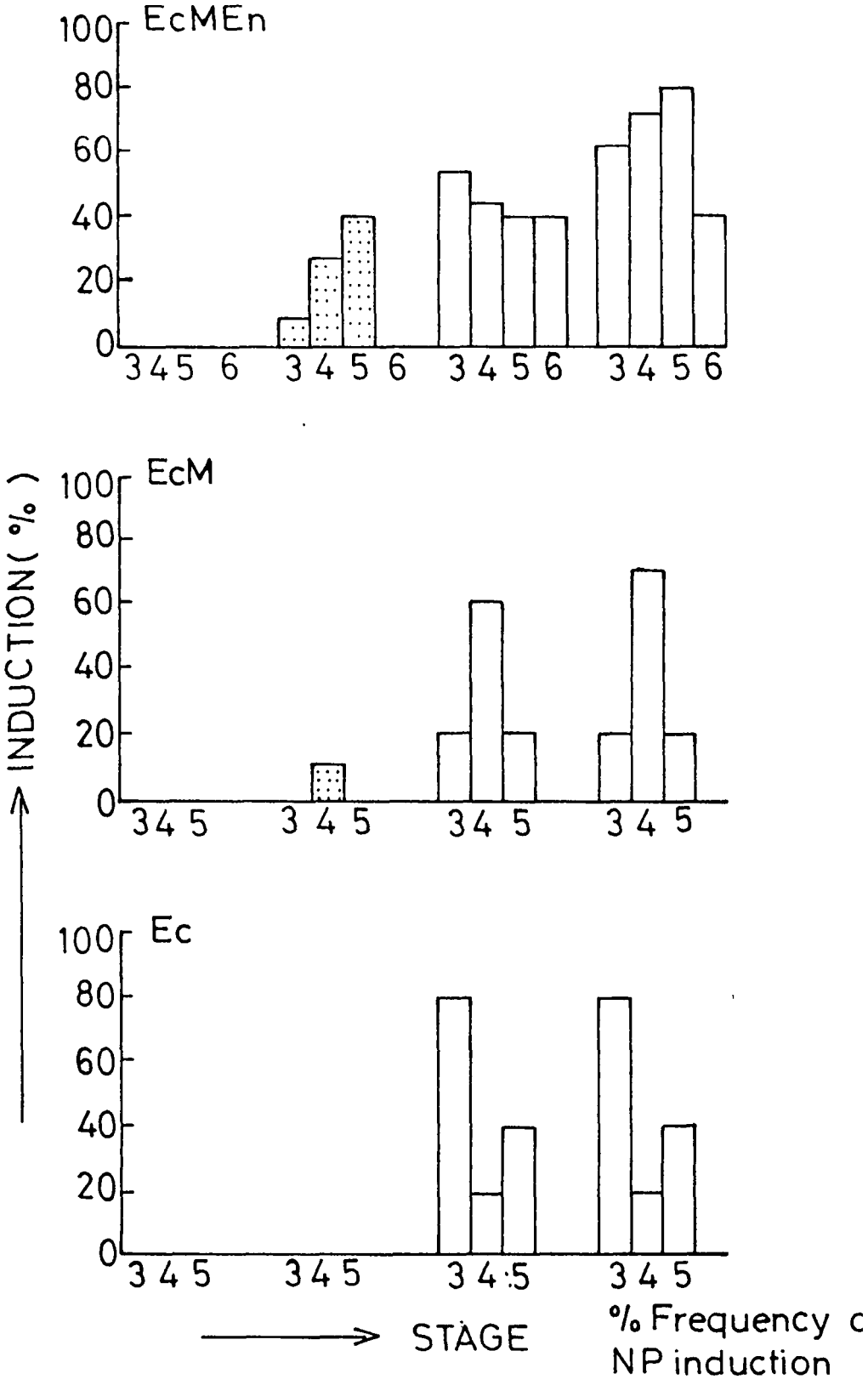

EcMEn
EcM
Ec
100
80
60
40
20
0
INDUCTION(%)
3 4 5 6
3 4 5
STAGE
% Frequency c
NP induction

FIG. 4.1C

Fig. 4.1D Histograms representing percentage of inductive capacity of various structures induced by Graft D at stages 3, 4, 5 and 6 with all germs layers, without endoderm, and without endoderm and mesoderm.

Abbreviations :

EcMEn - Grafts with all germ layers

Structures induced :

⊡ - Incomplete embryonic axis

□ - Induction of neural plate only

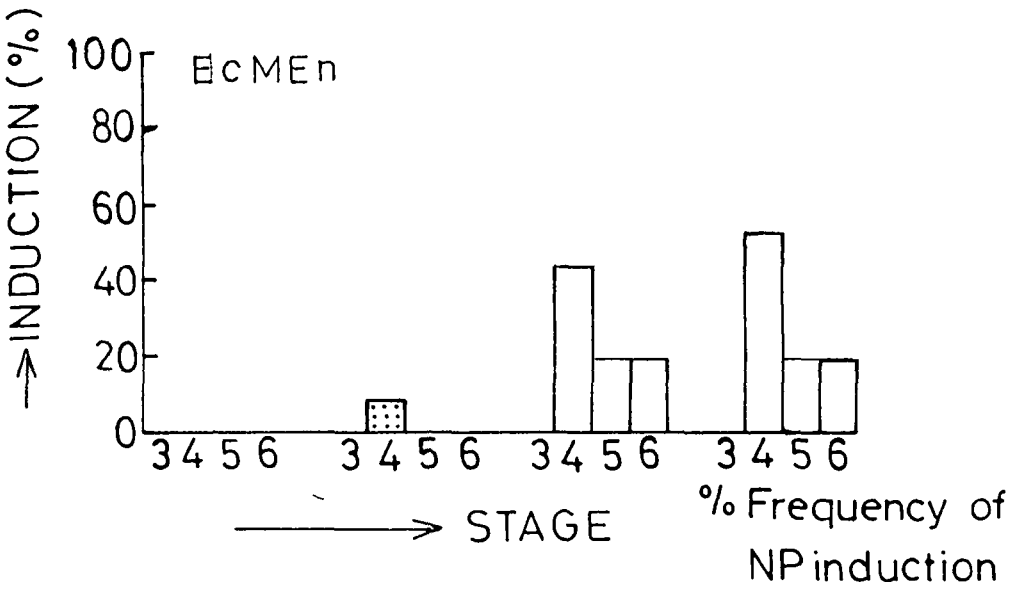

INDUCTION (%)
100
80
60
40
20
0
EcMEn
3 4 5 6
3 4 5 6
3 4 5 6
3 4 5 6
STAGE
% Frequency of
NP induction

FIG. 4·1 D

The three types of experiments performed showed that : (1) In the first set of experiments, the grafts A and B with all the germ layers at stages 3, 4, 5 and 6 induced complete embryonic axis. Incomplete embryonic axis was induced by A, B and C grafts, and by D grafts only at stage 4. Neural plate induction was achieved by all the grafts.

(2) In the second set of experiments when endoderm was removed from the inducing grafts, complete embryonic axis was not induced by any grafts. Incomplete embryonic axis was induced by A and B grafts at stages 3, 4 and 5 and by C grafts only at stage 4. Neural plate induction was induced by grafts A, B and C.

(3) In the third set of experiments when endoderm and mesoderm was removed from the inducing grafts, Complete embryonic axis was not induced by any grafts. Incomplete embryonic axis was induced only by graft A at stages 3, 4 and 5. Neural plate was induced by A, B and C grafts, and D graft of stage 4.

4.2 HISTOLOGICAL CHANGES IN THE NEURECTODERM INDUCED BY THE GRAFTS OF THE HENSEN'S NODE OF STAGES 4, 5 AND 6 AT DIFFERENT TIME INTERVALS

4.2.1 By the grafts having all germ layers.

The grafts of Hensen's node with all the germ layers designated as 4EcMEn, 5EcMEn and 6EcMEn with the number indicating the stage of the donor embryo were implanted below the ectoderm (Fig.3.7a) at the area pellucida almost at the level of the Hensen's node of the host embryo nearing H and H (1951) stage 4 and the cultures were returned to the incubator. The host embryos together intact with the grafts were removed from the incubator at different time intervals, fixed and processed for histological analysis. The histological changes in the reacting ectoderm were observed and recorded. The dimensions (length, width and nuclear diameter) of the difference cell types were measured. The different cell types were tall columnar cells whose length more than twice their breadth, cuboidal cells which appear more or less square in shape and their length is slightly longer than their

width; bottle-shaped cells which are narrow at their attached end and broad at the free end; rounded cells having a round or elliptical shape and irregular cells are irregular in shape.

A. Experiments with grafts of 4 EcMEn

The grafts 4 EcMEn were implanted in the hosts for time intervals of 5, 10, 15, 20, 25 and 30 minutes. The dimensions of length (L), width (W) and nuclear diameter (ND) of the different types of cells were measured and recorded in Table 4.2.1A and Fig. 4.2.1. Diagramatic representation of the histological changes in the induced ectoderm at different time intervals of contact with the grafts shown in Figs. 4.2.1(i) and 4.2.1(ii).

Contact period : 5 minutes : Even for 5 minutes of contact the induced portion of the ectodermal layer showed no neuroid response. The cells were seen to have undergone no morphological change when compared to uninduced parts. An average of 18 cells were present at the point of contact with the graft. The cells were bottle-shaped and cuboidal with distinct inter-cellular spaces in between them. Their length varied from 15 μm to 18 μm, width 6 μm to 12 μm and nuclear diameter 3 μm to 4.5 μm.

Contact period : 10 minutes : The resulting ectodermal layer in sections at 10 minutes of contact appeared as a thickened neural plate. There was an average of 20 cells at the point of contact with the graft in this layer. Many cells appeared bottle-shaped or pyramidal, some were elongated or cuboidal, a few were rounded or irregular shaped. Intercellular space was reduced. At the point of contact between the graft and the host ectoderm most of the cells were closely packed with one another with little intercellular spaces. The length of these cells varied from 12 µm to 15 µm, width 6 µm to 8 µm and ND 2.5 µm to 6 µm.

Contact period : 15 minutes : The induced neural plate is now a thickened strip of cells. At this stage an average of 20 cells were present. The cells of this area at this stage appeared bottle-shaped or cuboidal cells with very little intercellular space. After 15 minutes of contact the cells appear to have become more elongated, stratified and closely packed with one another (Plates 4.2.1a, 4.2.1b). The length of the cells varied from 9 µm to 16.0 µm, width 6 µm to 7.5 µm and their ND 1.5 µm to 6 µm.

Plate 4.2.1a Photomicrograph of a section of chick embryo showing induced neural plate when graft of stage 4 with all germ layers was kept in contact for 15 minutes (x 100).

Abbreviations :

G - Graft

Inp - Induced neural plate

Plate 4.2.1b Photomicrograph of the above induced neural plate under higher magnification (x 250).

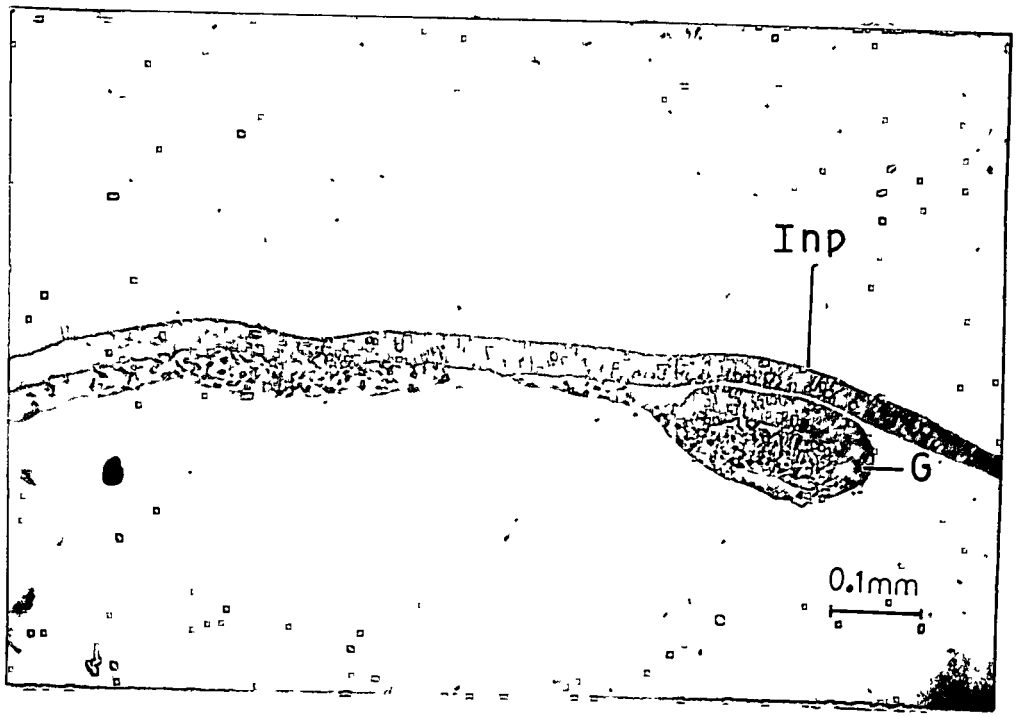


PLATE 4.2.1a

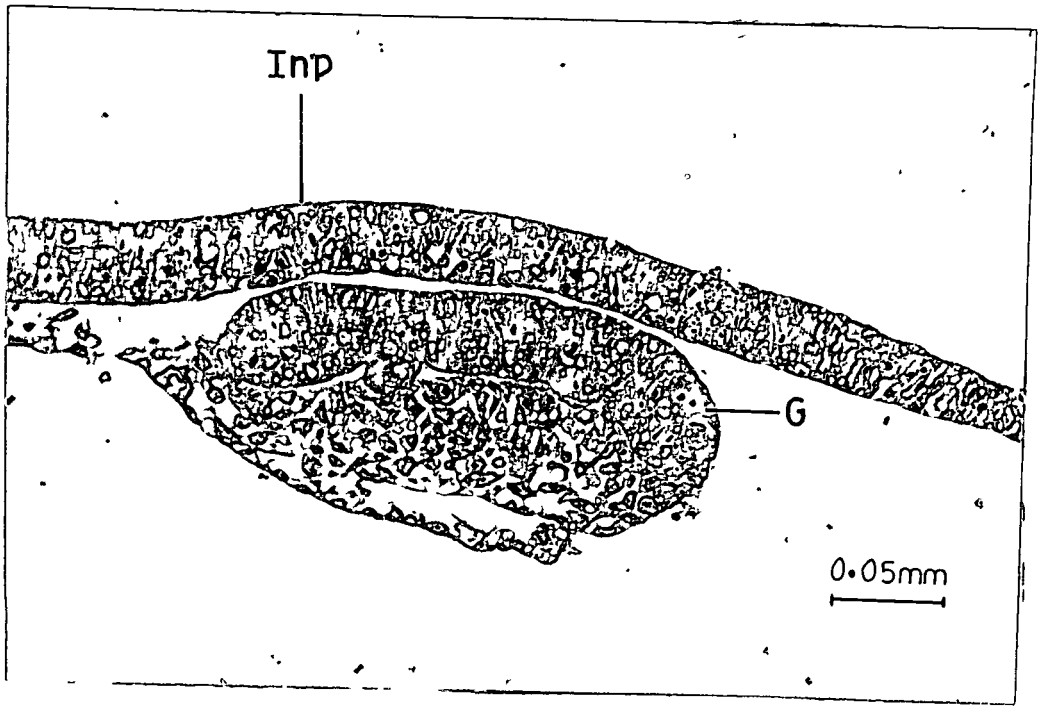


PLATE 4.2.1b

Contact period : 20 minutes : The entire reacting ectodermal layer appeared as a thickened neural plate with the formation of a groove, like neural groove. The average number of cells present was 27. The cells were of various shapes and sizes. Some were rounded, bottle-shaped but most were cuboidal Their length varied from 7.5 µm to 15 µm, width 6 µm and their ND 3 µm to 4.5 µm.

Contact period : 25 minutes : The entire reacting ectodermal layer appeared as a thickened neural plate. The average number of cells present was 23. Most of the cells were cuboidal or bottle-shaped and they were closely packed with less intercellular spaces between them. Their length varied from 9 µm to 15 µm, width 6 µm to 7 µm and their ND 3 µm to 4 µm.

Contact period : 30 minutes : The reacting ectodermal layer showed a distinct neural groove (Plates 4.2.1c, 4.2.1d). At this stage the average number of cells present in the induced neural plate was 30.Most of the cells at the particular point of contact with the graft appeared to be very much stratified with layers of cuboidal cells interspersed with few bottle-shaped cells. The base of some of the cells at the periphery was slightly broad and flattened and these cells were densely packed with no intercellular.

Plate 4.2.1c Photomicrograph of a section of chick embryo showing induced neural plate when graft of stage 4 with all germ layers was kept in contact for 30 minutes (x100).

Abbreviations :

G - Graft

Hnp - Host neural plate

Inp - Induced neural plate

Plate 4.2.1d Photomicrograph of the above induced neural plate under higher magnification (x250).

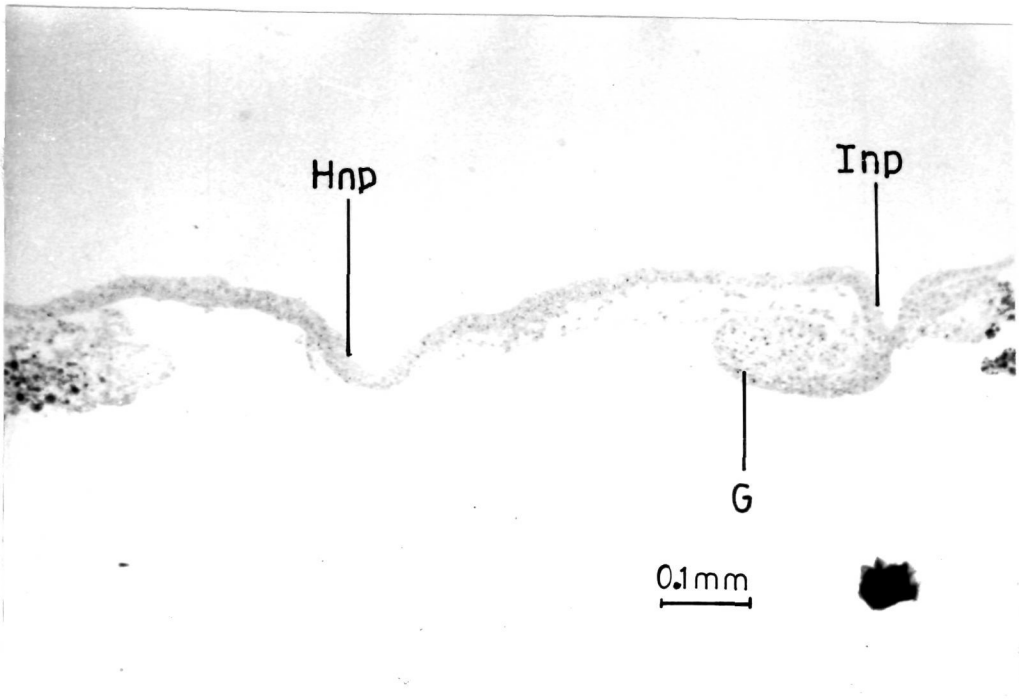


PLATE 4.2.1c

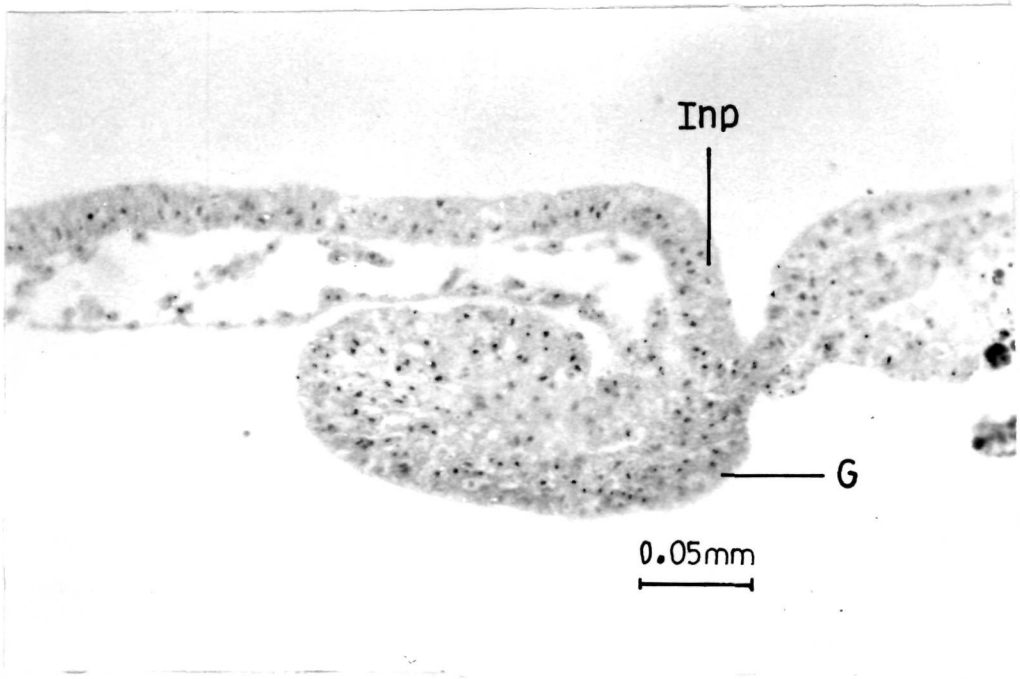


PLATE 4.2.1d

space. These different types of cells measured 9 μm to 16.0 μm in length, 6 μm in width and their ND was 3 μm.

B. Experiments with grafts 5 EcMEn

The changes induced in the host ectoderm by grafts of Hensen's node of stage 5 after a time interval of 5, 10, 15, 20, 25 and 30 minutes were observed. The type of cells observed and their dimensions has been illustrated in Table 4.2.1B and Fig. 4.2.1.

Contact period : 5 minutes : In comparison to uninduced portions, the host ectoderm in contact with the graft 5 EcMEn showed no neuroid response even at 5 minutes of contact. The number of cells present in reacting ectoderm at this stage were 14. It appeared stratified and formed a thin neural plate. The cells were of varying shapes and sizes. Most cells were cuboidal or bottle-shaped. The cells were not closely packed but had distinct intercellular spaces between them. The length of cells ranged from 9 μm to 12 μm, width 6 μm to 7.5 μm and their ND varied from 3 μm to 4.5 μm.

Contact period : 10 minutes : A prominent change was observed in the host tissue after contact with the graft tissue for this period. The host

ectodermal layer appeared as a thickened neural plate which showed formation of a neural groove (Plates 4.2.1e and 4.2.1f). It had 32 cells present at the point of contact with the graft. The cells were tightly packed without intercellular spaces in between them. Many cells were bottle-shaped or tall columnar and a few were round or irregular in shape. Their length varied from 6 µm to 15 µm, width 4.5 µm to 7 µm and their ND was 3 µm.

Contact period : 15 minutes : After 15 minutes of contact with the graft the host ectoderm showed the induction of a structure resembling neural groove on the right side of the host neural tube. There was 32 cells present at the point of contact with the graft. In this particular region most of the cells were bottle-shaped and tall columnar, and a few were broad and rounded. Large intercellular spaces were seen in between some irregular shaped cells. The length of cells varied from 10 µm to 16.5 µm, width 6 µm to 9 µm and their ND 3 µm to 4.5 µm.

Contact period : 20 minutes : The thickening of host ectoderm to form a neural plate under the inductive influence of the graft was prominent at

Plate 4.2.1e Photomicrograph of a section of chick embryo showing induced neural plate when graft of stage 5 with all germ layers was kept in contact for 10 minutes (x100).

Abbreviations :

G - Graft

Hnp - Host neural plate

Inp - Induced neural plate

Plate 4.2.1f Photomicrograph of the above induced neural plate under higher magnification (x400).

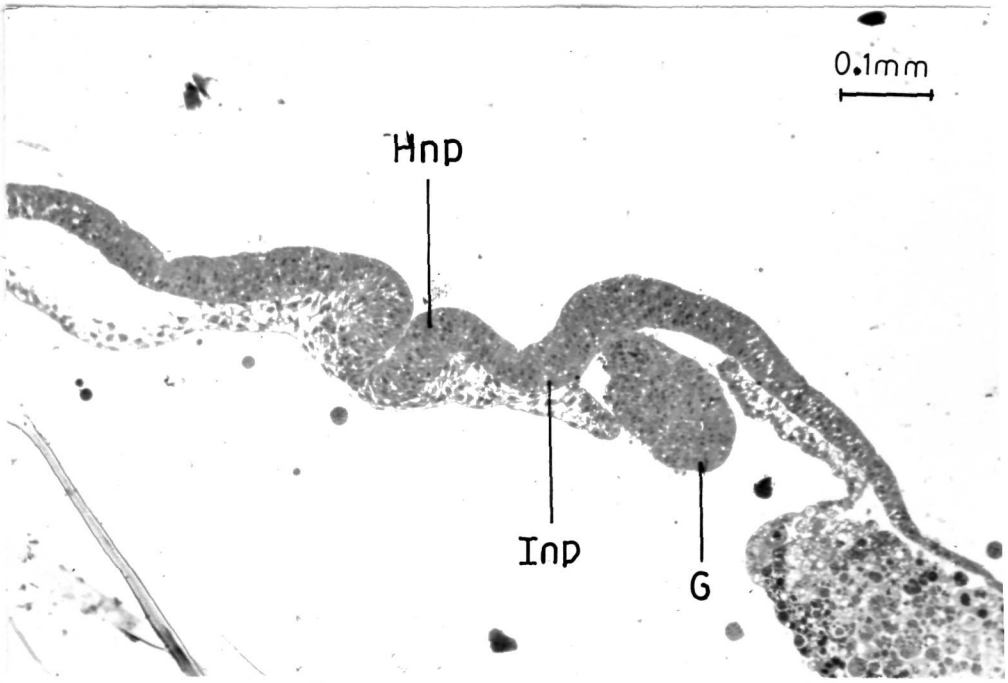


PLATE 4.2.1e

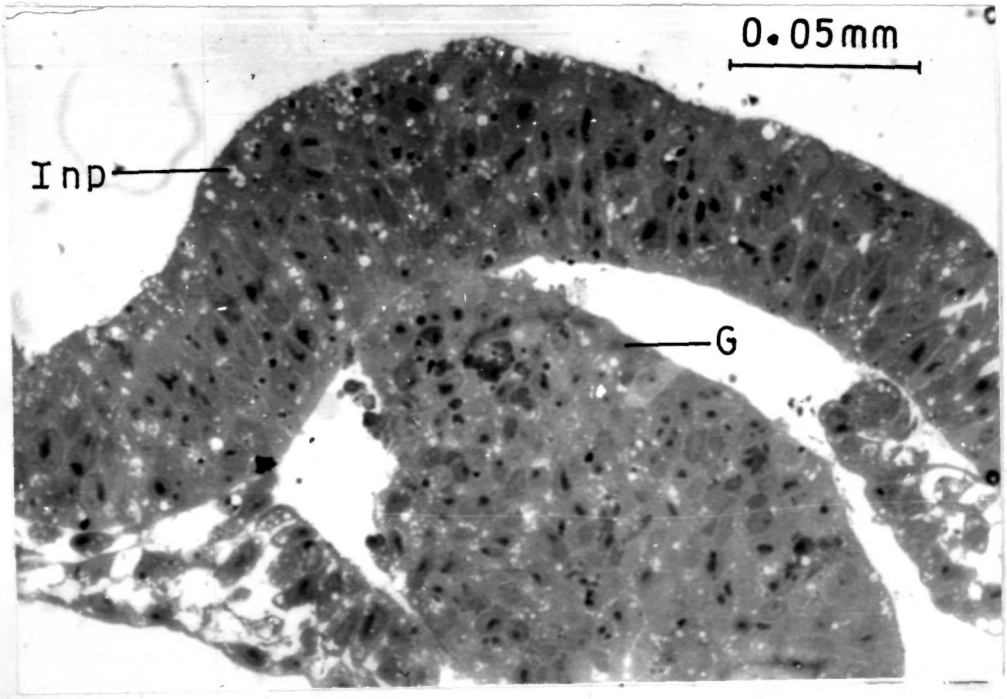


PLATE 4.2,1f

20 minutes contact. 20 cells were present at the point of contact. Some cells of the induced neural plate were large and columnar with large intercellular spaces in between them. Most of the cells were cuboidal. Their length varied from 7.5 µm to 16.5 µm, width 6 µm to 9 um and their ND 3 µm to 4.5 um.

Contact period : 25 minutes : The induced neural plate is now very prominent and thickened. The number of cells present at the point of contact with the graft was 27. These cells were cuboidal, bottle-shaped or rounded with large intercellular spaces in between them. The length of the cells varied from 7.5 µm to 14 µm, width 6 µm to 7 µm and their ND 3 µm to 4.5 µm.

Contact period : 30 minutes : The section of the host blastoderm after contact with the graft for 30 minutes showed a clear thickened neural plate with neural groove in the host ectoderm to the right side of the host neural groove. 16 cells were present. Most of the cells were elongated, bottle-shaped and closely packed without any intercellular spaces in between them. Their length varied from 10.5 µm to 16.5 µm, width 6 µm to 7 µm and their average ND was 3 µm.

C. Experiments with grafts 6 EcMEn

Since the pilot experiments performed with grafts 6 EcMEn did not elicit any neuroid response in the host tissues till after 50 minutes of contact. Only two experiments were performed (1) with 50 minutes of contact and (2) with 2 hours of contact only. The cellular morphology of the responding ectoderm was observed and the types of cells present and their dimensions is recorded in Table 4.2.1C.

Contact period : 50 minutes : The responding ectoderm did not show any neuroid response and remained as a thin layer of cells. The host cells at the point of contact with the graft were loosely attached with one another. The number of cells present in this area was 18. These were largely broad and short and a few were rounded in shape. Intercellular spaces were seen between them. The length of these cells was seen to vary from 9 µm to 15 µm, width 6 µm to 8 µm and their ND was 3 µm to 4 µm.

Contact period : 2 hours : The responding host ectoderm had formed a thickened neural plate with slight depressions at some regions. At the point of contact between the graft and the host ectoderm the number of cells present was 25. The cells in the induced ectoderm were of varying shapes. Some

TABLE 4.2.1 A

Changes in cell size during neuroid response induced by grafts of the Hensen's node of stage 4 embryo having all germ layers at different time interval of contact with the graft.

		DIMENSIONS (μm)																	
TIME INTERVAL (MINUTES)		5			10			15			20			25			30		
TYPES OF CELLS		L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND
BOTTLE-SHAPED CELL	C	14	8	3	14	8	4	14	6	3	12	8	3	12	6	3	14	6	3
	I	15	6	2	15	8	2.5	16	7.5	6	15	6	4	15	6	4	16	6	3
CUBOIDAL CELL	C	8	7	4	9	6	4	9	7	4	12	8	3	9	7	4	12	8	3
	I	10	6	2	12	8	4	12	7.5	4	9	6	3	9	7	4	9	6	3
TALL COLUMNAR CELLS	C	18	4	3	18	4.5	3	18	4.5	3	-	-	-	12	6	4	10	6	3
	I	18	6	3	15	6	6	15	6	1.5	-	-	-	15	6	3	9	6	3
ROUNDED CELLS	C	12	10	3	-	-	-	-	-	-	7	6	3	-	-	-	-	-	-
	I	15	12	4.5	-	-	-	-	-	-	7.5	6	3	-	-	-	-	-	-
IRREGULAR SHAPED CELLS	C	-	-	-	10	4	3	8	6	3	8	6	3	-	-	-	-	-	-
	I	-	-	-	12	6	3	9	6	3	9	6	4.5	-	-	-	-	-	-
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I	18			20			20			27			23			30		
NEUROID RESPONSE		(-)			(+)			(+)			(+)			(+)			(+)		

L - Length; W - Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm; (+) Presence of Neuroid response; (-) Absence of Neuroid response.

TABLE 4.2.1B

Changes in cell size during neuroid response induced by grafts of the Hensen's node of stage 5 embryo having all germ layers at different time interval of contact with the graft.

		DIMENSIONS (μm)																	
TIME INTERVAL (MINUTES)		5			10			15			20			25			30		
TYPES OF CELLS		L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND
BOTTLE-SHAPED CELL	C	10	5	4	12	7	3	12	6	3	13	8	3	12	6	3	12	6	3
	I	12	6	4.5	14	7	3	16	7	3	16	8	3	14	7	4	16	7	3
CUBOIDAL CELL	C	8	7	4	8	6	3	8	6	3	9	3	1.5	9	6	4.5	-	-	-
	I	10	7	4	9	6	3	10	8	3	9	7	4	12	6	3	12	6	3
TALL COLUMNAR CELLS	C	9	6	4.5	14.5	6	3	16	6	3	15	9	4	12	6	3	15	6	3
	I	12	6	3	15	6	3	16.5	6	3	16.5	6	4.5	12	0	4.5	16.5	6	3
ROUNDED CELLS	C	8	6	3	8	6	3	6	6	3	6	6	3	-	-	-	-	-	-
	I	9	7.5	4.5	9	6	3	6	6	4.5	7.5	6	3	7.5	6	3	10.5	6	3
IRREGULAR SHAPED CELLS	C	8	7	3	-	-	-	9	6	3	-	-	-	-	-	-	-	-	-
	I	9	6	3	6	4.5	3	10.5	9	3	-	-	-	-	-	-	-	-	-
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I		14			32			32			20			27			16	
NEUROID RESPONSE			(-)			(+)			(+)			(+)			(+)			(+)	

L - Length; W - Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm; (+) Presence of Neuroid response; (-) Absence of Neuroid response.

TABLE 4.2.1C

Changes in cell size during neuroid response induced by grafts of the Hensen's node of stage 6 embryo having all germ layers at different time interval of contact with the graft

		DIMENSIONS (μm)					
TIME INTERVAL		50 mins.			2 hrs.		
TYPES OF CELLS		L	W	ND	L	W	ND
BOTTLE-SHAPED CELLS	C	14	6	4	14	8	3
	I	12	6	3	15	8	3
CUBOIDAL CELLS	C	14	8	4	12	6	3
	I	15	8	3	12	6	3
TALL COLUMNAR CELLS	C	16	6	3	-	-	-
	I	-	-	-	-	-	-
ROUNDED CELLS	C	8	6	3	6	6	3
	I	9	6	4	9	6	3
IRREGULAR SHAPED CELLS	C	7	4	3	6	6	3
	I	9	4.5	3	12	6	3
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I		18			25	
NEUROID RESPONSE			(-)			(+)	

L - Length; W - Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm;
(+) Presence of Neuroid reponse; (-) Absence of Neuroid response

Fig. 4.2.1 Changes in the length of bottle-shaped cells in the reacting ectoderm after varying periods of contact with EcMEn grafts at stage 4 and stage 5. (with all germ layers intact).

O—O Length in the host induced neurectoderm.

●--● Length in the host normal neurectoderm.

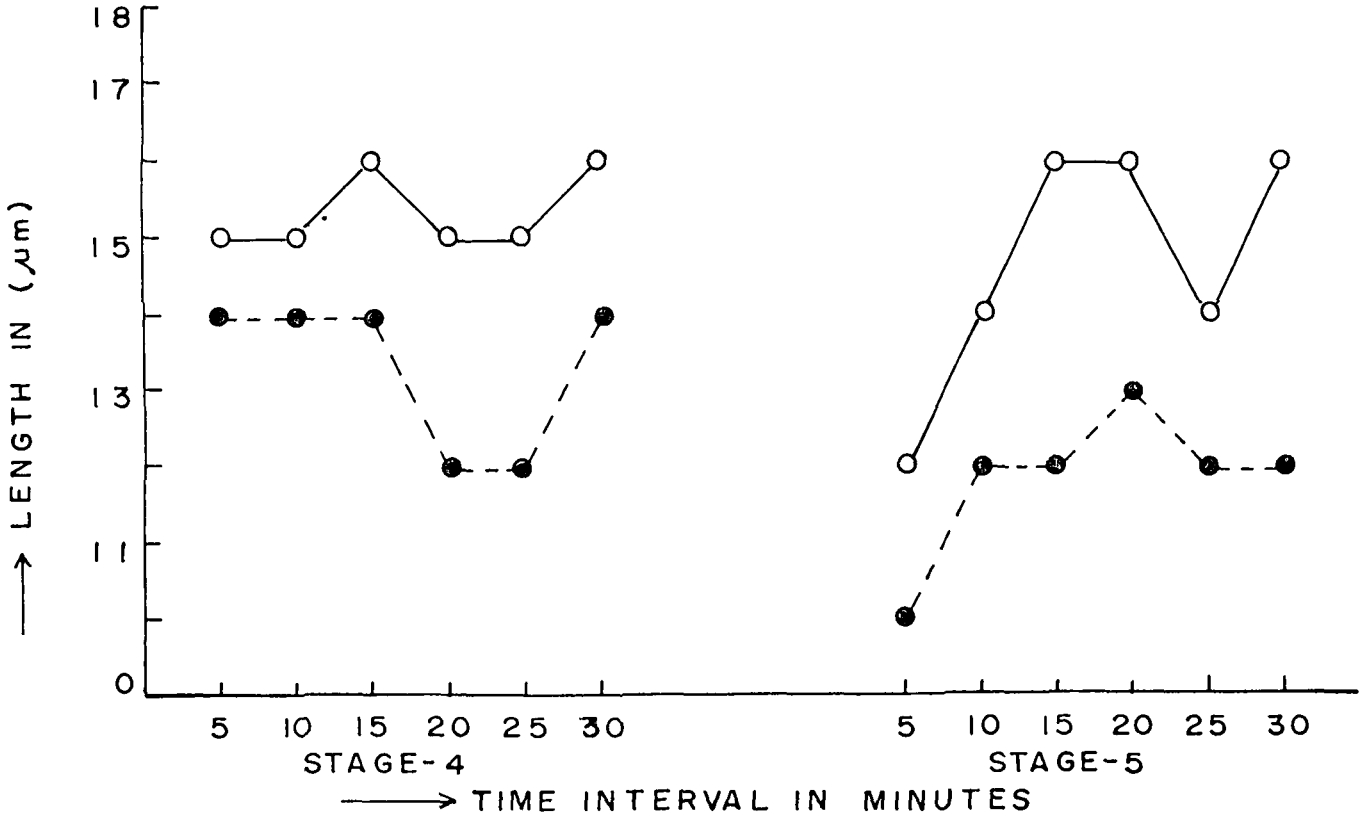

18
17
15
13
11
0
LENGTH IN (μm)
5 10 15 20 25 30
STAGE-4
5 10 15 20 25 30
STAGE-5
TIME INTERVAL IN MINUTES

FIG. 4·2·1

Fig. 4.2.1 (i) Diagramatic representation of the changes in the reacting ectoderm when graft was kept in contact at different time intervals (A) for 5 minutes (B) 10 minutes (C) 15 minutes.

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

G - Graft

RC - Rounded cells

TCC - Tall columnar cells

r - reacting ectoderm

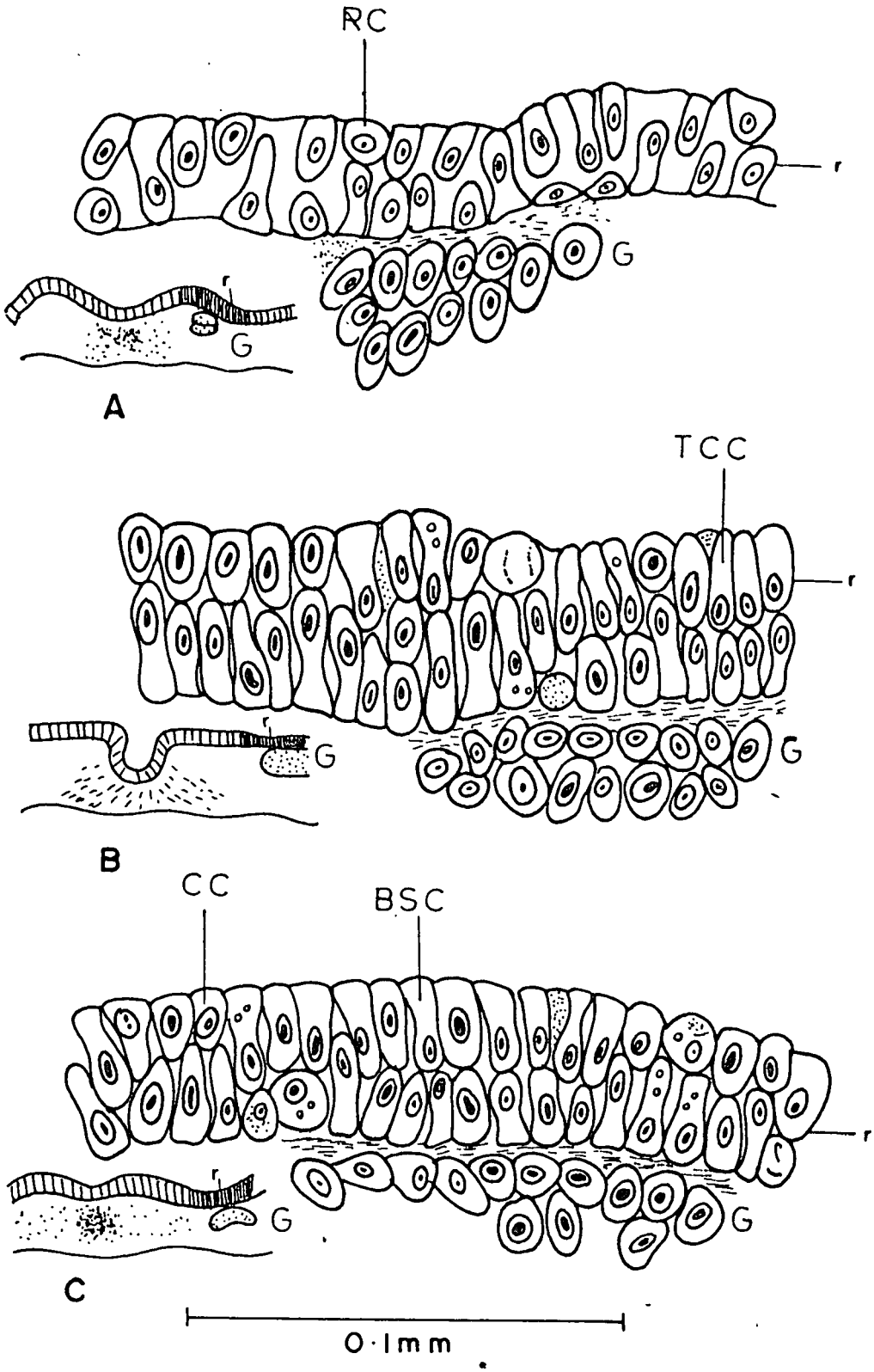


FIG. 4.2.1(i)

Fig. 4.2.1 (ii) Diagramatic representation of the changes in the reacting ectoderm when graft was kept in contact at different time intervals (A) for 20 minutes (B) 25 minutes (C) 30 minutes.

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

G - Graft

RC - Rounded cells

TCC - Tall columnar cells

r - reacting ectoderm

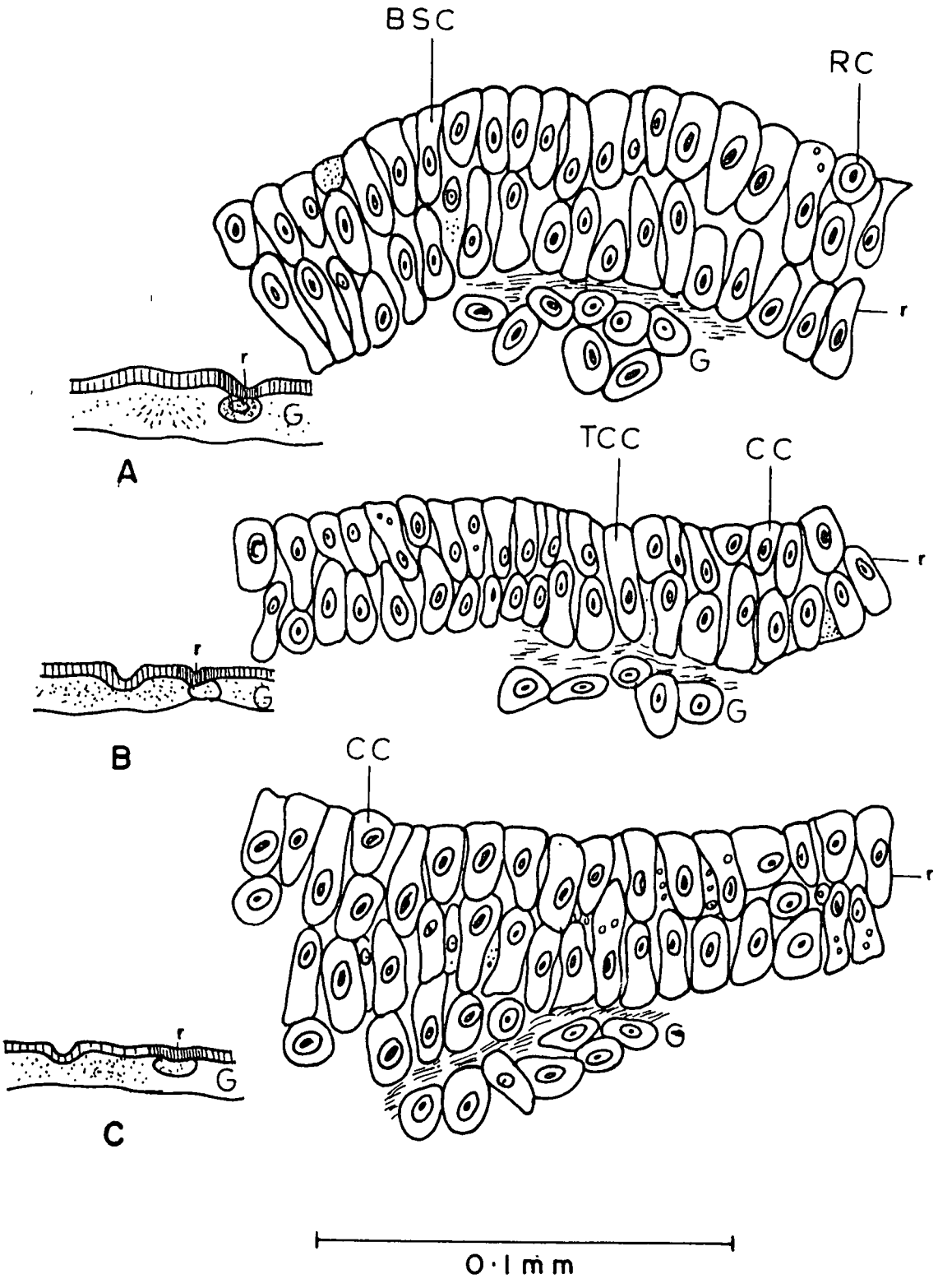


FIG. 4.2.1 (ii)

were cuboidal, others rounded and few were irregular in shape. The cells were closely packed with little intercellular space. Their length varied from 9 µm to 15 µm, width 6 µm to 8 µm and their average ND was 3 µm.

4.2.2. By the grafts without endoderm

The grafts of Hensen's node without endoderm designated as 4 ECM, 5ECM and 6 EcM with the number indicating the stage of the donor embryo were implanted below the ectoderm (Fig.3.7a) at the area pellucida almost at the level of the Hensen's node of the host embryo nearing H and H (1951) stage 4 and the cultures were placed back in the incubator at different time intervals. The host embryo together intact with the grafts were removed from the incubator at different time interval, fixed and processed for embedding in plastic section and sectioned for histological analysis. The histological changes in the induced ectoderm were observed. The dimensions of different cell types were measured.

A. Experiments with grafts 4 EcM

Following is the account of the changes observed in the responding host after the grafts of

Hensen's node at stage 4 without endoderm removed, were placed in contact with competent host ectoderm for varying periods 10, 15, 20, 25 and 30 minutes. In pilot experiments, no neuroid response was observed when the contact period was observed less than 10 minutes. The histological changes observed and the types of cells present and their dimensions are given in Table 4.2.2A and Fig. 4.2.2.

Contact period : 10 minutes : The host ectodermal layer remained very thin as a single strip of cells. The average number of cells present in this layer at the point of contact with the graft was 6. Most of the cells were cuboidal, some slightly broader at their base and tapered towards the free end and had intercellular spaces in between them. Their length varied from 9 µm to 10 µm, width 4.5 µm to 8 µm and their average ND was 3 µm.

Contact period : 15 minutes : The reacting host ectoderm was now seen as a thickened layer of ectodermal cells. The number of cells present in this layer was 20. The cells were elongated and were arranged with some intercellular spaces in between them (Plates 4.2.2a and 4.2.2b). The length of the cells varied from 9 µm to 12 µm, average width 6 µm and their average ND 3 µm.

Plate 4.2.2a Photomicrograph of a section of chick embryo showing induced neural plate when graft of stage 4 without endoderm was kept in contact for 15 minutes (x100).

Abbreviations :

G - Graft

Inp - Induced neural plate

Plate 4.2.2b Photomicrograph of the above induced neural plate under higher magnification (x400).

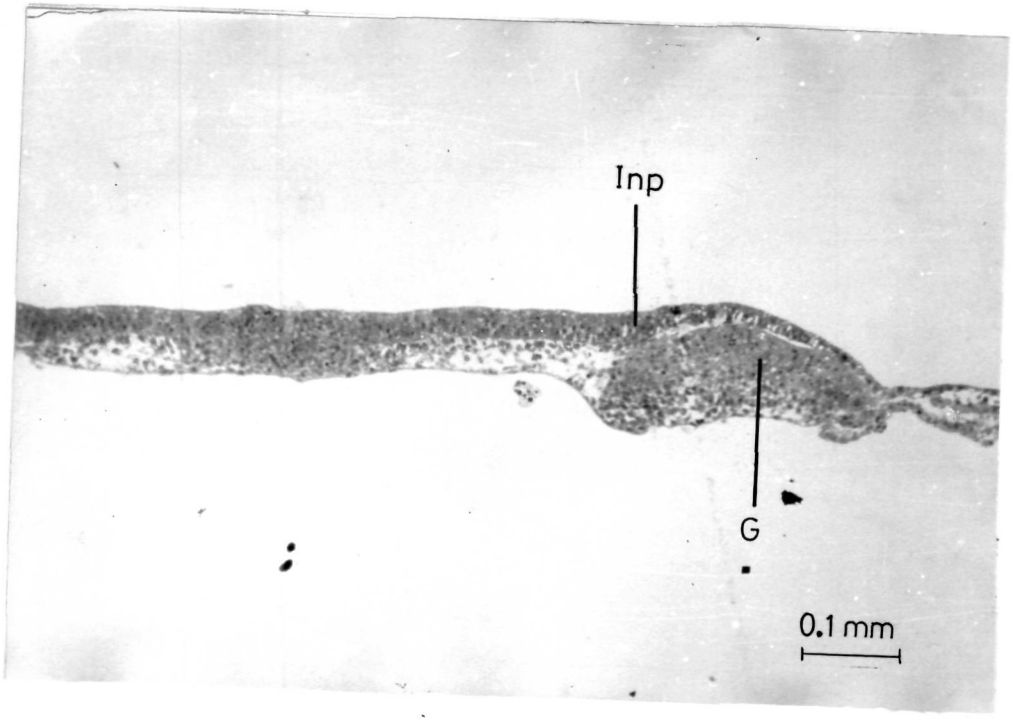


PLATE 4.2.2a

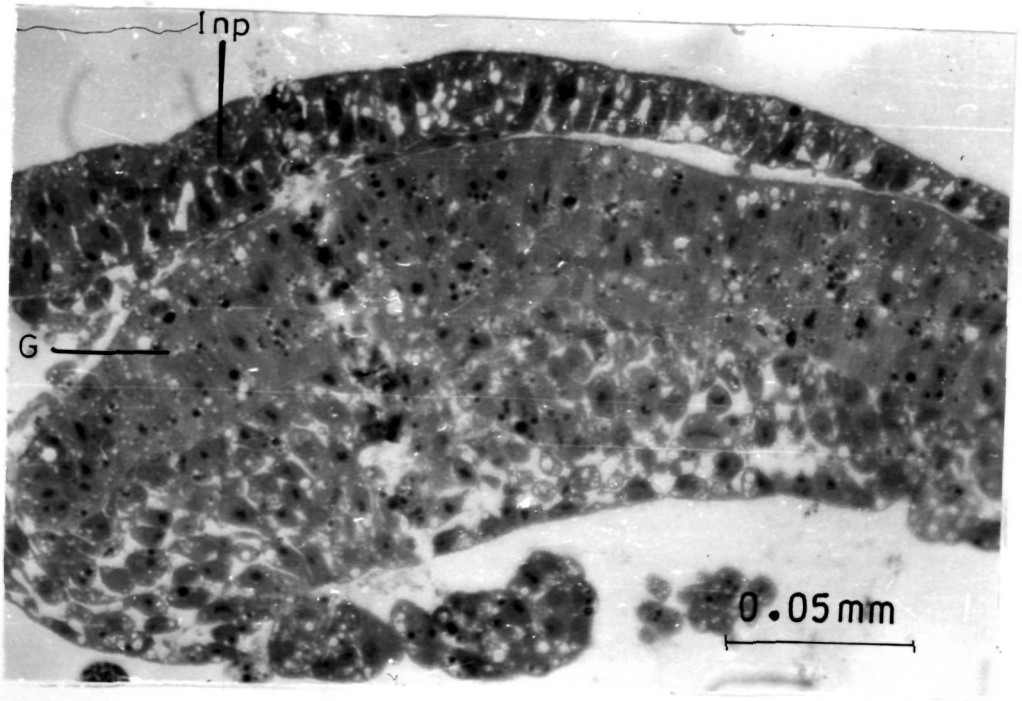


PLATE 4.2.2b

Contact period : 20 minutes : The host ectoderm appeared as a thickened layer with 13 cells at the point of contact with the graft. The cells were large, elongated and bottle-shaped with large intercellular spaces in between them. The length of the cells varied from 9 µm to 15 µm, width 6 µm and their ND 2.5 µm to 3 µm.

Contact period : 25 minutes : The host ectoderm layer was now somewhat thickened with the formation of slight depression at the point of contact with the graft. The number of cells present at this point in the induced ectoderm was 21. The cells were densely packed without any intercellular space. Their length varied from 7.5 µm to 15 µm, width 4 µm to 9 µm and their ND was 3 µm to 4.5 µm.

Contact period : 30 minutes : The entire induced neurectoderm had thickened to form a neural plate. At the point of contact between the graft and the host ectoderm, a slight depression representing a neural groove was formed. The number of cells present at this point in the induced ectoderm was 30. The cells in this area were generally large, and elongated, cuboidal or bottle-shaped with a few rounded cells. Some cells were densely packed without any intercellular space. The cells varied in length from 6 µm to 15 µm, width 6 µm to 8 µm and their ND 1.5 µm to 4 µm.

B. Experiments with grafts 5 EcM

The graft of Hensen's node was prepared without endoderm at stage 5 and kept in contact with the ectoderm of the host embryo nearing stage 4. The host were fixed after 10, 15, 20, 25 and 30 minutes after the implantation of the graft. There was no neuroid response when the period of contact was less than 10 minutes. The changes in the induced ectoderm have been observed. Different types of cells observed and their dimensions have been illustrated in Table 4.2.2 B and Fig. 4.2.2.

Contact period : 10 minutes : The reacting ectoderm remained as a thin strip of cells. There was no change in the cellular morphology of the cells. The number of cells present in this layer was 14. Most cell were cuboidal and they are not stratified. The length of the cells varied from 9 µm to 14 µm, width 6 µm to 10.5 µm and their ND 3 µm to 1.5 µm.

Contact period : 15 minutes : At 15 minutes of contact with the graft, the cells of the reacting host ectodermal layer acquire various shapes. Most cells were elongated, columnar and densely packed. The number of cells present was 13. Their length varied from 9 µm to 15 µm, width 6 µm to 8 µm and their ND 1.5 µm to 4.0 µm.

Contact period : 20 minutes : The entire induced neuroectodermal layer appeared to be thickened and had 30 cells. The cells were elongated but slightly smaller in size and closely packed without any intercellular spaces. The length of the cells varied from 9 µm to 15 µm, width 6 µm to 8 µm and their ND 1.5 µm to 3.0 µm.

Contact period : 25 minutes : At 25 minutes of contact with the graft, the reacting host ectodermal layer formed a thickened neural plate. The number of cells was 28. The cells were mostly columnar and closely packed without intercellular space. Morphologically, the cells at the region of the formation of a groove were flat and broad. The length of the cells varied from 7.5 µm to 15 µm, their width was 6 µm to 8 µm and ND varied from 3 µm to 4.5 µm.

Contact period : 30 minutes : At this period of contact with the graft the entire reacting ectodermal layer was seen as a thickened neural plate (Plates 4.2.2c and 4.2.2d). The number of cells present in it was 30. The cells were slightly larger in size and elongated. The bases of some of the cells were broad. Many irregular shaped cells were also observed. The entire induced tissue was stratified. The length of cells ranged from 9 µm to 18 µm, width 6 µm to 7 µm and ND 1.5 µm to 4 µm.

Plate 4.2.2c Photomicrograph of a section of chick embryo showing induced neural plate when graft of stage 5 without endoderm was kept in contact for 30 minutes (x100).

Abbreviations :

G - Graft

Hnp - Host neural plate

Inp - Induced neural plate

Plate 4.2.2d Photomicrograph of the above induced neural plate under higher magnification (x250).

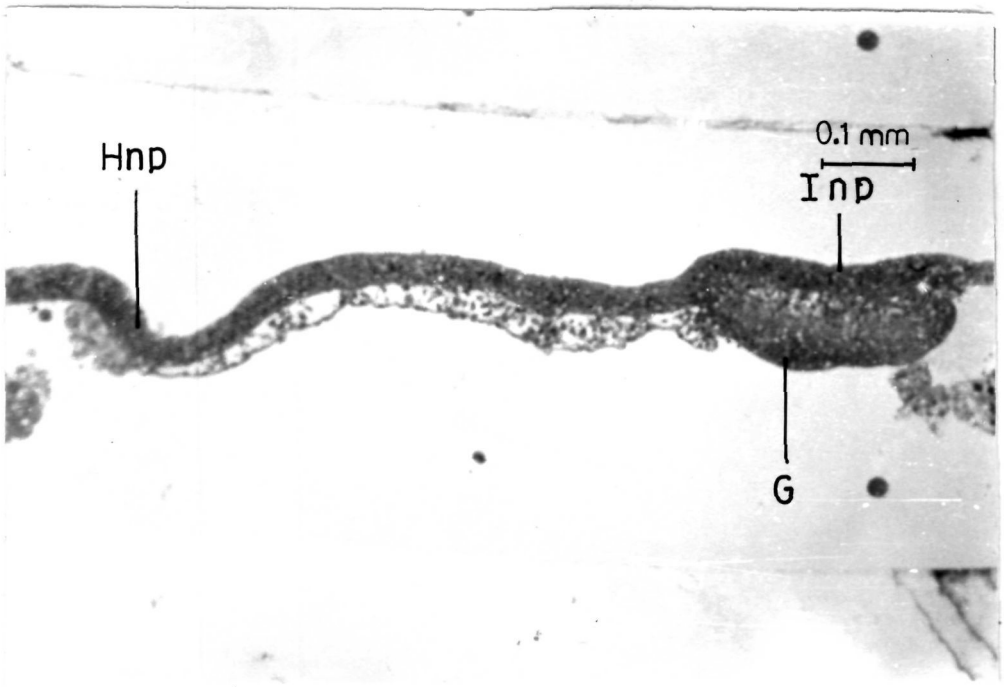


PLATE 4.2.2c

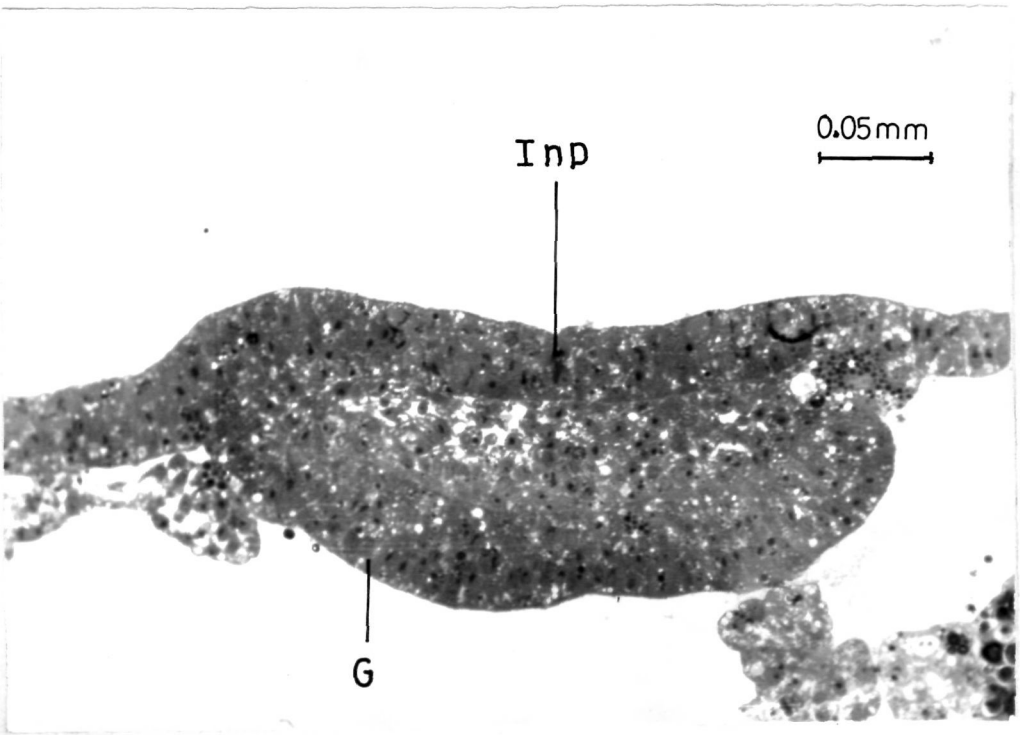


PLATE 4.2.2d

C. Experiments with grafts 6 EcM

Since pilot experiments with grafts of Hensen's node without endoderm at stage 6 showed very feeble neuroid response even after 2 hours of contact, present experiment was conducted with 2hours and 2 hours 30 minutes contact only. The cellular morphology of the reacting ectoderm was observed and the types of cells present along with their dimensions are given in Table 4.2.2C

Contact period - 2 hours : The sections of the reacting ectoderm at this stage showed that it was comparatively thickened than uninduced ectoderm and it had 10 number of cells present at the point of contact with the graft. The cells were mainly columnar and a few were bottle-shaped. The cells had a broad base and were pointed at the other end. At certain regions the cells appeared to be stratified. The length of the cells varied from 10 µm to 13 µm, width 6 µm to 9 µm and their ND 1.5 µm to 4.5 µm.

Contact period - 2 hours 30 minutes : The reacting host ectoderm had formed a thickened neural plate with 17 cells at the point of contact with the graft. The cells were mainly elongated and columnar with very few rounded cells. They were closely packed without any intercellular space. The length

of the cells varied from 9 µm to 15 µm, width 6 µm to 8.0 µm and their ND 3 µm to 4.5 µm

TABLE 4.2.2A

Changes in cell size during neuroid response induced by grafts of the Hensen's node of stage 4 embryo without endoderm at different time interval of contact with the graft.

		DIMENSIONS (μm)														
TIME INTERVAL (MINUTES)		10			15			20			25			30		
TYPE OF CELLS		L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND
BOTTLE-SHAPED CELLS	C	10	9	3	10	6	3	11	6	3	13	8	4	14	6	3
	I	10	6	3	12	6	3	12	6	3	15	7	4	15	8	4
CUBOIDAL CELLS	C	10	8	4	10	7	4	8	6	4	10	8	3	10	8	4
	I	10	8	3	11	6	3	12	6	3	12	8	4.5	12	8	4
TALL COLUMNAR CELLS	C	9	6	3	12	6	4.5	15	9	4.5	12	6	4.5	12	6	3
	I	9	4.5	3	12	6	3	15	6	2.5	12	9	3	12	6	3
ROUNDED CELLS	C	6	6	3	6	6	3	6	6	3	6	4	3	7	6	3
	I	9	5	3	9	6	3	9	6	3	7.5	4	3	7.5	6	3
IRREGULAR SHAPED CELLS	C	-	-	-	-	-	-	-	-	-	-	-	-	6	4	3
	I	-	-	-	-	-	-	-	-	-	-	-	-	6	3	1.5
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I		6			20			13			21			30	
NEUROID RESPONSE			(-)			(+)			(+)			(+)			(+)	

L - Length; W-Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm; (+) Presence of neuroid response; (-) Absence of neuroid response.

TABLE 4.2.2B

Changes in cell size during neuroid response inddduced by grafts of the Hensen's node of stage 5 embryo without endoderm at different time interval of contact with the graft.

		DIMENSIONS (μm)														
TIME INTERVAL (MINUTES)		10			15			20			25			30		
TYPE OF CELLS		L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND
BOTTLE-SHAPED CELLS	C	14	6	3	14	8	3	14	6	3	14	7	4	13	6	3
	I	14	6	3	15	8	4	15	8	3	15	6	3	16	6	3
CUBOIDAL CELLS	C	10	6	3	10	7	3	9	6	3	10	8	3	12	6	3
	I	10	8	3	12	8	3	12	8	3	12	8	4.5	15	7	4
TALL COLUMNAR CELLS	C	10	9	9	10	6	3	12	9	3	12	9	4.5	15	6	3
	I	10.5	6	4.5	13.5	6	3	9	6	3	15	6	3	18	6	1.5
ROUNDED CELLS	C	10	9	3	8	6	3	8	6	3	7	6	3	6	6	3
	I	12	10.5	3	9	6	1.5	9	6	1.5	7.5	6	3	9	6	3
IRREGULAR SHAPED CELLS	C	8	6	3	-	-	-	-	-	-	-	-	-	-	-	-
	I	9	6	3	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I		14			13			30			28			30	
NEUROID RESPONSE			(-)			(+)			(+)			(+)			(+)	

L - Length; W-Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm; (+) Presence of neuroid response; (-) Absence of neuroid response.

TABLE 4.2.2C

Changes in cell size during neuroid response induced by grafts of the Hensen's node of stage 6 embryo without endoderm at different time interval of contact with the graft

TIME INTERVAL		DIMENSIONS (µm) 2 hrs.			2 hrs 30 mins		
TYPES OF CELLS		L	W	ND	L	W	ND
BOTTLE-SHAPED CELLS	C	12	6	3	12	6	3
	I	13	6	4	15	6	3
CUBOIDAL CELLS	C	10	6	4	10	6	4
	I	12	8	3	12	8	3
TALL COLUMNAR CELLS	C	9	6	3	12	6	4.5
	I	12	6	4.5	12	6	3
ROUNDED CELLS	C	10	8	1.5	9	7	3
	I	10	9	1.5	9	7	4.5
IRREGULAR SHAPED CELLS	C	-	-	-	-	-	-
	I	-	-	-	-	-	-
TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT	I		10			17	
NEUROID RESPONSE			(+)			(+)	

L - Length; W - Width; ND - Nuclear Diameter; I - Host Reacting Ectoderm; C - Host Normal Ectoderm; (+) - Presence of Neuroid reponse; (-) - Absence of Neuroid response

Fig. 4.2.2 Changes in the length of bottle-shaped cells in the reacting ectoderm after varying periods of contact with EcM grafts at stage 4 and stage 5. (without endoderm).

O—O Length in the host induced neurectoderm.

●--● Length in the host normal neurectoderm.

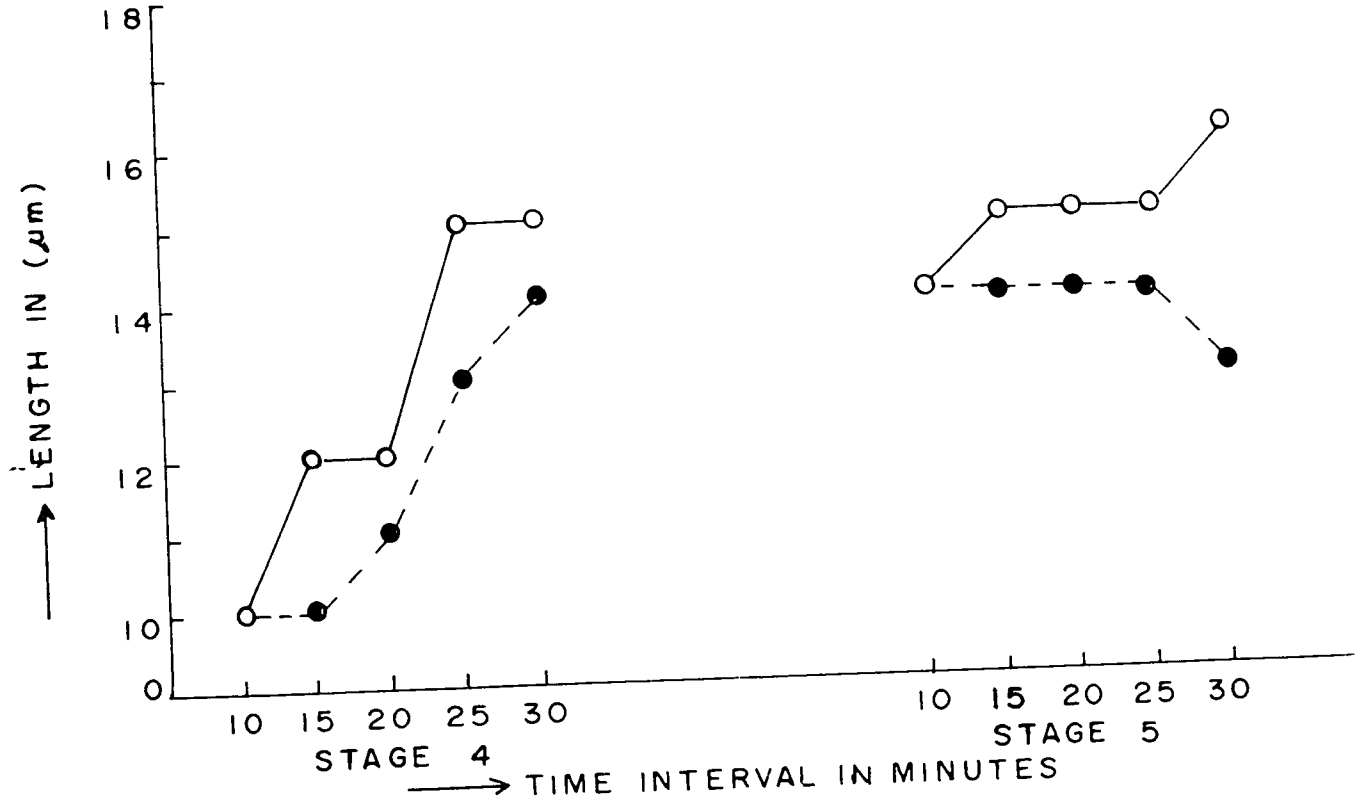

18
16
14
12
10
0
LENGTH IN (μm)
10 15 20 25 30
STAGE 4
10 15 20 25 30
STAGE 5
TIME INTERVAL IN MINUTES

FIG. 4·2·2

4.3 HISTOLOGICAL CHANGES IN THE NORMAL NEURECTODERM AT STAGES 3, 4, 5 and 6

In the present investigation, embryos at stages 3, 4, 5 and 6 were taken, fixed and processed for embedding in epoxy resins and then sectioned in the normal neurectoderm region (Figs. 3.8a, 3.8b) for histological analysis of thin and semithin sections. The histological sections of the neurectoderm of the chick embryo at stages 3, 4, 5 and 6 have been studied in order to follow the histological changes that occur as development proceeds from stage 3 to 6. An assessment of such changes was made by measuring the dimensions of various types of cells, that is, length (L), width(W), nuclear diameter (ND) of 2 to 5 cells in each cell type (Table 4.3 and Fig.4.3).

The various types of cells present were tall columnar cells, cuboidal cells, bottle-shaped cells, rounded cells and irregular - shaped cells. The tall columnar cells are those whose length is more than twice their width, cuboidal cells appear more or less square in shape and their length is only slightly longer than their width. Rounded cells are round or elliptical in shape. Irregular cells are, as their name suggests, irregular in shape.

Histology of neurectoderm at stage 3 : In histological sections the neurectoderm is revealed as a thin strip composed of different types of cells with large

intercellular spaces between them (Plates 4.3a, 4.3b). The cells were of varied shapes and sizes bottle - shaped, tall columnar, cuboidal cells, a few were round or irregular in shape. The dimensions are recorded in Table 4.3.

Histology of neurectoderm at stage 4 : Histological sections compared with stage 3, the neurectoderm now shows a slight increase in the degree of stratification, though the cellular composition showed no change and the intercellular spaces remained large (Plates 4.3c, 4.3d). The cells present were mostly tall columnar, irregular-shaped cells, bottle-shaped cells, cuboidal cells and few rounded cells (Table 4.3).

Histology of neurectoderm at stage 5 : The whole neurectodermal layer appeared as a thickened strip of cells. Compared with stage 4, the cells were closely packed with less intercellular space (Plate 4.3e) and most of the cells were bottle - shaped or tall columnar cells with few cuboidal and rounded cells (Table 4.3)

Histology of neurectoderm at stage 6 : The neurectoderm at this stage appeared as a stratified strip of cells. The cells were closely packed with less intercellular spaces and appeared mainly to be tall columnar cells with a few cuboidal cells

(Plate 4.3f). The cells at the neural fold region appeared stratified with presence of intercellular spaces between them (Plate 4.3g). The dimensions of these cells are recorded in Table 4.3.

Remarks :

A comparative study of sections of neurectoderm at stages 3, 4, 5 and 6 reveals the following changes :

(1) The sections of neurectoderm at stages 3 and 4 appeared similar, without much difference and with large intercellular spaces.

(2) At stages 5 and 6 these cells appeared to have become elongated and they form accordingly a thickened strip of cells arranged to one another with less intercellular spaces. The neurectodermal layer appears stratified, only in the neural fold region it showed presence of intercellular spaces.

(3) The tall columnar cells increased in length continuously as the embryo developed from stage 3 to stage 6. Of all the cell types, the tall columnar cells showed the greatest increase in length. Starting from an initial length of 14 µm at stage 3 they reached 22 µm at stage 6. The bottle-shaped cells show some changes in their length. The average length of 12 µm at stage 3 remained unchanged at stage 4, reached 16 µm at

stage 5 but showed no further increase at stage 6. Rounded cells at stage 3 they measured 6 μm, at stage 4, 7 μm; at stage 5, 9 μm and stage 6, 10 μm. The cuboidal cells showed variations in average length. At stages 3 and 4 these cells measured 10 μm which increased to 11 μm at stage 5 but at stage 6 the average length was again 10 μm as illustrated in Table 4.3 and Fig. 4.3.

TABLE 4.3

Different cell types present in the normal neurectoderm and their dimensions at stages 3, 4, 5 and 6

TYPES OF CELLS	STAGE 3						STAGE 4						STAGE 5						STAGE 6					
	DIMENSIONS (μm)			AVERAGE			DIMENSIONS (μm)			AVERAGE			DIMENSIONS (μm)			AVERAGE			DIMENSIONS (μm)			AVERAGE		
	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND	L	W	ND
TALL COLUMNAR CELLS	14	7	3				19	6	5				20	8	4				22	8	4			
	13	6	2				21	4	3				20	8	4				23	9	5			
	15	7	4	14	6	3	20	5	4	20	5	4	20	7	4	20	8	4	21	7	3	22	8	4
	14	5	3				20	5	4				21	9	5				22	8	4			
	14	5	3				20	5	4				19	8	3				22	8	4			
BOTTLE-SHAPED CELLS	12	6	4				12	5	3				16	7	4				16	6	4			
	13	7	3				12	6	3				16	6	3				16	7	4			
	11	5	2	12	6	3	12	5	3	12	5	3	15	5	2	16	6	3	17	7	4	16	6	4
	12	7	3				12	5	3				17	7	3				15	5	4			
	12	5	3				12	4	3				16	5	3				16	5	4			
CUBOIDAL CELLS	10	5	4				10	8	4				11	8	3				10	8	4			
	10	5	4				10	8	4				11	8	3				10	8	4			
	10	5	4	10	5	4	11	9	5	10	8	4	10	7	2	11	8	3	11	9	4	10	8	4
	9	4	3				9	7	3				12	9	4				9	7	5			
	11	6	5				10	8	4				12	8	3				10	8	3			
ROUNDED CELLS	6	6	3				7	5	2				9	9	4				10	6	3			
	6	6	3	6	6	3	7	5	2	7	5	2	9	9	4	9	9	4	10	6	3	10	6	3
IRREGULAR SHAPED CELLS	9	6	2				9	4	3				6	6	3					-				
				9	6	2	9	4	3	9	4	3	6	6	3	6	6	3		-			-	

Fig. 4.3 Changes in the length of different types of cells in the normal neurectoderm of stage 3, stage 4, stage 5 and stage 6 embryos.

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

TCC - Tall columnar cells

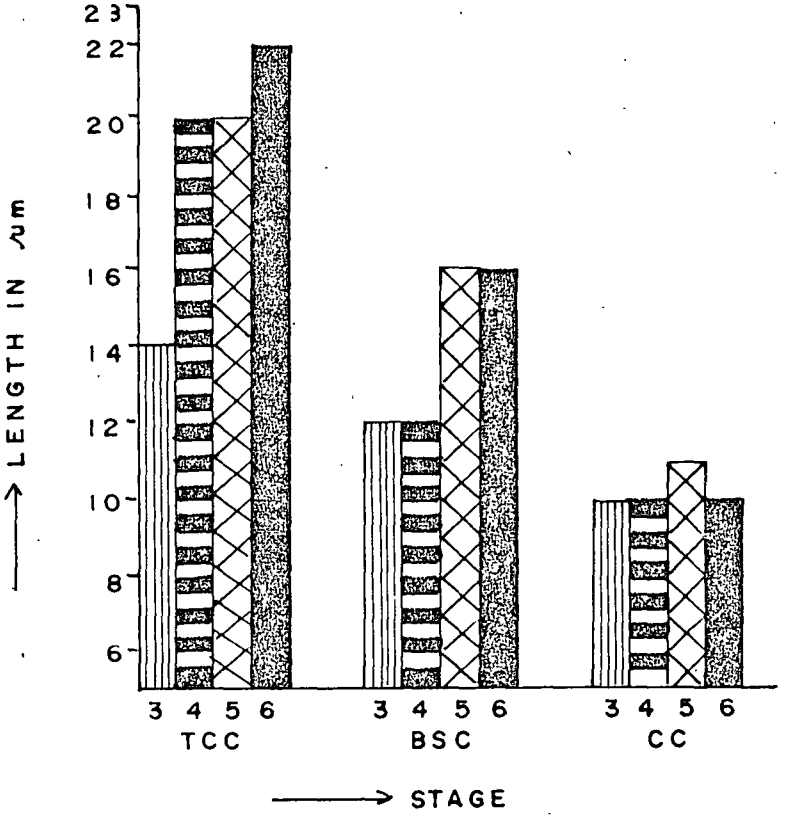

23
22
20
18
16
14
12
10
8
6
LENGTH IN μm
3 4 5 6
TCC
3 4 5 6
BSC
3 4 5 6
CC
STAGE

FIG. 4·3·

Plate 4.3a Photomicrograph of a section of chick embryo at stage 3 sectioned in the normal neurectoderm showing presence of different cell types (x250).

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

IS - Intercellular spaces

ISC - Irregular shaped cells

RC - Rounded cells

TCC - Tall columnar cells

Plate 4.3b Photomicrograph of a section of chick embryo at stage 3 sectioned in the normal neurectoderm showing presence of different cell types (x400).

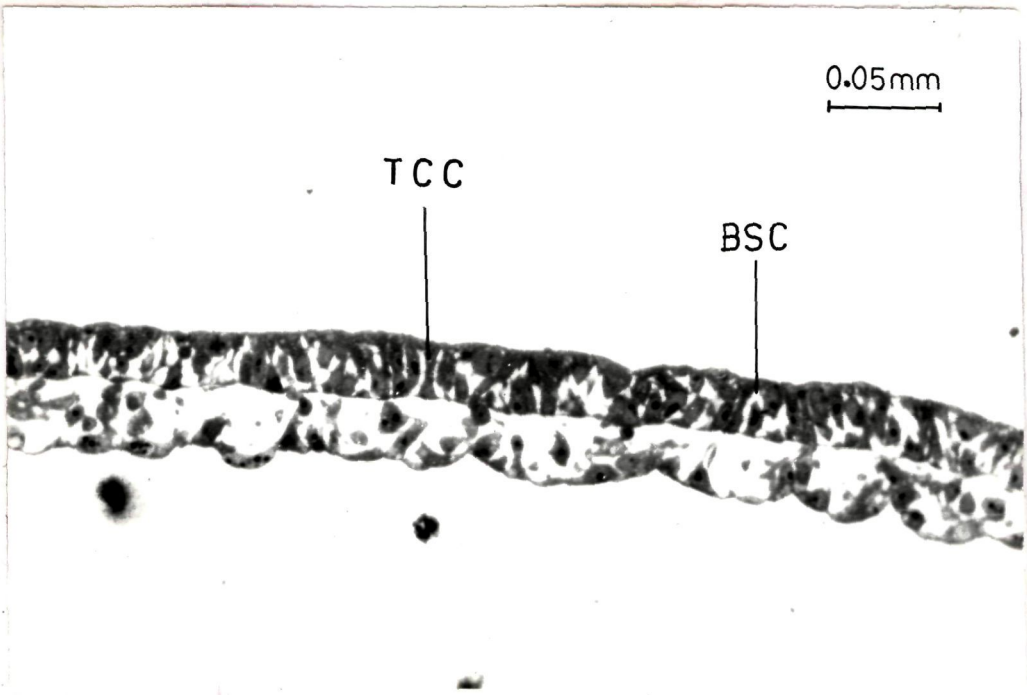


PLATE 4.3a

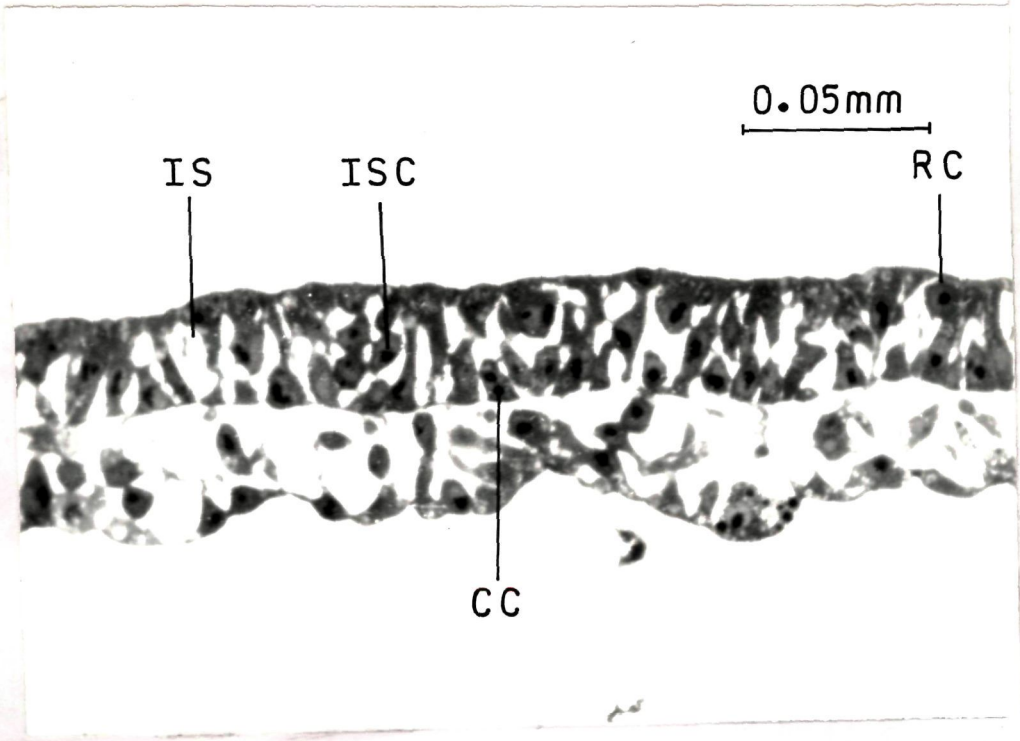


PLATE 4.3b

Plate 4.3c Photomicrograph of a section of chick embryo at stage 4 sectioned in the normal neurectoderm showing presence of different cell types (x400).

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

IS - Intercellular spaces

ISC - Irregular shaped cells

RC - Rounded cells

TCC - Tall columnar cells

Plate 4.3d Photomicrograph of a section of chick embryo at stage 4 sectioned in the normal neurectoderm showing presence of different cell types (x400).

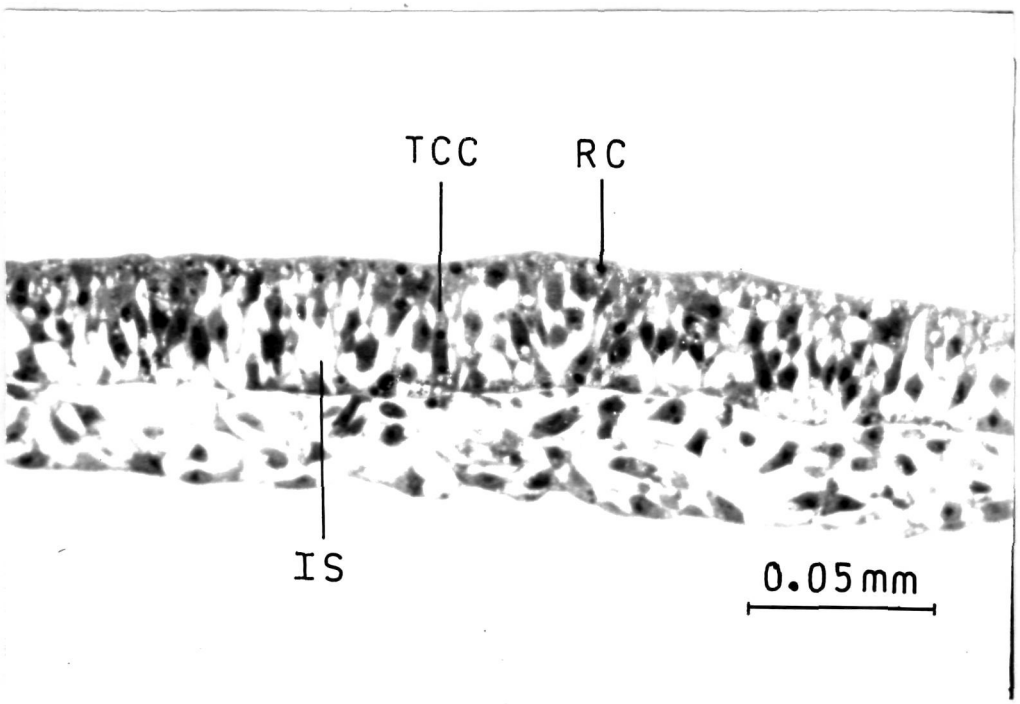


PLATE 4.3c

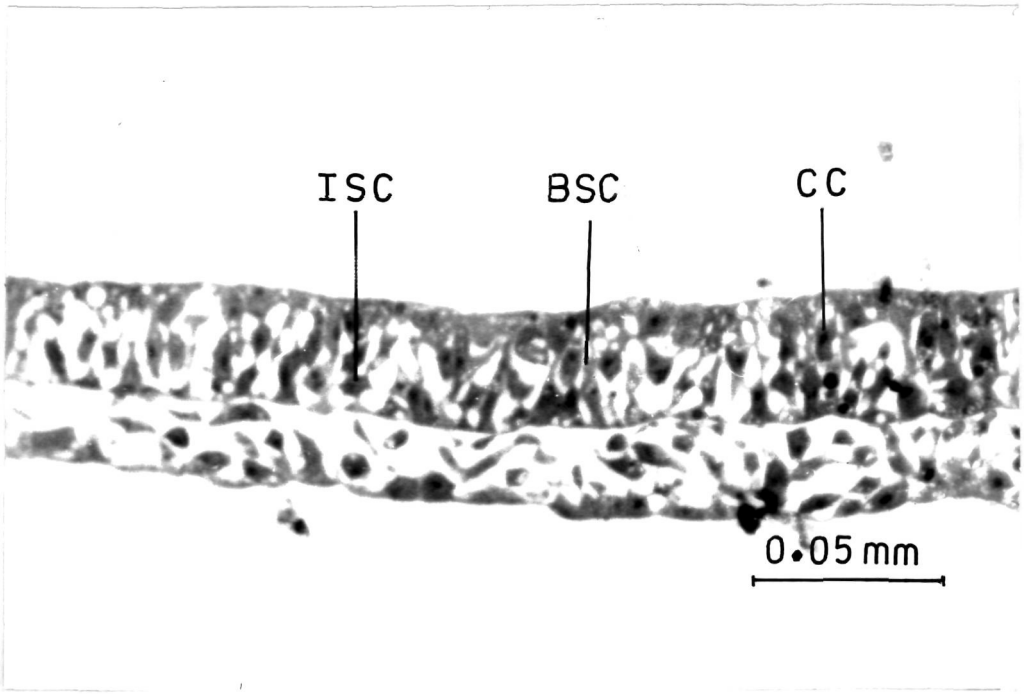


PLATE 4.3d

Plate 4.3e Photomicrograph of a section of chick embryo at stage 5 sectioned in the normal neurectoderm showing presence of different cell types (x 250).

Abbreviations :

BSc - Bottle-shaped cells

CC - Cuboidal cells

RC - Rounded cells

TCC - Tall columnar cells

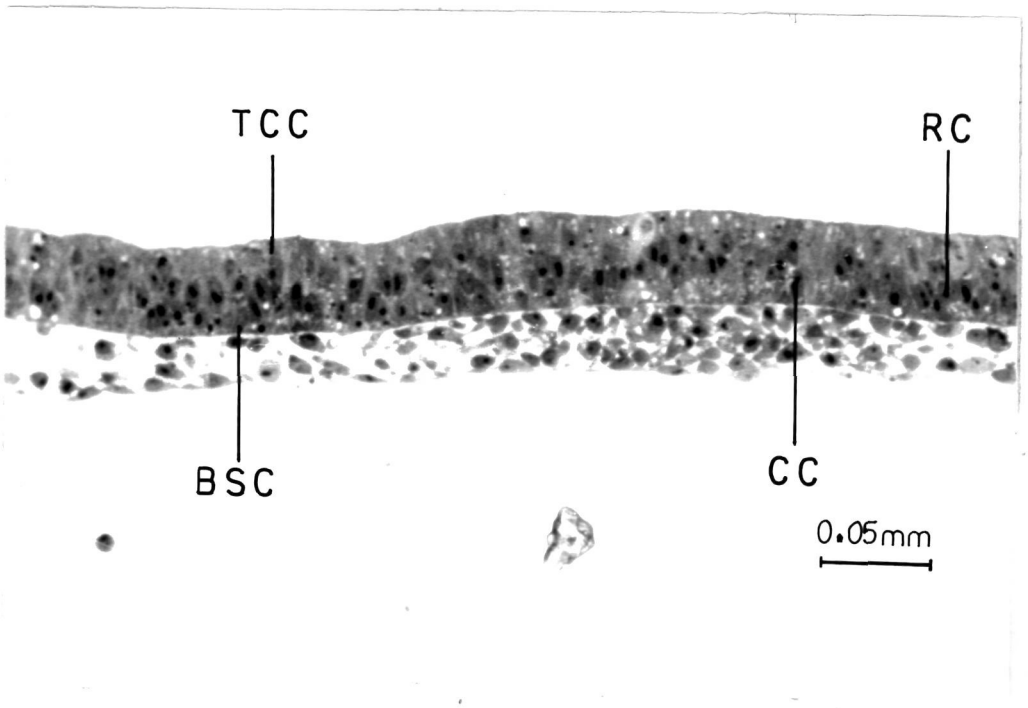


PLATE 4.3e

Plate 4.3f Photomicrograph of a section of chick embryo at stage 6 sectioned in the normal neurectoderm showing presence of different cell types (x400).

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

RC - Rounded cells

TCC - Tall columnar cells

ISC - Irregular shaped cells

Plate 4.3g Photomicrograph of a section of chick embryo at stage 6 sectioned in the neural fold showing presence of different cell types (x400).

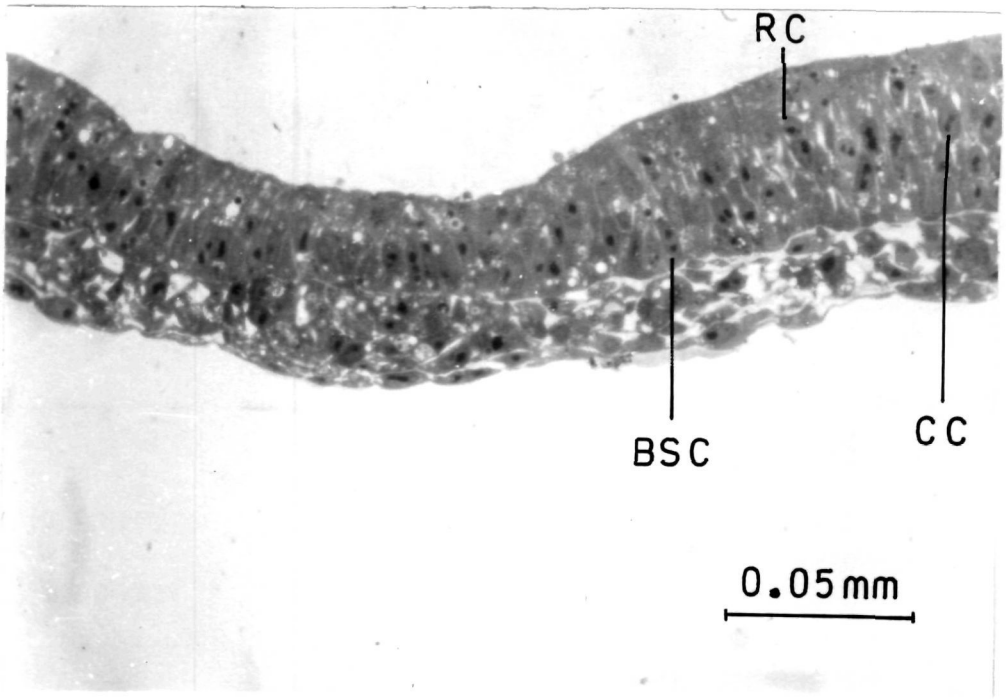


PLATE 4.3f

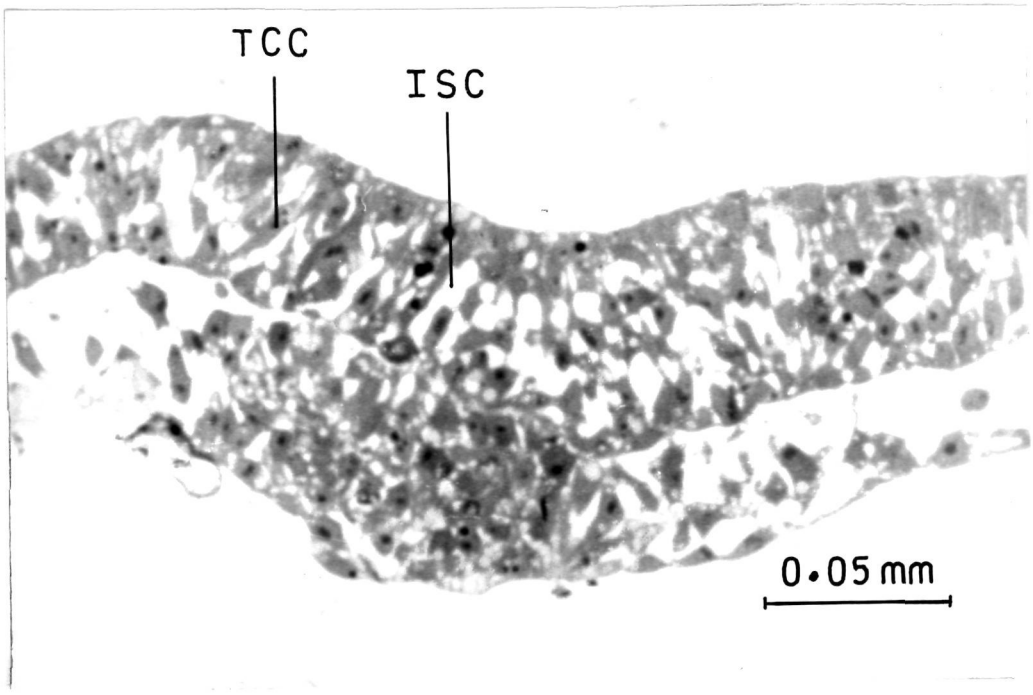


PLATE 4.3g

4.4 ULTRA-STRUCTURAL CHANGES IN THE NORMAL NEURECTODERM AT STAGES 4, 5 AND 6

The embryos at H and H (1951) stages 4, 5 and 6 were removed in Tyrode solution, the vitelline membrane was separated and the desired portion of the embryo was cut out. The embryos were fixed and processed for embedding in plastic for Transmission Electron Microscopy. The blocks were trimmed and sectioned at the desired position (Figs. 3.9a, 3.9b). The thickness of these sections was judged by interference colours. Gold colour sections (thickness ± 600 Å to 900 Å) were picked up on 300 mesh grids. The sections were stained in 2% aqueous uranyl acetate for 5 to 10 minutes followed by lead citrate for 2 minutes. The sections were then examined with the TEM, JEM 100C X II.

In the present investigation, sections of the normal neurectoderm of the chick embryo at stages 4, 5 and 6 were studied. The photomicrographs of the sections show the changes in the arrangement and presence of different types of cells observed at these stages.

Stage 4 : At stage 4, the study of the sections of the normal neural ectoderm at 1000 X magnification (plate 4.4a) reveals that it consists of two types

Plate 4.4a Transmission Electron Microscope Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 4 (x 1000) showing the two types of cells (1) elongated TCC (2) irregular shaped cells.

Abbreviations :

ISc - Irregular shaped cells

TCC - Tall columnar cells

`` - Yolk granules

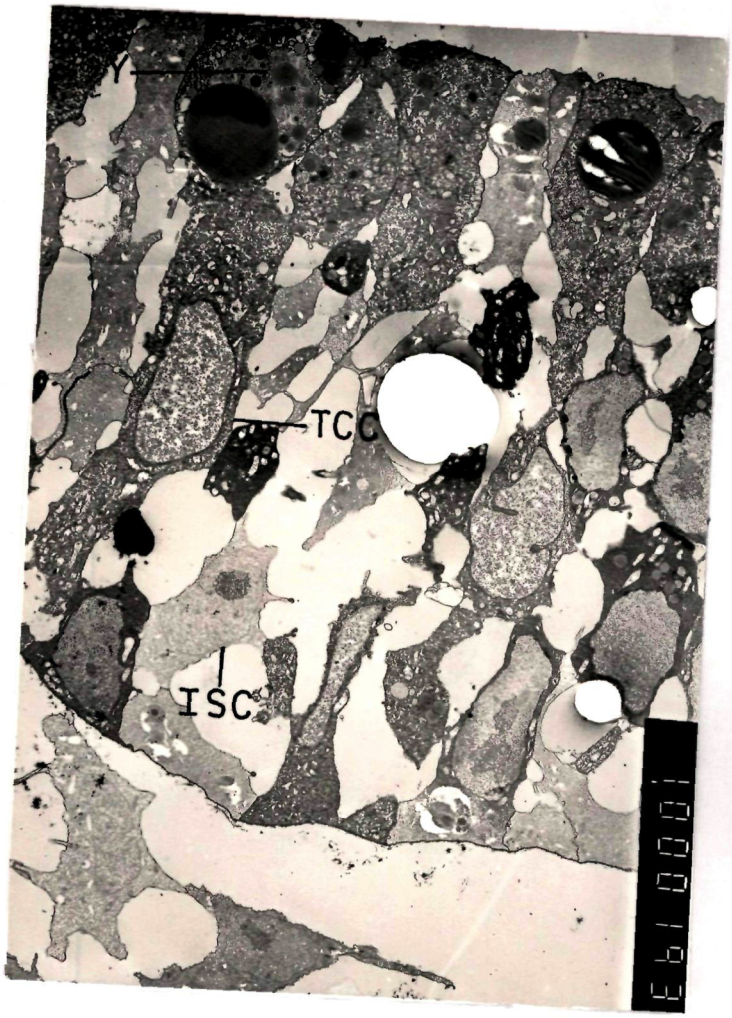


PLATE 4.4a

of cells : (1) deeply staining elongated cells (2) lightly staining irregular shaped mesenchymal cells each with a number of pseudoped - like processes.

The deeply staining cells are those which arranged or aligned later themselves to form the neural plate. They are firmly attached to the dorsal or upper surface of the neurectoderm and interspersed with irregularly shaped mesenchymal cells in the groups of three or four ectodermal cells. Towards the ventral or lower surface of the neurectoderm the cells are sparse and are loosely distributed on a ground matrix. The number of mesenchymal cells in the ventral or lower surface is larger. The elongated ectodermal cells appear to elongate from the dorsal or upper surface towards the ventral basal lamina. The elongated ectodermal cells have large prominent nuclei. The cytoplasm is densely packed with mitochondria, ribosomes, rough and smooth endoplasmic reticulum, lipid droplets and yolk granules. The presence of these organelles are clearly seen at 2700 x and 4000 x magnification (Plate 4.4b, 4.4c, 4.4d) respectively.

The propective normal neurectodermal cells appear to be stretching from the dorsal or upper surface towards the ventral basal lamina with the

Plate 4.4b TEM Photomicrograph of a section of chick embryo showing two types of cells, bottle-shaped cells and cuboidal cells (x 2700).

Abbreviations :

BSC - Bottle-shaped cells

CC - Cuboidal cells

m - mitochondria

n - nucleus

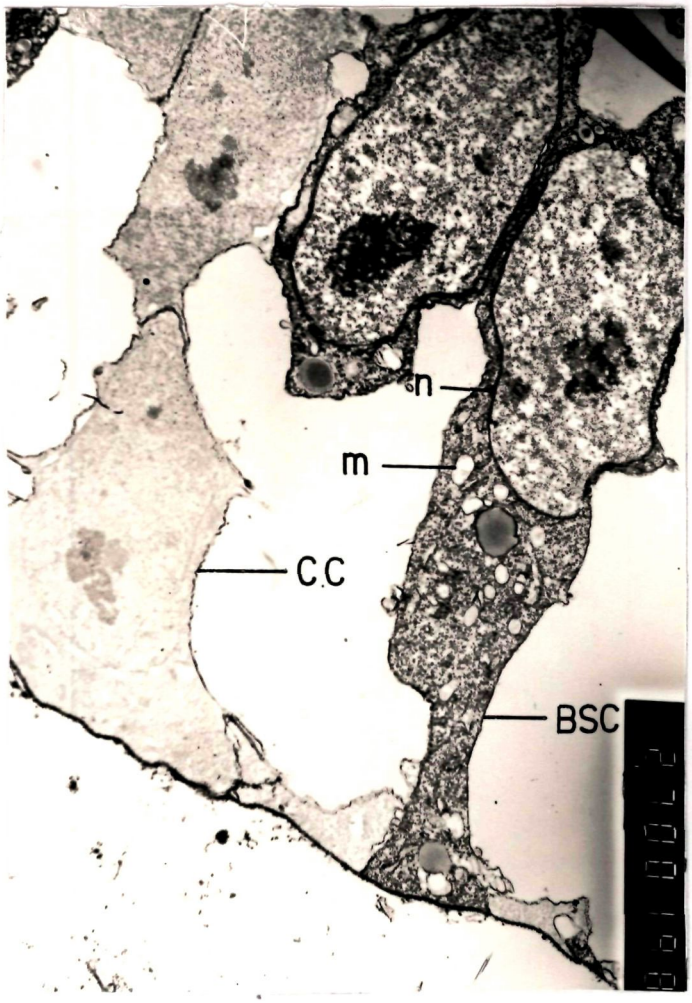


PLATE 4.4b

Plate 4.4c TEM Photomicrograph of a section of chick embryo showing presence of different types of cell organelles (x 4000).

Abbreviations :

G - Golgi

m - mitochondria

n - nucleus

y - Yolk granules

Plate 4.4d TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 4 (x 4000).

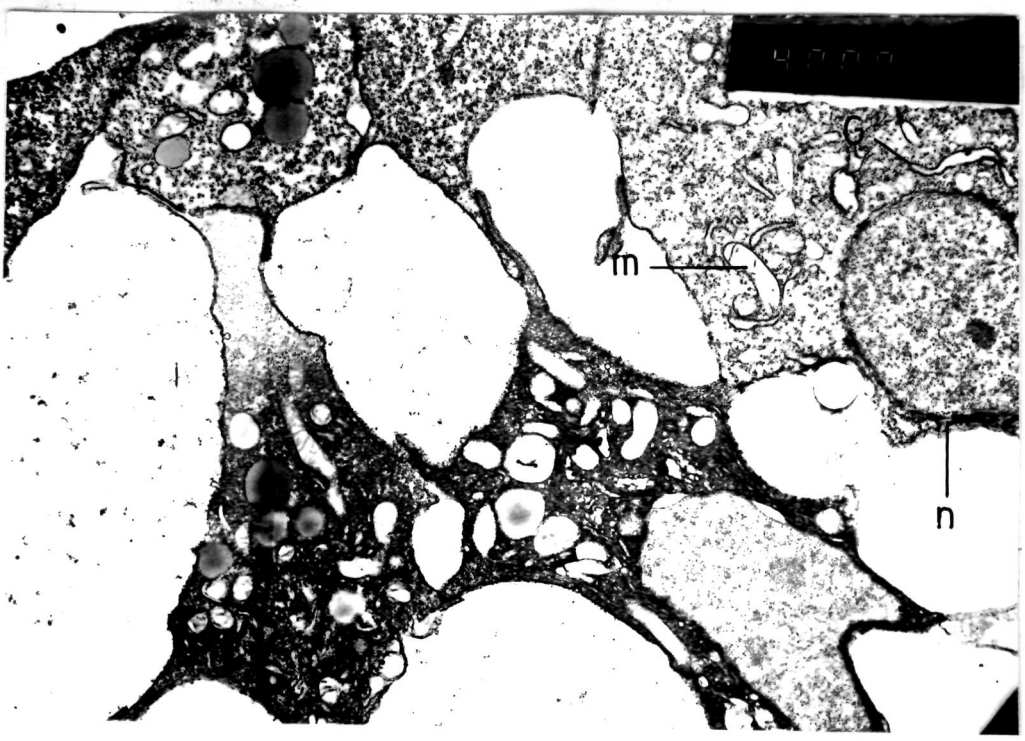


PLATE 4.4c

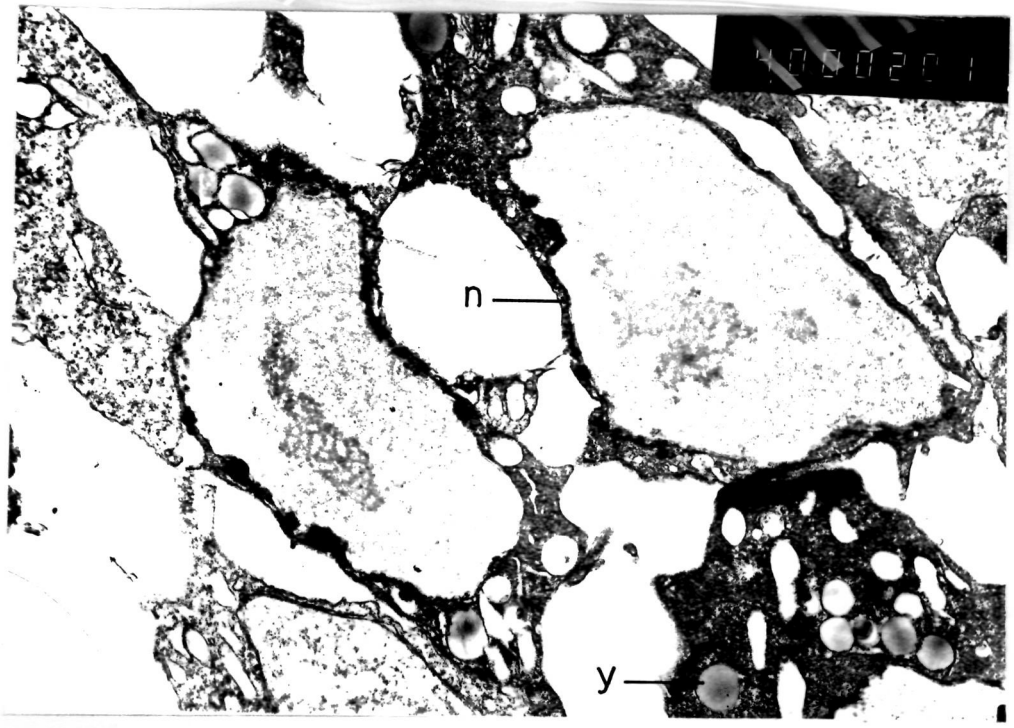


PLATE 4.4d

nucleus in the middle. Though mitochondria and yolk granules showed dense concentration on the cytoplasm, both towards the ventral or lower portion and dorsal or upper portion of the cells, their concentration is however more in the ventral or lower leading end. The neighbouring ectodermal cells are attached to each other by demosomes and gap junctions, their upper part are more closely packed with the neighbouring cells than the lower parts. At certain places (Plate 4.4a) the yolk granules are surrounded by groups of lysosomes.

At 4000 X and 2000 X magnification (Plates 4.4e, 4.4f) it is clearly seen that the mitochondria and lipid droplets occur abundantly throughout the length of the elongated cells.

At stage 4, yolk granules and lipid droplets are present in high concentration along with mitochondria in the elongated cells both at the dorsal or upper and ventral or lower portions (Plate 4.4g). However, they appear to exhibit a sort of streaming movement. In these cells (Plate 4.4g) whose leading ventral or lower portions are firmly attached with the ventral basal lamina the distribution of the lipid droplets is diffused.

In certain cells phagocytic lysosomal vesicles are visible e.g. surrounding the lipid

Plate 4.4e TEM Photomicrograph of a section of chick embryo showing presence of mitochondria and yolk granules in the elongated cell (x 4000).

Abbreviations :

m - mitochondria

n - nucleus

R - Ribosomes

y - Yolk granules

Plate 4.4f TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 4 (x 2000).

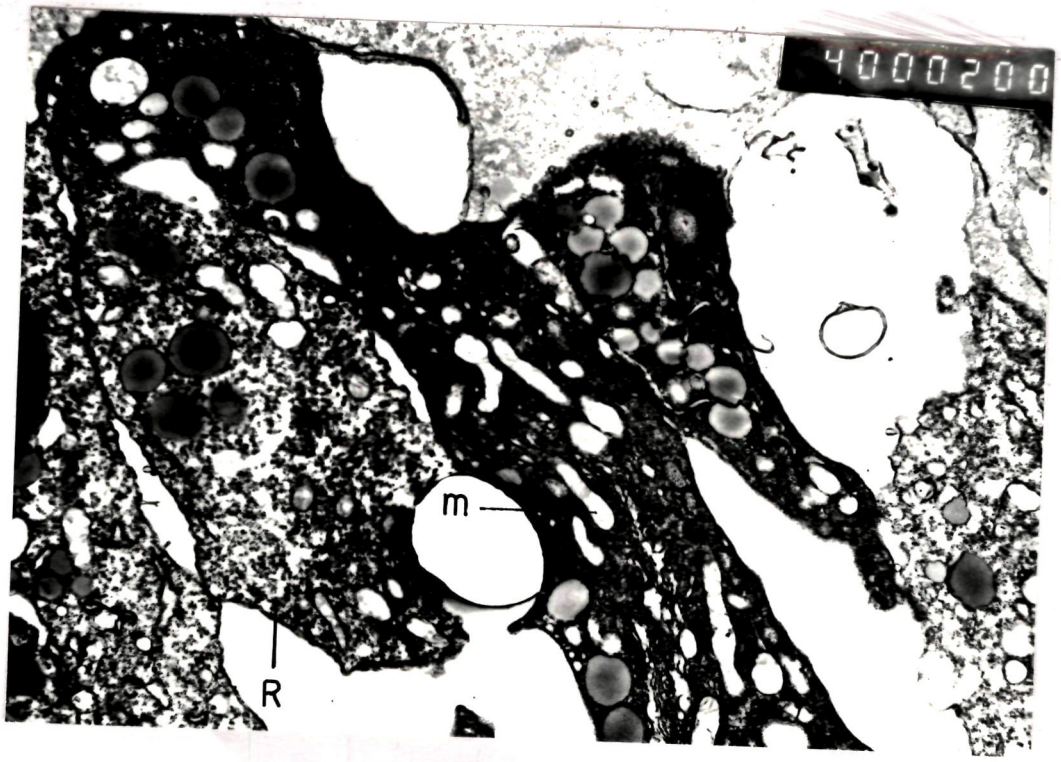


PLATE 4.4e

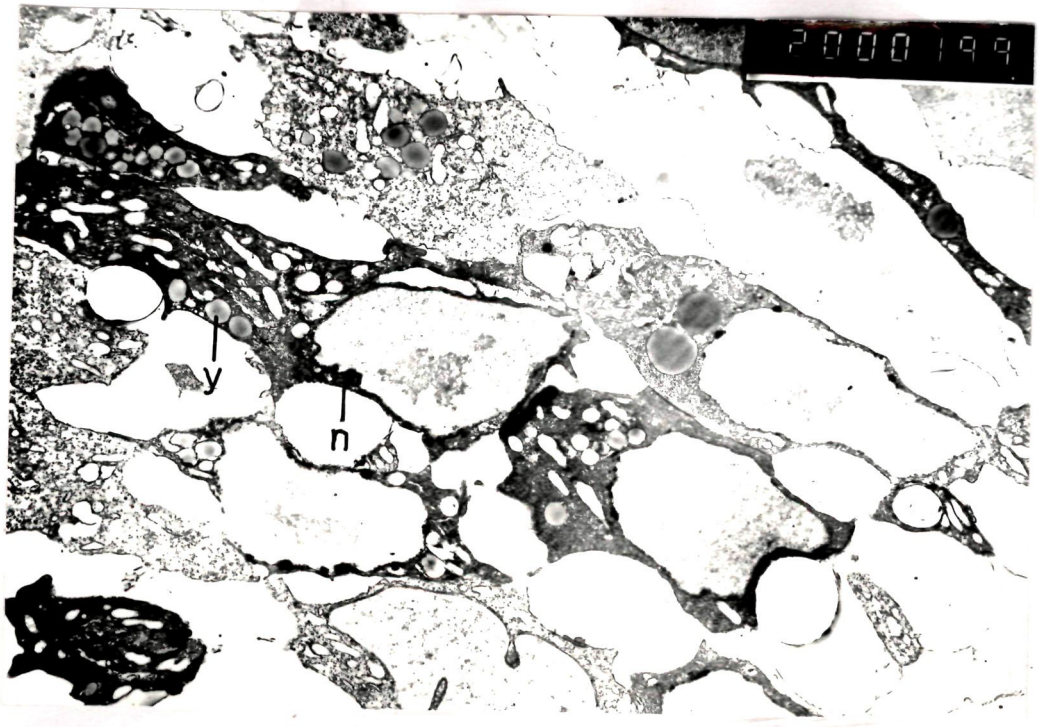


PLATE 4.4f

Plate 4.4g TEM Photomicrograph showing a tall columnar cell (x 4000).

Abbreviations :

m - mitochondria

n - nucleus

R - Ribosomes

Y - Yolk granules

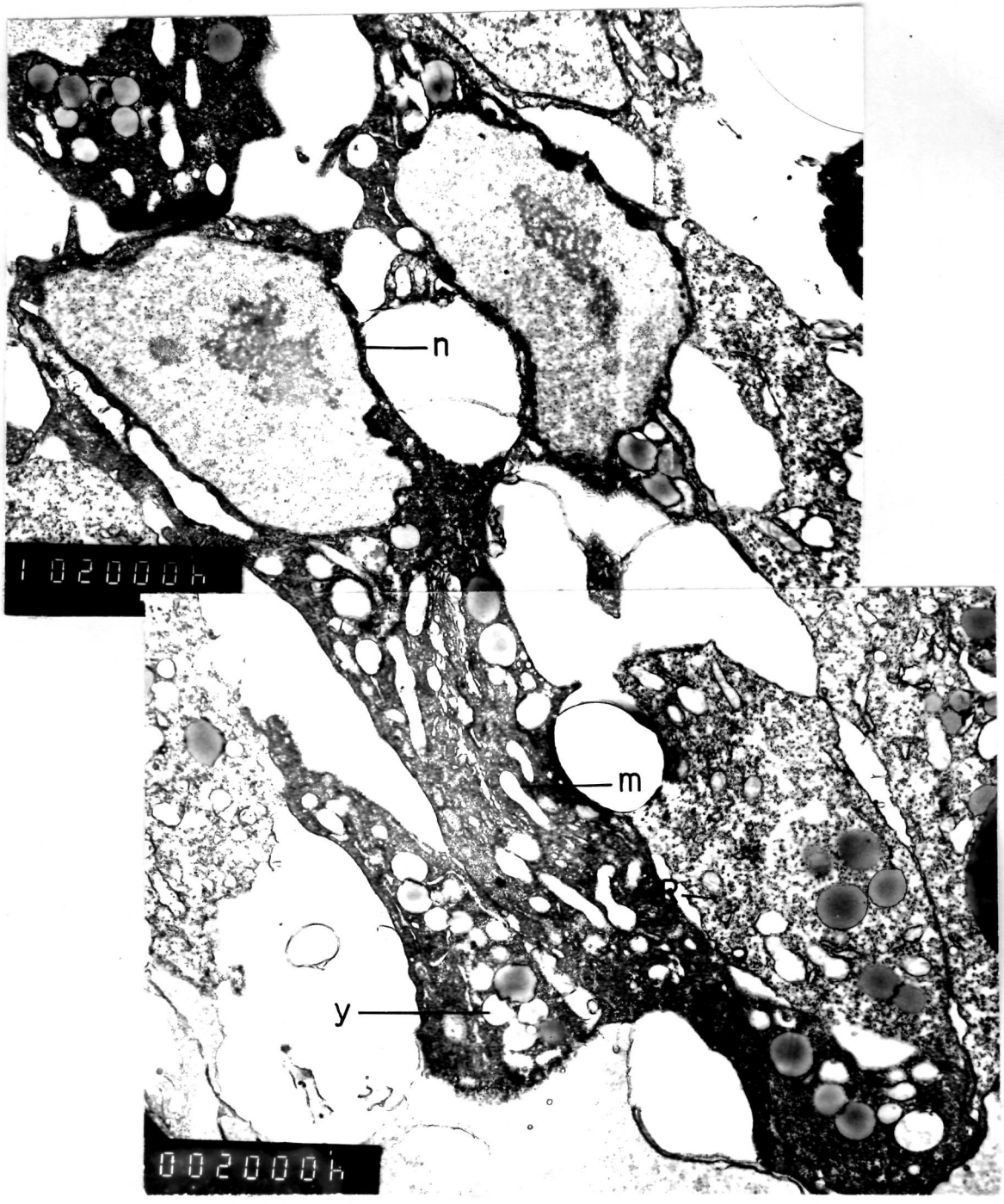


PLATE 4.4g

droplets or yolk granules (Plate 4.4a). This indicates some on going digestive processes in the cells. Occurrence of cell death is likely during early differentiation of neural plate.

Stage 5 : At stage 5, the cells are seen at 1400 X magnification to be nicely aligned and densely packed throughout the thickness of the neurectoderm. Their density increases in the dorsal or upper portion of the neurectoderm (Plate 4.4h, 4.4i)

The lightly staining mesenchymal cells are very few in the dorsal or upper portion of the neurectoderm but they occur more frequently in its ventral or lower portion. All cytoplasmic organelles, mitochondria, ribosomes, rough and smooth (ER) endoplasmic reticulum, golgi, lipid droplets, yolk granules are clearly seen at a magnification 2000 X in the dorsal or upper aspect and at 5000 X is the ventral or lower portion (Plates 4.4j, 4.4k) Concentration of mitochondria and yolk granules are seen both at dorsal or upper and ventral or lower portions of the neurectodermal cells. These cells are observed at 5000 X to be firmly attached to the ventral basal lamina (Plate 4.4k).

At stage 5, the cells are elongated as well as being compacted with adjacent cells. The degfee of compaction appears to be much higher in the

Plate 4.4h TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 5 (x 1400).

Abbreviations :

m - mitochondria

n - nucleus

TCC- Tall columnar cells

y - Yolk granules

Plate 4.4i TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 5 (x 1400).

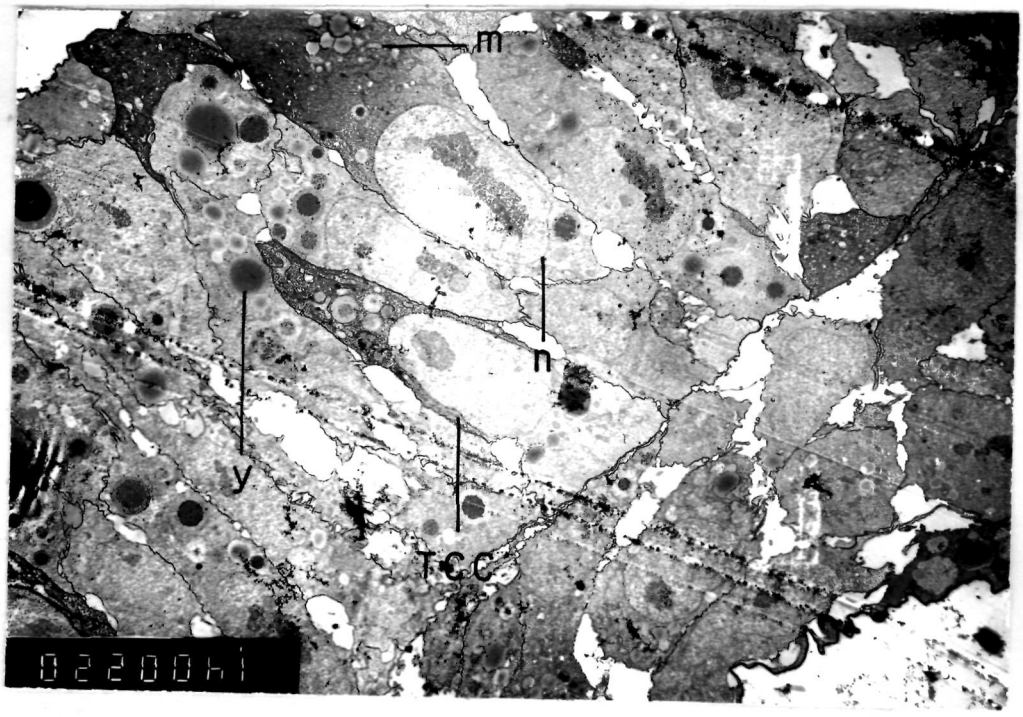


PLATE 4.4h

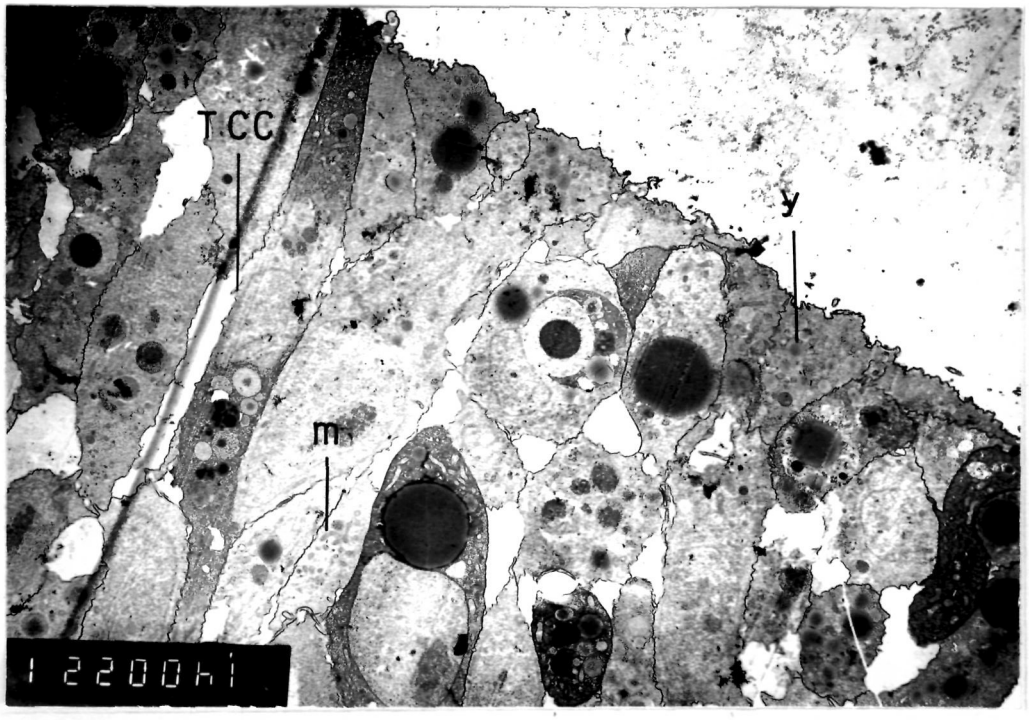


PLATE 4.4i

Plate 4.4j TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 5 showing the elongated TCC and densely packed cells (x 2000).

Abbreviations :

m - mitochondria

TCC - Tall columnar cells

Y - Yolk granules

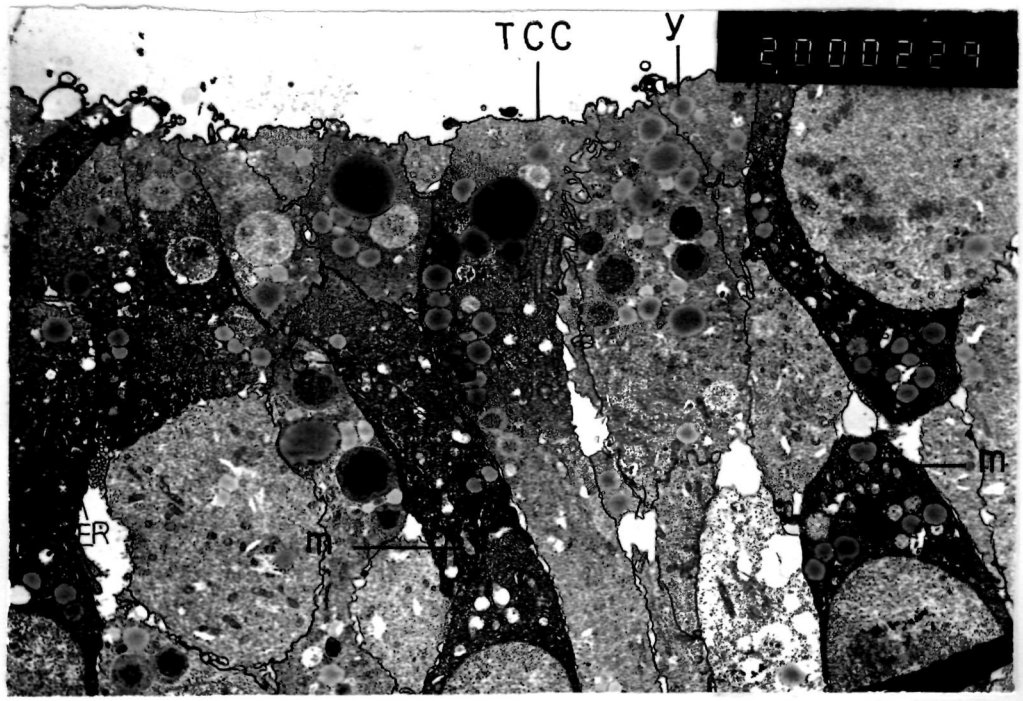


PLATE 4.4j

Plate 4.4k TEM Photomicrograph of a section of chick embryo showing attachment of the cells to the basal lamina (x 5000).

Abbreviations :

BL - Basal lamina

TCC - Tall columnar cells

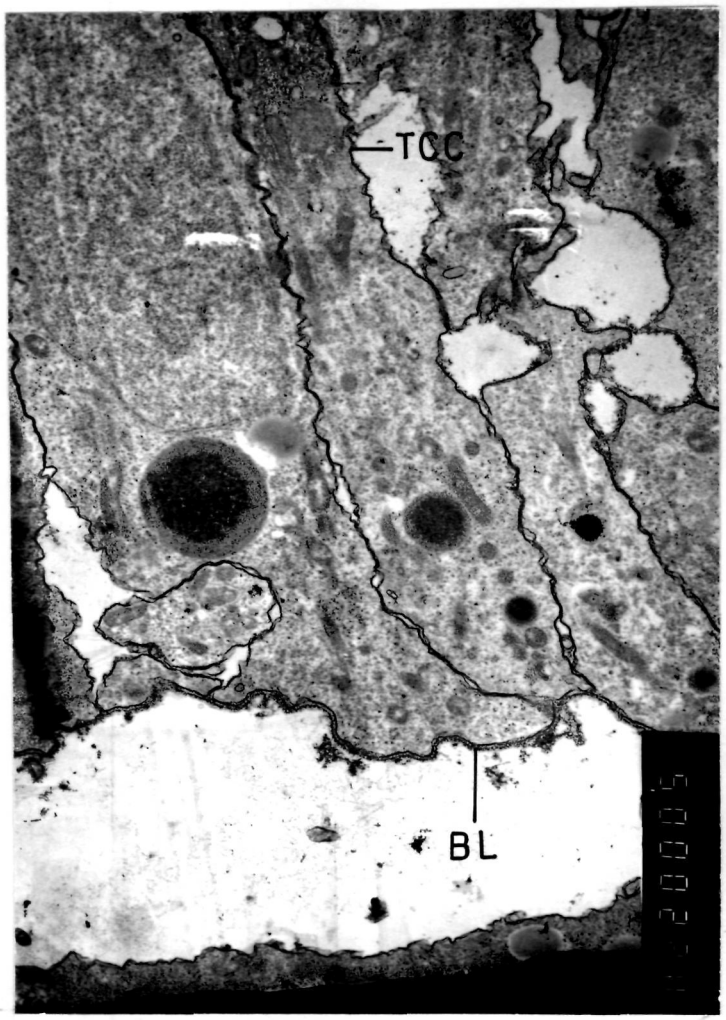


PLATE 4.4k

dorsal or upper portion (Plate 4.4j) of the neural plate than in its ventral or lower portion (plate 4.4k). Here in the dorsal or upper compacted portions of the cells high density of lipid droplets and yolk granules is seen but the mitochondria however are not present in equal abundance. At the ventral or lower parts of the cells, the density of mitochondria is higher than that of lipid droplets or yolk granules.

At 4000 X magnification (Plate 4.4l) the cuboidal cells showed a large prominent nucleus. Adjacent to the nucleus are large yolk granules and many mitochondria, golgi and ribosomes.

In Plate 4.4m shows a typical bottle-shaped cell which are firmly attached to the dorsal or upper surface of the neurectoderm. The cells are closely packed with their neighbouring cells. Mitochondria occur abundantly throughout the whole cell. Other organelles such as ribosomes, golgi and endoplasmic reticulum are scattered throughout the length of the cell. Adjacent to these bottle-shaped cells there are many large yolk cells.

Plate 4.4n shows a tall columnar cell firmly attached to the basal lamina. It is also shows that the cells are closely apposed to neighbouring cells throughout their length. Many

Plate 4.41 TEM Photomicrograph of a section of chick embryo showing cuboidal cell and presence of different cell organelles and large yolk granule (x 4000).

Abbreviations :

BSC - Bottle-shaped cells
CC - Cuboidal cells
G - Golgi
m - mitochondria
n - nucleus
RER - Rough endophlasmic reticulum
y - Yolk granules

Plate 4.4m TEM Photomicrograph of a section of chick embryo showing a bottle-shaped cell and presence of different cell organelles (x 5000).

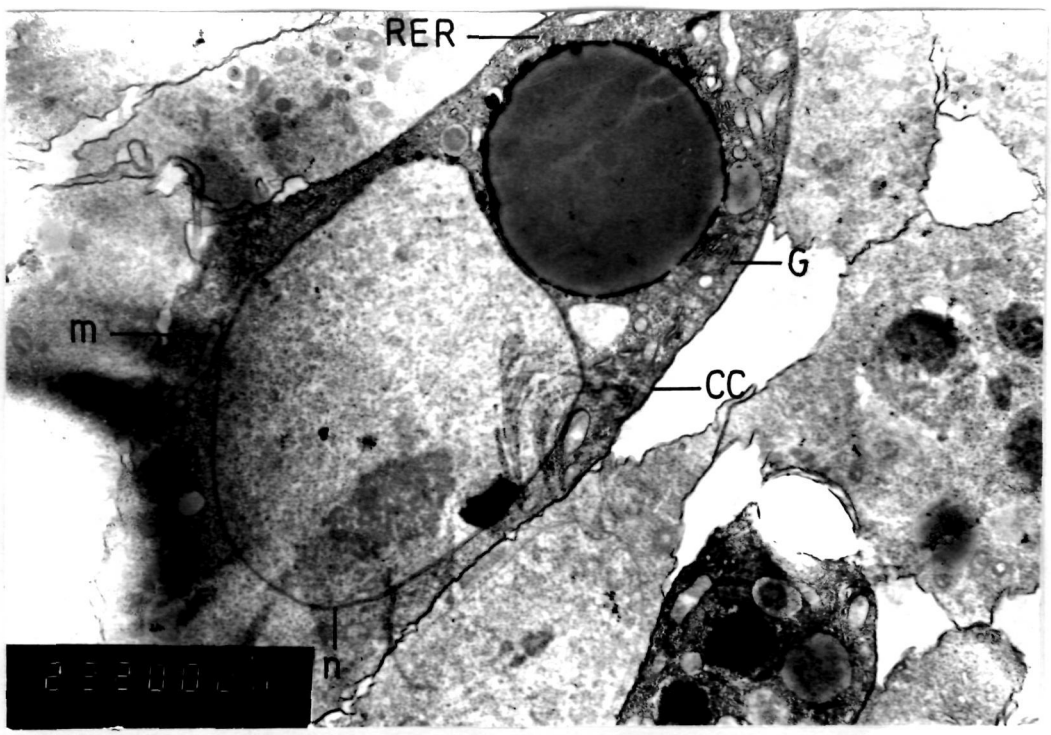


PLATE 4.4l

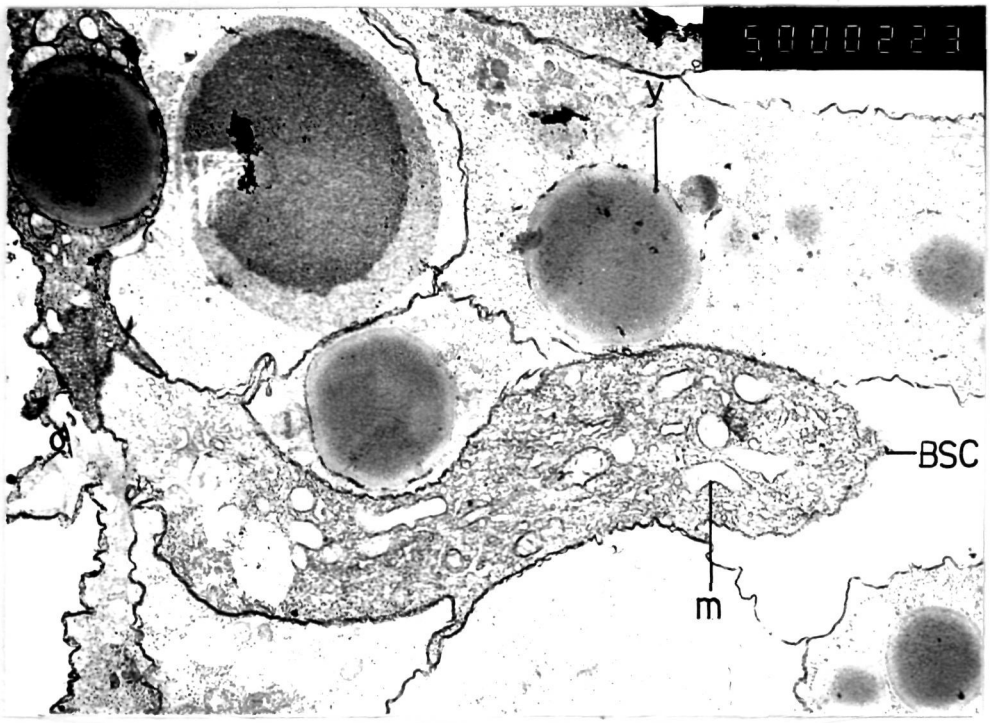


PLATE 4.4m

Plate 4.4n TEM Photomicrograph of a section of chick embryo showing a tall columnar cell (x 5000).

Abbreviations :

G - Golgi

m - mitochondria

TCC - Tall columnar cells

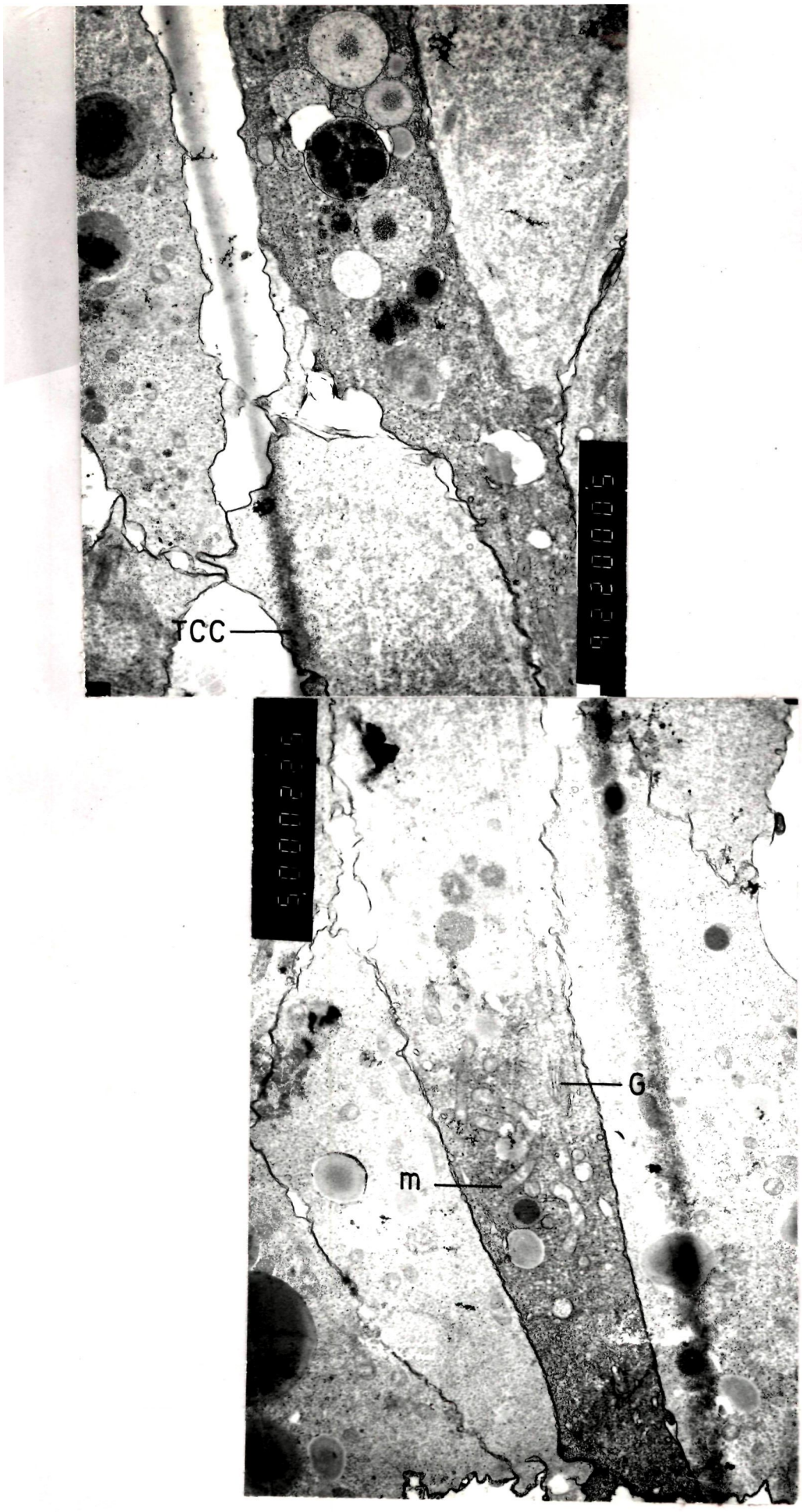


PLATE 4.4n

cell organelles such as golgi bodies endoplasmic reticulum and mitochondria are seen concentrated towards the posterior side.

At stage 5 a few cuboidal cells were present among the densely compacted columnar cells in the neural plate region. Plate 4.4o shows one such cell at 5000 X magnification. In this cell, distinct cell organelles such as golgi, ribosomes, rough endoplasmic reticulum and mitochondria are observed. Compared with the tall columnar cells mitochondria present are less in number.

At 2000 X magnification (Plate 4.4p) the cells are seen to be densely packed and the yolk granules are highly concentrated both at the upper and lower portions of the cells.

Stage 6 : The study of ultrathin sections of the neurectoderm at stage 6 at 1400 X magnification (Plate 4.4q) reveals that cells are distinctly elongated and attached to dorsal or upper portion of the neurectoderm. They are densely packed with little intercellular space and ground material.The mesenchymal cells are rarely present. The cells are closely apposed to the neighbouring cells generally over a large part of their length. Plate 4.4r at 1400 X shows the ultrathin sections

Plate 4.4o TEM Photomicrograph of a section of chick embryo showing cuboidal cell and presence of different cell organelles and large yolk granule (x 5000).

Abbreviations :

G - Golgi

m - mitochondria

R - Ribosomes

RER- Rough endophlasmic reticulum

y - Yolk granules

Plate 4.4p TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 5 (x 2000).

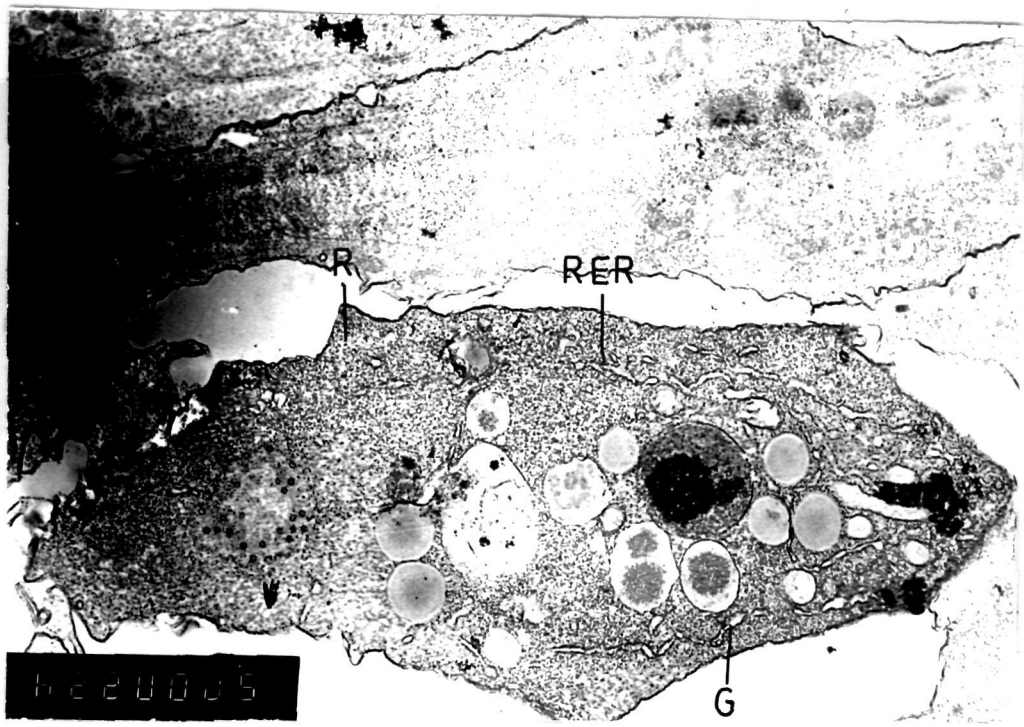


PLATE 4.4o

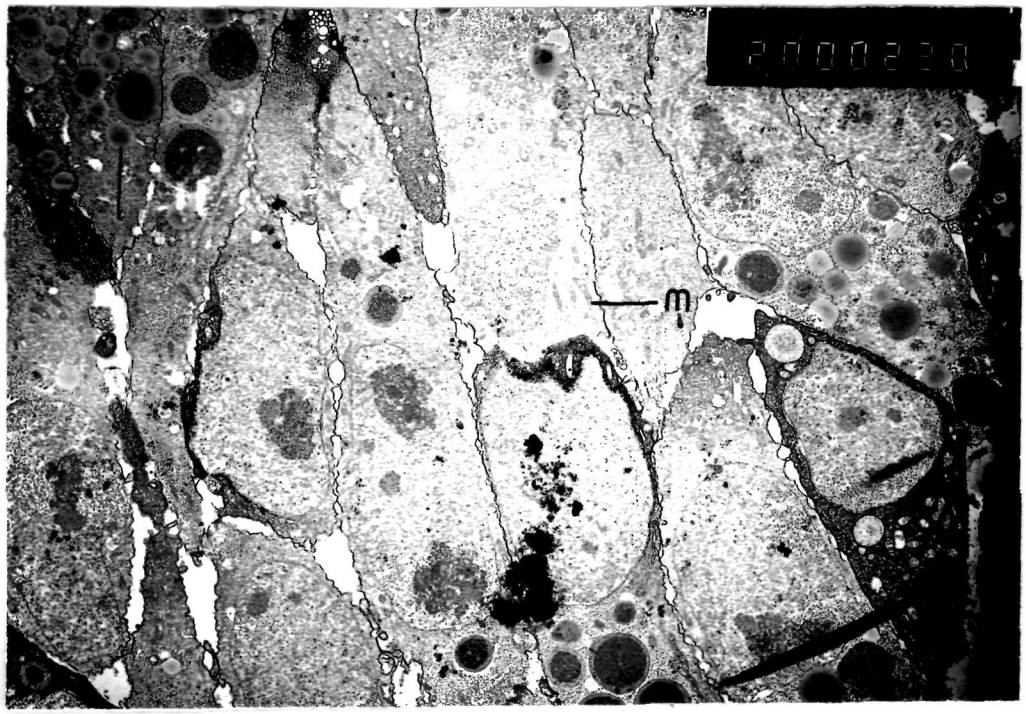


PLATE 4.4p

Plate 4.4q TEM Photomicrograph of a section of chick embryo in the normal neurectoderm at stage 6 (x 1400).

Abbreviations :

TCC - Tall columnar cells

y - Yolk granules

Plate 4.4r TEM Photomicrograph of a section of chick embryo in the neural fold region at stage 6 (x 1400).

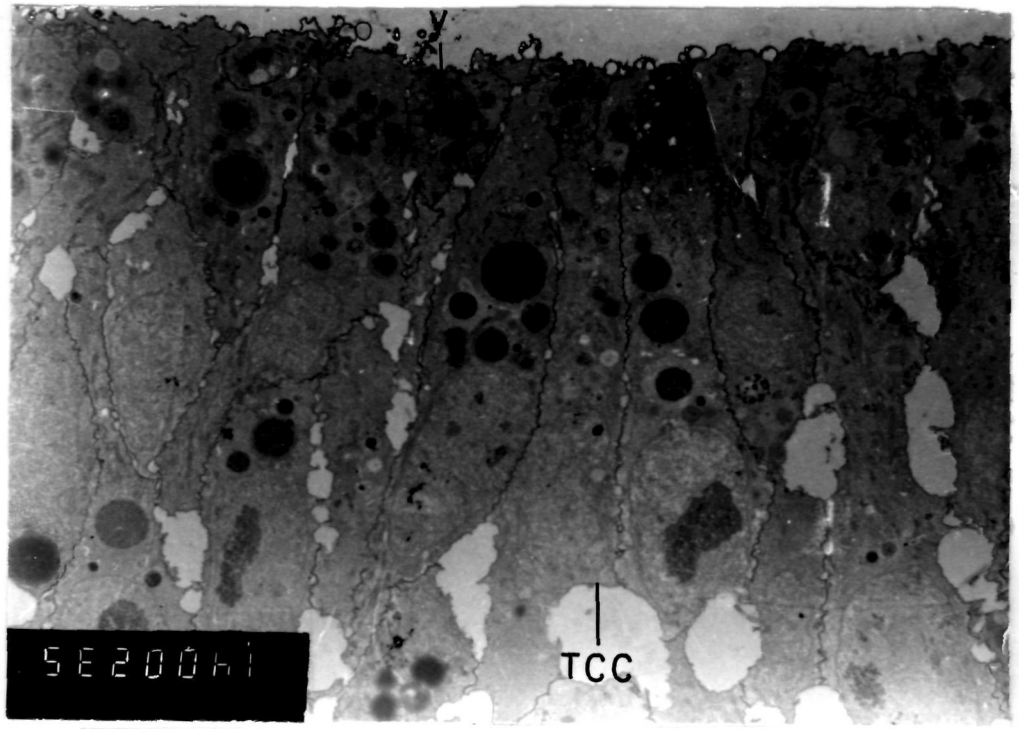


PLATE 4.4q

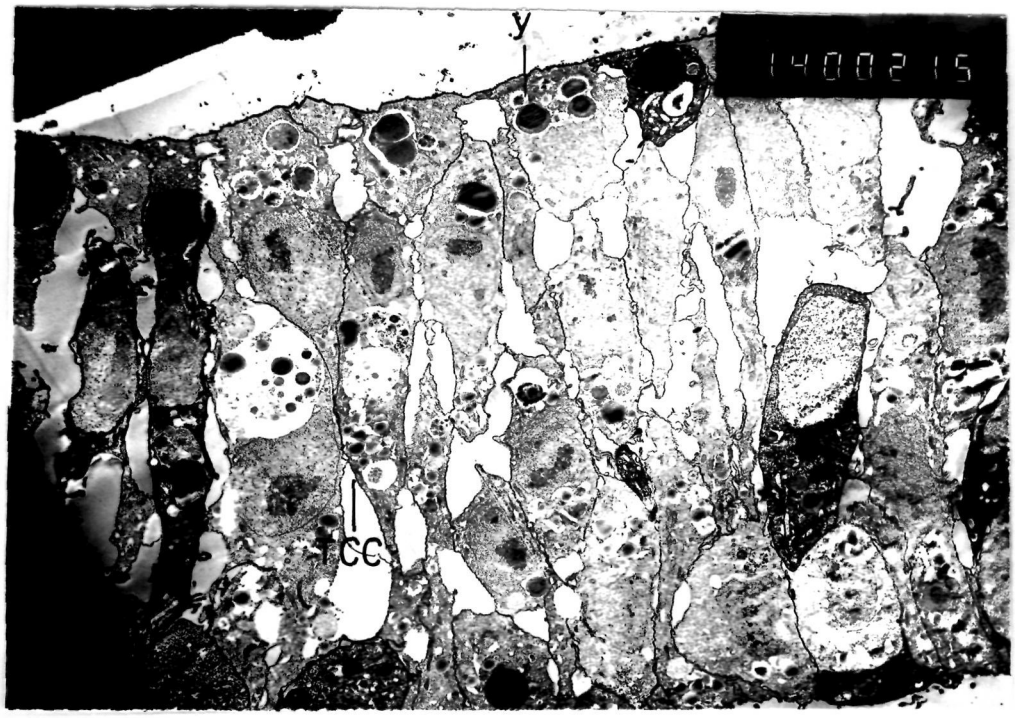


PLATE 4.4r

at the neural fold region with presence of some intercellular spaces. A study of individual cells at a magnification of 5000 x (Plate 4.4s) reveals the concentration of yolk granules and some mitochondria at the lower portion of the cell and many mitochondria are present in the neighbouring cell.

At 5000 X magnification (Plate 4.4t) shows the structure of a tall columnar cell and comparatively abundance of lipid droplets, mitochondria, golgi, endoplasmic reticulum and ribosomes in the deeply staining cells.

At stage 6, the upper portion of the cells are seen to be well compacted in the neural plate but their lower portions are separated by intercellular spaces. The cells are elongated but are more or less symmetrical. The nuclei of most cells are centrally located. The lipid droplets, yolk granules and mitochondria are observed to present in both the upper and lower portions of these cells. At higher magnification of 10,000 X and 20,000 X (Plates 4.4u, 4.4v) respectively the endoplasmic reticulum, golgi and the mitochondria are also clearly seen.

Plate 4.4s TEM Photomicrograph of a section of chick embryo showing a tall columnar cell (x 5000).

Abbreviations :

G - Golgi

m - mitochondria

TCC - Tall columnar cells

y - Yolk granules

Plate 4.4t TEM Photomicrograph of a section of chick embryo showing a tall columnar cell and presence of different cell organelles and yolk granules (x 5000).

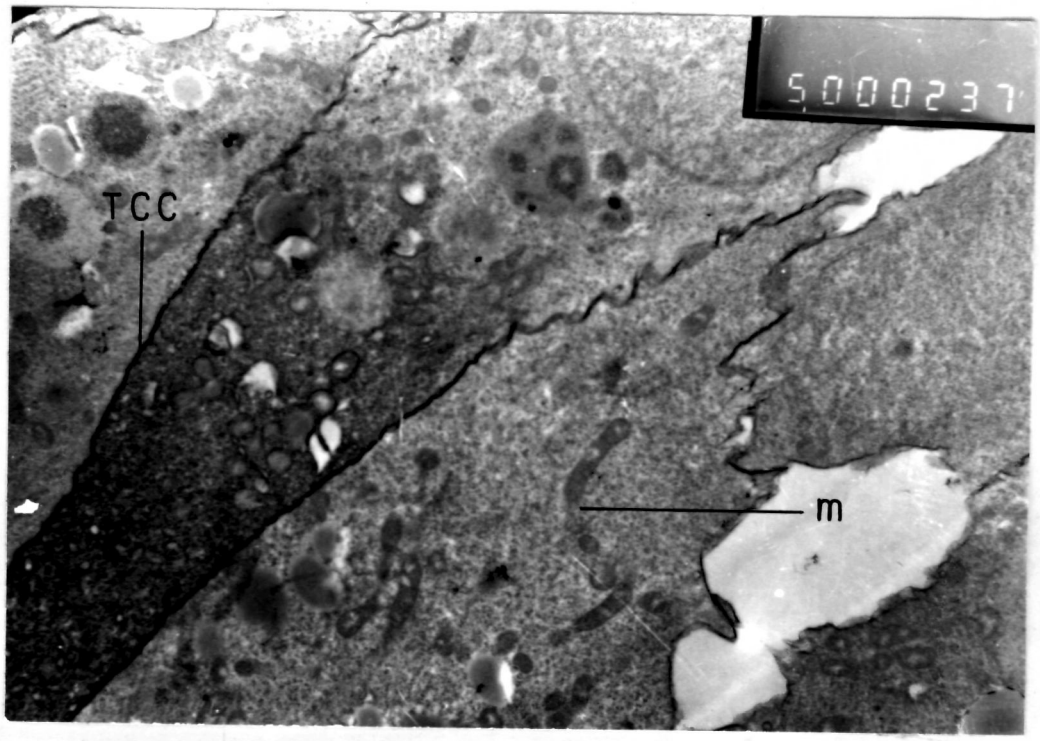


PLATE 4.4s

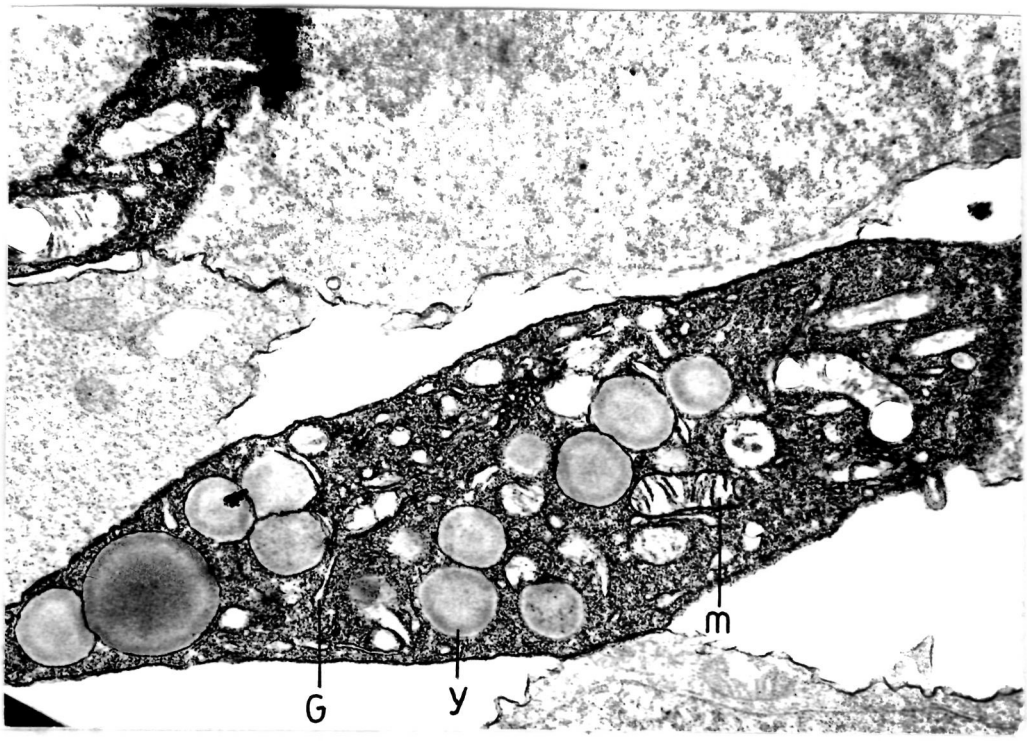


PLATE 4.4t

Plate 4.4u TEM Photomicrograph of a section of chick embryo showing part of a structure of a tall columnar cell and presence of different cell organelles (x 10,000).

Abbreviations :

G - Golgi

m - mitochondria

y - Yolk granules

Plate 4.4v TEM Photomicrograph of a section of chick embryo showing part of a structure of a tall columnar cell and presence of different cell organelles (x 20,000).

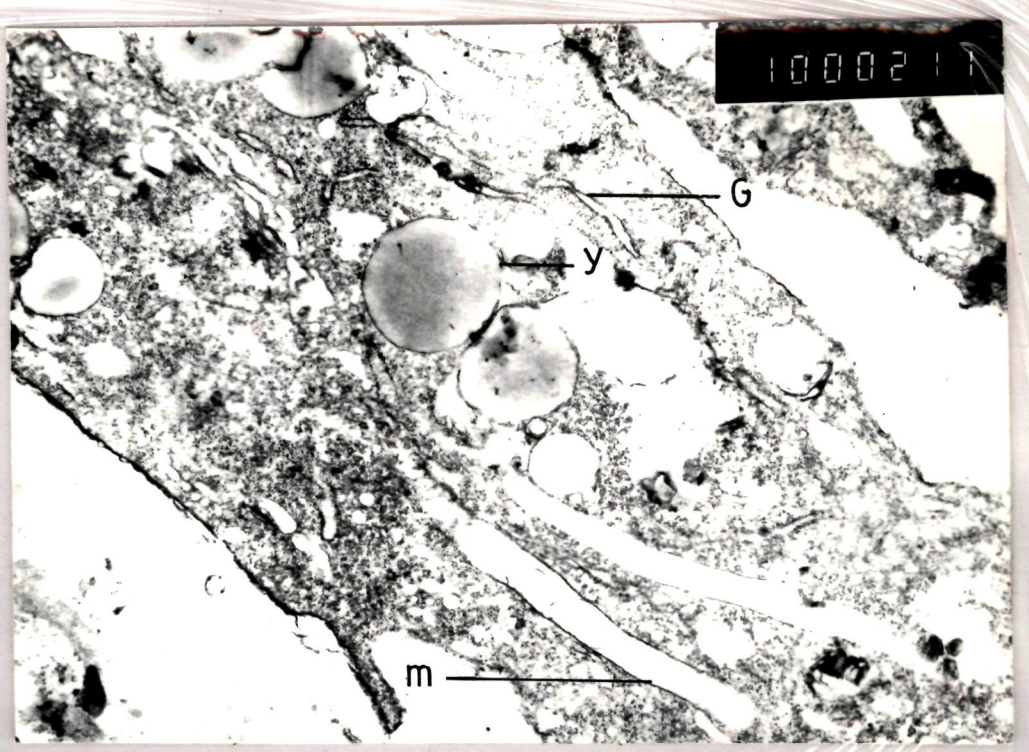


PLATE 4.4u

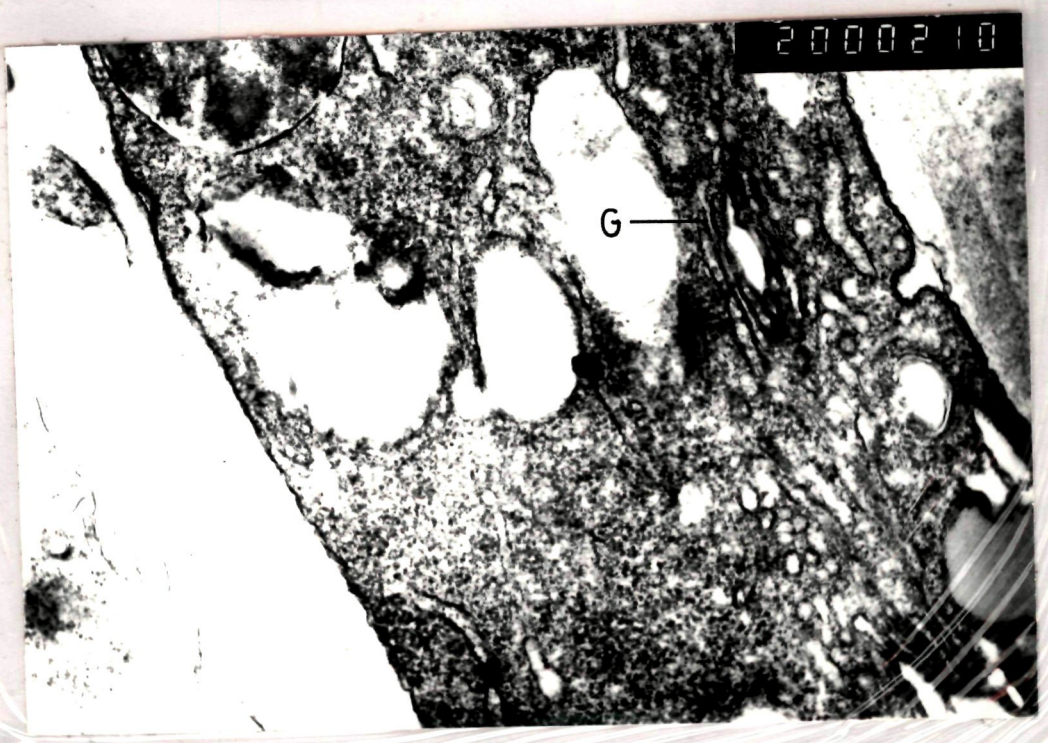


PLATE 4.4v

Chapter 5

DISCUSSION

D I S C U S S I O N

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.

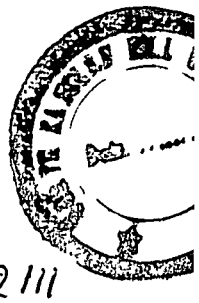
The phenomenon of neural induction brings about the transformation of ectoderm lying over the developing notochord into neural plate during gastrulation. This was first demonstrated by Spemann (1918) in an amphibian embryo, in the course of his investigation on the role of dorsal lip of blastopore as 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; Nieukopp, 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) demonstated 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 Nieukoop, 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 embryo 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 stagewise analysis

Waddington and his collaborators during (1930-1940) notably, Abercrombie, Taylor and Schmidt already discussed and demonstrated that the anterior part of the primitive streak 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 Nicolet (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 193 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 except at stage 4. 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.

Waddington, 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, 1979, 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 occured 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 one 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 8½ hours or longer.

Leikola and McCallion (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 intact 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 histo-morphology of the cells at time interval of 5, 10, 15, 20, 25 and 30 minutes. The cells become slightly elongated with interceullular 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 neuroid 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 contact but when the graft was isolated at stage 5, 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.

3. At stage 5, and 6, 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 neurectodermal 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 neurectoderm 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 tissues. 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. Neighbouging 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 on going 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 also larger amount of debris in the neural plate region than laterally (Baneroft 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 neurectodermal cells.

5.7 Embryonic Endoderm-Origin and Formation

Earlier workers (Kionka, 1894; Wetzel, 1929; Merbach, 1935, Hunt,1937, Pasteels, 1937; Peter, 1938; Jacobson, 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 Mareel 1964, Modak,1963, 1965, 1966, Rosenguist 1966, Nicolet,1965, 1967 have conclusively demonstrated that the embryonic endoderm arises from the base of Hensen's node at stage 3, 4 and 5. The original hypoblast, also called primary hypoblast,is pushed anteriorly in the germinal crescent area. The now secondary hypoblast arising from the base of Hensen'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 immunefluorescence 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. Gallera (1971) writes that "the, first stimulus for the neural induction, then, would originate in the presumptive embryonic endoblast, 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 (Gallera 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 these 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; Gallera, 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°, 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 millipore 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.

SUMMARY

SUMMARY

This work includes an analytical investigations on the neural inducing capacity of the primitive streak of the chick embryo with and without endoderm at (Hamburger and Hamilton, 1951) stages 3, 4, 5 and 6. The changes in the neurectoderm induced by grafts of Hensen's node of stages 4, 5 and 6 at - different time intervals were observed. The histological and the ultra - structural changes in the normal neurectoderm of the chick embryo were carried out respectively at stages 3, 4, 5 and 6 and stages 4, 5 and 6.

(1) In the first part of the investigation, the host and donor blastoderms were incubated for the desired period of time at 37.5°C (± 1°C) and the technique of New (1955) was followed for culturing the grafts for 24 hours. The graft pieces were taken from the anterior to the posterior portion of the primitive streak designated as A, B, C and D and, they were isolated from the primitive streak region of the blastoderm at stages 3, 4, 5 and 6 (Figs. 3.5a, 3.5b). Three types of grafts were prepared and implanted:

(a) Grafts pieces with all germ layers intact at stages 3, 4, 5 and 6 designated as EcMEn grafts.

(b) Graft pieces without the endoderm at stages 3, 4 and 5 designated as EcM grafts.

(c) Graft pieces without endoderm and mesoderm at stages 3, 4 and 5 designated as Ec grafts.

Each graft so prepared was implanted below the ectoderm of the host embryo nearing stage 4 in the antero - lateral margin of the area pellucida (Figs. 3.5a, 3.5b). The host and graft were cultured *in vitro* for a maximum of 24 hours, after that they were fixed and processed for morphological and histological analysis. The structures differentiated were categorised as 'Complete' embryonic axis when all three axial structures namely, neural tube, notochord and somites were present; 'Incomplete' embryonic axis when only two axial structures, namely, neural plate and either notochord or somites were present; and instances where only the neural plate is formed.

The following is a summary of morphological and histological analysis :

(a) In the first set of experiments a total of 183 grafts were implanted of which 48 (25%) died. Complete embryonic axis was induced only by A and B grafts, the anterior portion of the primitive streak; while C and D grafts did not show any induction. Incomplete embryonic axis was induced by A, B and C grafts (Figs. 4.1A - EcMEn, 4.1B - EcMEn, 4.1C - EcMEn). D grafts did not show any induction from stage 3 to 6 except at stage 4. This shows that anterior portion of primitive streak have greater inducing power than the posterior region. This supports the earlier findings

of Waddington, Abercrombie, Taylor and Schmidth (1930-1940), Mulherkar (1958), Gallera (1964) and Vakeet (1964). Neural plate induction was achieved by all grafts at stages 3, 4, 5 and 6. The present work indicates that posterior half is not devoid of inducing capacity since at least a neural plate is induced by posterior portion in some cases.

(b) In the second set of experiments when endoderm was removed from the primitive streak, a total of 95 grafts of stages 3, 4 and 5 were implanted of which 42 (44%) died. Complete embryonic axis was not induced by any of the grafts. Incomplete embryonic axis was induced by A and B grafts, and by C graft only at stage 4, C grafts of stages 3, and 5 and D graft of all the three stages did not show any induction. Neural plate was always induced by A, B, C grafts but not by D grafts. This shows that in the absence of endoderm, the formation of complete embryonic axis does not occur, thereby indicating that the presence of endoderm might be essential for the induction of other axial structures other than the neural plate.

(c) In the third set of experiments when the primitive streak was stripped free of the endoderm and mesoderm a total of 80 grafts of stages 3, 4 and 5 were implanted of which 32 (40%) died. Complete embryonic axis was not induced by any of the grafts. Incomplete embryonic axis was induced only by graft A at stages 3, 4 and 5. This shows that in the absence of both endoderm and

mesoderm, only the anterior part of primitive streak has the greater inducing capacity of all the graft types. However, neural plate was induced by A, B, C grafts and by D graft at stage 4. This shows that except anterior graft A, other grafts are capable of inducing only the neural plate.

(2) In the second part of the investigation, grafts of only Hensen's node of stages 4, 5 and 6 at different time intervals were implanted below the ectoderm of the host embryo nearing stage 4 (Fig. 3.7a). The host embryo together with the graft were incubated for different periods of time after which they were fixed and processed to study the histological changes in the induced neurectoderm over time. Two types of grafts were prepared and implanted :

(a) Grafts of Hensen's node of stages 4, 5 and 6 with all the germ layers, designated as 4 EcMEn, 5 EcMEn and 6 EcMEn. At Stages 4 and 5, grafts were implanted for periods ranging from 5 to 30 minutes while stage 6 grafts were left in contact for 50 minutes and 2 hours.

(b) Grafts of Hensen's node of stages 4, 5 and 6 without endoderm, designated as 4 EcM, 5 EcM and 6 EcM. At stages 4 and 5, grafts were left in contact for periods ranging from 10 to 30 minutes and at stage 6, grafts were left in contact for 2 hours and also for 2 hours 30 minutes.

In the present investigation, comparative analysis was made to study the effect of difference in the timing of induction under experimental condition by grafts of Hensen's node at stage 4, 5 and 6 with all the germ layers and without endoderm.

(a) A distinct difference was noted with stage 4 grafts, a thickened neural plate in the responding ectoderm was seen at 15 minutes but at stage 5, a clear histological change was observed at 10 minutes of contact. Fully formed neural plate was seen at 30 minutes of contact. While at stage 6, the inducing power of Hensen's node was much reduced and no induction occured even at 50 minutes of contact. These results indicate that at stage 5, the Hensen's node has greater inducing capacity than at stage 4 and 6.

(b) When endoderm is removed from the grafts, they could evoke only a slow and weak response. There was not much difference in the timing of neural induction at stage 4 but at stage 5, it took twice as long for the formation of a thickened neural plate. At stage 6, there was a weak response in the responding tissue even at 2 hours of contact. Thus, it is clear that at stage 4, Hensen's node has prospective embryonic endoderm and chordal mesoderm cells. At stage 5, chordal mesoderm cells have emigrated anteriorly from Hensen's node to give rise to

head process and the endoderm cells have emigrated to the base of Hensen's node to give rise to embryonic endoderm, this may be the reason why the grafts with all germ layers intact caused induction in the reacting ectoderm faster than those without endoderm. At stage 6, all endoderm cells have emigrated to the base of the Hensen's node. Instructive investigations in this field has been carried out by Gallera (1964, 1965, 1966). The experiments performed showed that at stages 4 and 5, fully formed neural plate was seen at 30 minutes of contact.

(3) In the third part of the investigation, histological changes in the normal neurectoderm at stages 3, 4 and 5 have been studied. The embryos were excised, fixed, processed and embedded in epoxy resin and sectioned in the normal neurectoderm for histological analysis (Figs. 3.8a, 3.8b). An assessment of histological changes was made by recording the different cells types present and their dimensions. The sections of neurectoderm at stage 3 appeared as a thin strip of cells composed of different cell types with intercellular spaces. At stage 4, the neurectoderm showed a slight increase in the degree of stratification with intercellular spaces between the different cell types. At stage 5, the entire layer appeared as a thickened strip with less inter - cellular spaces, cells were bottle - shaped, tall columnar,

cuboidal and some rounded cells. Similarly, at stage 6 the neurectoderm appeared thickened with cells closely packed except in a section at neural fold region where some intercellular spaces appeared. The different cell types also show an increase in their length as development proceeds.

(4) In the fourth part of the investigation, the ultra - structural changes of the normal neurectoderm at stages 4, 5 and 6 were studied (Figs. 3.9a, 3.9b). The embryos were excised and processed for TEM, thin sections were observed (thickness 600 Å to 900 Å). At stage 4, the sections revealed that normal neurectoderm consists of two types of cells (a) elongated and deeply staining cells and (b) irregular shaped and lightly staining cells, mesenchymal in character with a number of pseudopod - like processes. The deeply staining cells were firmly attached to the upper or dorsal surface of the neurectoderm and had prominent nucleus, and a dense concentration of yolk granules and mitochondria. At stage 5, the cells are nicely aligned, elongated and densely packed throughout the thickness of the neurectoderm. The mesenchymal cells present in the upper or dorsal surface of the neurectoderm are very few in number. Similarly, at stage 6 the cells were distinctly elongated and densely packed, closely apposed to neighbouring ectodermal cells.

Mesenchymal cells were rarely seen.

Observation of dense concentration of yolk granules and mitochondria in elongated cells may be on account of the greater energy required for morphogenesis. Once their shapes is established, the function of these organelles is also reduced to some extent. The large amount of lipid droplets or yolk granules seen, store raw material for providing energy during the elongation and morphogenesis of these neurectodermal cells.

REFERENCES

REFERENCES

Abercrombie, M. 1937. The behaviour of epiblast grafts beneath the primitive streak of the chick. J. exp. Biol. 14 : 302-318.

_____ 1950. The effects of antero-posterior reversals of lengths of the primitive streak in the chick. Phil. Trans. R. Soc. Ser B234 : 317-338.

Azar, Y. and Eyal-Giladi, H. 1979. Marginal zone cells the primitive streak inducing component of the primary hypoblast in the chick. J. Embryol. exp. Morph. 52 : 79-88.

_____ 1981. Interaction of epiblast and hypoblast in the formation of the primitive streak and the embryonic axis in chick, as revealed by hypoblast-rotation experiments. J. Embryol. exp. Morph. 61 : 133-144.

_____ 1983. The retention of primary hypoblastic cells underneath the developing primitive streak allows for their prolonged inductive influence J. Embryol. exp. Morph. 77 : 143-151.

Balinsky, B.I. 1976, An Introduction to Embryology (5th edition). Saunders, Philadelphia.

Bancroft, M. and Bellairs, R. 1974. The onset of differentiation in the epiblast of the chick blastoderm (SEM and TEM). Cell. Tiss. Res. 155 : 399-418.

_____ 1975. Differentiation of the neural plate and neural tube in the young chick embryo. Anat Embryol. 147 : 309-335.

Bellairs, R. 1971. Developmental Processes in Higher Vertebrates. Logos. London.

_____ 1986. The primitive streak. Anat. Embryol 174 : 1-14.

Bellair, R., Curtis, A.S.G. and Sanders, E.J. 1978, Cell adhesiveness and embryonic differentiation. J. Embryol. exp. Morph, 46 : 207-213.

Bellairs, R., Sander, E.J. and Portch, P.A. 1978. In vitro studies on the development of neural and ectodermal cells from young chick embryos. Zoon 6 : 39-50.

Berril, N.J. 1974. Developmental Biology. Tata Mc. Graw Hill Publishing Company.

Brachet, J. 1967. Behaviour of nucleic acids during early development. Comprehensive Biochemistry 28 : 23-54.

Brown, M.G., Hamburger, V. and Schmidt, F.O. 1941. Density studies on amphibian embryos with special reference to the mechanism of organiser action. J. exp. Zool. 88 : 353-372.

Butros, J. 1960. Induction by deoxyribonucleic acids of axial structures in post nodal fragments of chick blastoderm. J. exp. Zool. 143 : 259-282.

Choudhury, S. and Khare, M.K. 1978. Differentiation tendencies of eye potency field in early chick (Gallus domesticus) embryo. Ind. J. Exp. Biol. 16 : 555-557.

Constantinides, P. 1974. Functional Electronic Histology. Elsevier Scientific Publishing Company.

*Curtis, A.S.G. 1967. The cell surface : Its molecular role in morphogenesis. London. Logos press.

Dixan, B.A. 1966. A study of the effects of colchicine on the process of morphogenesis and induction in chick embryos. J. Embryol. exp. Morph. 16 : 245-258.

Duprat, A.M., Kan, P., Gualandris, L., Foulquier, F., and Marty, J. 1985. Neural induction : embryonic determination elicits full expression of specific neuronal traits. J. Embryol. exp. Morph. 89(suppl) : 167-183.

Ede, D.A. and Agarbak, G.S. 1968. Cell adhesion and movement in relation to the developing limb pattern in normal and talpid^3 mutant chick embryos. J. Embryol. exp. Morph. 20 : 81-100.

Emanuelsson, H. 1961. Mitotic activity in chick embryos at the primitive streak stage. Acta. Physiol. Scand. 52 : 211-233.

England, M.A. 1973. The occurrence of a band of nuclei in primary neural induction in the chick embryos. Experientia 29 : 1267.

_____ 1974. Cytoplasmic changes in primary neural induction. Experientia 30 : 808-809.

England, M.A. 1981. Application of the Scanning Electron Microscope to the analysis of morphogenatic events. Journal of Microscopy. Vol. 123, Pt-2, pp 133-146.

England, M.A. and Cowper, S.V. 1975. Primary neural induction as studied by Scanning electron microscopy. Experientia 31 : 1449-1451.

_____ 1976. A transmission and scanning electron microscope study of primary neural induction. Experientia 32 : 1578-1580.

England, M.A. and Wakely, J. 1977. Scanning electron microscope of the mesoderm layer in chick embryos. Anat. Embryol. 150 : 291-300.

_____ 1978. Endoderm regeneration in the chick embryo studied by SEM Anat. Embryol. 154 : 55-66.

Eyal-Giladi, H. 1970. Differentiation potencies of the young chick blastoderm as revealed by different manipulations. II. Localized damage and hypoblast removal experiments. J. Embryol. exp. Morph. 23(3) : 739-749.

_____ and Kochav, S. 1976. From cleavage to primitive streak formation : A complementary normal table and a new look at the first stages of the development of the chick. 1. General morphology. Devl. Biol. 49 : 321-337.

Eyal-Giladi, H. and Wolk, M. 1970. The Inducing Capacities of the primary hypoblast as revealed by Transfilter Induction Studies. Wilhelm Roux's Archiv. 165 : 226-241.

Eyal-Giladi, H. and et al 1975. Protein synthesis in the epiblast versus hypoblast during the critical stages of induction and growth of the definitive primitive streak in the chick embryo. Devl. Biol. 45 : 358-365.

Eyal-Giladi, H. and Kochav, S. 1976. From cleavage to primitive streak formation : A complementary normal table and a new look at the first stage of the development of the chick. I. General morphology. Devl. Biol. 49 : 321-337.

*Fell, H.B. and Robinson, R. 1929. The growth, development and phosphatase activity of embryonic avian femora and limb buds cultivated in vitro Biochem. Jour. 23 : 767-784.

*Flickinger, R.A. 1961. Formation, biochemical composition and utilization of amphibian egg yolk. In Symposium on germ cells and Development, Ed. S. Ranzi. Instut. Intern. d'Embryol. and Fondazione. A. Baselli, Milano, pp. 29-48.

Gallera, J. 1964. Exision et transplantation des differentes regions de la ligne primitive chez le poulet. Bull. de l'Assoc. d'Anat. 49 : 632-639.

Gallera, J. 1966. Le pouvoir inducteur de la chorde et du mesoblaste parachordal chez les oizeaux en function du facteur 'temps', Acta. Acta. 63 : 388-397.

_____ 1968. Induction neurale chez les oiseaux Rapport temporal entre la neuralation du blastoderme hote et l'appararition de l'ebauche neurale induite par un fragment de la ligne primitive. Rev. Suisse Zool. 75 : 227-234.

Gallera, J. 1970. L'action de l'actinomycine D sur le pouvoir inducteur du noeud de Hensen et la competence neurogene de l'ectoblaste de poulet. J. Embryol. exp. Morph. 23 : 473-489.

_____ 1971. Primary induction in Birds. Adv. Morphogen. 9 : 149-180.

Gallera, J. and Nicolet, G. 1969. Le pouvoir inducteur de l'endoblastepre'somptif contenu dans la ligne primitive jeune de poulet. J. Embryol. exp. Morph. 21 : 105-118.

Grunz, H. 1985. Information transfer during embryonic induction in amphibians. J.Embryol. exp. Morph. 89(supol) 349-364.

Grunz, H. and Tacke, L. 1986. The inducing capacity of the presumptive endoderm of Xenopus laevis studied by transfilter experiments. Wilhelm Roux. Arch. Dev. Biol. 195 : 467-473.

Gurdon, J.B. 1987. Embryonic induction - molecular prospects. Development. 99 : 285-306.

Hamburger, V. and Hamilton, H.L. 1951. A series of normal stages in the development of the chick embryo. J. Morph. 88 : 49-92.

Hamilton, H.L. 1952. Lillie's Development of chick. Henry Holt, New York.

Hara, K. 1961. Regional neural differentiation induced by prechordal and presumotive chordal mesoderm in the chick embryo. Ph.D. thesis. University of Utrecht.

Hara, K. 1978. "Spemann's organizer in Birds" in organizer - A milestone of half century from Spemann' edited by O. Nakumara and S. Toiyonen, Elsevier/North Holland, Amsterdam. pp 221-265.

Hayat, M.A. 1970. Principles and Techniques of Electron Microscopy. Biological Applications Vol.I. Van. Nastrand Reinhold Company.

Hay, E.D. 1968. Organization and fine structure of epithelium and mesenchymal interactions. In Flischmajor R. Billengham, R B (eds) Williams and Wilkins, Baltimore, pp 31-55.

Humason, G.L. 1979. Animal Tissue techndques W.H. Freeman and Company.

Hunt, T. 1937. The developmental of the gut and its derivatives from the mesectoderm of the early chick blastoderm. Anat. Rec. 68 : 349-370.

*His, W. 1874. Unsere Koerperform und das physiologische Problem ihrer Entstehung. F.C.W. Vogel Leipzig.

Ishiziya-Oka, A. 1983. Electron microscopical study of cell differentiation potency in the chick embryonic endoderm cultured in vitro. Wilhelm Roux's Arch. Dev. Biol. 192(3/4) : 171-178.

Jacobson, W. 1938a. The early development of avian embryo. I. Endoderm formation, J. Morph. 62 : 415-444.

Jaffe, L.F. and Stern, C.D. 1982. Strong electrical currents leave the primitive streak of the chick embryos. Nature 206 : 569-571.

Johnson, K.E. 1970. The role of changes in cell contact behaviour in amphibian gastrulation. J. exp. Zool. 175 : 391-428.

Khare, M.K. and Choudhury, S. 1985. Establishment of the neural differentiation pattern in the prospective prosencephalic ectoderm of the chick embryo (Gallus domesticus). Develop. Growth and Differ. 27(1) : 83-93.

*Kionka, H. 1894. Die Furchung des Huhnereies Anat. Hefte 3 : 391-445.

Kochav, S., Ginsburg, M. and Eyal-Giladi, H. 1980. From cleavage to primitive streak formation : A complementary normal table and a new look at the first stages of the development of the chick. II Microscopic anatomy and cell population dynamics. Devl. Biol 79 : 296-308.

Litke, L. 1978. Development and hypoblast formation In early chick, embryos as observed by scanning electron microscopy. J. Submicrose Cytol 10(1) : 1-14.

Mitrani, E. and Eyal-Giladi, H. 1981. Hypoblastic cells can form a disk inducing an embryonic axis in chick epiblast. Nature. 289(5800) : 800-802.

_____1984. Differentiation of dissociated Reconstituted Epiblast of the chick under the influence of a normal hypoblast. Differentiation 26(2) : 107-111.

Mitrani, E. and Farberov, A. 1982. Fibronectin expression during the pfocesses leading to axis formation in the chick embryo. Devl. Biol. 91 : 197-201.

Mitrani, E., Shimoni, Y., and Eyal-Giladi, H. 1983. Nature of the hypoblastic influence on the chick embryo epiblast. J. Embryol. exp. Morph. 75 : 21-30.

Mitrani, E. 1984. Mitosis in the formation and function of the primary hypoblast of the chick. Wilhelm Roux's Arch Dev. Biol 193(6) : 402-405.

*Merbach, H. 1935. Beobachtungen an der Keimscheibe des Huhnchens vor dem Er-Scheinen des Primitivstreinfens. Z. Anat. Entwgesch 104 : 635-652.

Mulherkar, L. 1958. Induction by regions lateral to the streak in the chick embryo. J. Embryol. exp. Morph. 6 : 1-14.

*Modak, S.P. 1963. L'endoblaste se forme-t-il par invagination a travers la ligne primitive dans l'embryon de poulet? International Congress Series No.70 : Excerpta Medica Foundation, Amsterdam, London, pp 122-124.

_____ 1965. Sur l'origine de l'hypoblaste chez les oiseaux, Experientia 21 : 273-274.

_____ 1966. Analyse experimentale de l'origine de l'endoblaste embryonnaire chez les oiseaux. Rev Suisse Zool. 73 : 877-908.

New, D.A.T. 1966. The Culture of Vertebrate Embryos Logos Press, Academic Press. London.

Nicolet, G. 1965a. Etude autoradiographique de la destination des cellules invaginees au niveau du noeud de Hensen de la ligne primitive achevee de l'embryon de poulet. Acta. Emb. Morph. 8 : 213-220.

*_____ 1965b. Action du chlorure de lithuim sur la morphogenese du jeune embryon de poulet. Acta. Emb. Morph. 8 : 32-85.

Nicolet, G. 1967. La chronologie d'invagination chez le poulet : Etude a l'aide de la thymidine tritiee. Experientia 23 : 576.

_____ 1970. Analyse autoradiographique de la localisation des differentes ebauches presomptive dans la ligne primitive de l'embryon de poulet. J. Embryol. exp. Morph. 23 : 79-108.

_____ 1971. Avian Gastrulation. Adv Morphogenesis 9 : 231-262.

Nieuwkoop, P.D. et al. 1952. Activation and organisation of the central nervous systems in amphibians. I. Induction and Activation. II. Differentiation and organisation. III. Synthesis of a new working hypothesis. J. exp. Zool. 120 : 1-108.

_____ 1973. The organisation centre of the amphibian embryo its origin spatial organisation and morphogenetic action. Adv. Morphogen 10 : 1-37.

Patten, B.M. 1971. Early Embryology of the chick (5th Edition). Tata McGraw Hill Publishing Company.

Pasteels, J. 1937. Etudes sur la gastrulation des vertebrees meroblastiques. III. Oiseaux. IV. Conclusions generales. Arch. Biol. Paris 48 : 381-488.

*Pasternak, L. and McCallion, D.J. 1962. Heterogenous inductions in the chick embryo. Canad. J. Zool. 40 : 585-591.

Penner, P.I. and Brick, I. 1984. Acetylcholisterase and polyingression in the epiblast of the primitive streak chick embryo. Roux's Arch. Dev. Biol. 193 : 234-241.

*Peter, K. 1938. Die Entwicklung des ento derms being Huhnchen Z. Mikrosk-anat. Frosch. 43 : 362-415.

Rao, B.R. 1968. The appearance and extension of neural differentiation tendencies in the neurectoderm of the early chick embryo, Roux' Arch. EntwMech. org. 160 : 187-236.

Rasilo, M.L. and Leikola, A. 1976. Neural induction by previously induced epiblast in avian embryo in vitro. Differentiation 5 : 1-7.

*Rauber, A. 1876. Primitive rinne und Urmund. Morph. Jhr p 2 : 550.

Rawles, M.E. 1936. A study in the localization of organ-forming areas in the chick blastoderm of the head process stage. J. exp. Zool. 72 : 271-315.

_____ 1943. The heart forming areas of the early chick blastoderm. Physiol. Zool. 16 : 22-42.

Romanoff, A.L. 1960. The Avian Embryo. Macmillan, New York.

Rosenquist, G.C. 1966. A radioautographic study of labelled grafts in the chick blastoderm : development from primitive streak stages, to stage 12. Contr. Embryol. Carneg. Instn. 38 : 71-110.

_____ 1972. Endoderm movements in the chick embryo between the early short streak and head process stages. J. exp. Zool. 180 : 95-104.

Rugh, R. 1962. Experimental Embryology (3rd Edition) Burgess Publishing Company. Minneopolis 15, Minnesota.

Rudnick, D. 1932. Thyroid forming potencies of the early chick blastoderm. J. exp. Zool. 62 : 287-313.

Sanders, E.J., Bellairs, R. and Portch, P.A. 1978. In vivo and in vitro studies on the hypoblast and definitive endoblast of Avian embryos. J. Embryoyol. exp. Morph. 46 : 187-205.

Shertbet, G.V. 1963. Studies on transplantations of amphibian anterior pituitary into chick embryo. Analysis of induction capacity by differential solubility and precipitation method J. Embryol. exp. Morph. 11 : 227-253.

Sherbet, G.V. and Mulherkar, L. 1963. The morphogenetic action of follicle stimulating hormone on post nodal fragments of early chick blastoderm. Arch. EntwMech. Org. 154 : 506-512.

_____ 1965. A study of the inductive capacity of post nodal-primitive streak pieces after treatment with follicle stimulating hormone. Arch. EntwMech. Org. 155 : 701-708.

Smith, J.C., Dale, L. and Slack, J.M.W. 1985. Cell lineage labels and region specific markers in the analysis of inductive interactions. J. Embryol. exp. Morph. 89 (Supl) : 317-331.

Solursh, M. and Revel, J.P. 1978. A Scanning electron microscope study of cell shape and cell appendages in the primitive streak region of the rat and chick embryo. Differentiation 11 : 185-190.

Spemann, H. 1918. Uber die Determination der ersten Organanlagen des Amphibien-embryos. Roux : EntwMech. Org. 43 : 448-555.

Spemann, H. and Mangold, H. 1924. Ueber Induction von Embryonalanlagen durch Implantation artfremder Organisatoren. Roux' Arch. EntwMech. Org. 100 : 599-638.

Spratt, N.T. Jr. 1952. Localization of the prospective neural plate in the early chick blastoderm. J. exp. Zool. 120 : 109-130.

Spratt, N.T. and Haas, H. 1960a. Morphogenetic movements in the lower surface of the unincubated and early chick blastoderm. J. exp. Zool. 144 : 139-157.

_____ 1960b. Importance of morphogenetic movements in the lower surface of the young chick blastoderm. J. exp. Zool. 144 : 257-276.

Steinberg, M.S. 1970. Does differential adhesion govern self assembly processes in histogenesis? Equilibrium configurations and the emergence of a hierarchy among populations of embryonic cells. J. exp. Zool. 173 : 395-434.

Tiedemann, H. 1967a. Inducers and inhibitors of embryonic differentiation : their chemical nature and mechanism of action. In Morphological and Biochemical Aspects of Cytodifferentiation, Eds. E. Hagen, W. Wechsler, and P. Zilliken. S. Karger, Basel, pp.8-21.

_____1967b. Biochemical aspects of primary induction and determination. In the Biochemistry of Animal Development, 2. Ed. R. Weber. Academic Press, New York and London, pp.4-55.

Tindall, A.R. 1960. Tungsten needles for microdissection stain. Technol. 35 : 105-106.

Toivonen, S. 1967. Mechanisms of primary embryonic induction. In Morphological and Biochemical Aspects of Cytodifferentiation, Eds. E. Hagen, W. Wechsler and P. Zilliken. S. Karger, Basel, pp 1-7.

Toivonen, S. and Wartiovaara, J. 1976. Mechanisms of cell interaction during Primary Embryonic Induction studied in transfilter experiments. Differentiation 5 : 61-66.

*Townes, P.L. and Holtfreter, J. 1955. Directed movements and selective adhesion of embryonic amphibian cells. J. exp. Zool. 128 : 53-120.

Trinkaus, J.P. 1984. Cells into organs (2nd edition). Prentice Hall. Inc. NJ.

Vakaet, L. 1962. Some new data concerning the formation of the definitive endoblast in the chick embryo. J. Embryol. exp. Morph. 10 : 38-57.

Vakaet, L. 1964. Diversite fonctionelle de la ligne primitive du blastoderme de poulet. C.R. Soc. Biol. 158 : 1964-1966.

_____ 1965. Resultats de la greffe de noeuds de Hensen dage different sur le blastoderme de poulet. C.R. Soc. Biol., 159 : 232-233.

Vakaet, L. 1967. Contribution a l'etude de la pregastrulation et de la gastrulation de l' embryon de poulet en culture in vitro. Mem. Acad. Roy. Med. Biol. 5 : 235-237.

_____ 1970. Cinephoto micrographic investigations of gastrulation in the chick blastoderm. Archs. Biol (Liege) 81 : 387-426.

Vakaet, L. and Mareel, M. 1964. Queles precisions sur la regeneration de l'endoblaste du blastoderm de poulet. C.R. Soc. Biol., Paris. 158 : 902-903.

Vanroelen, C.H.Verplanken, P. and Vakaet, L.C.A. 1982. The effects of partial hypoblast removal on the cell morphology of the epiblast in the chick blastoderm. J. Embryol. exp. Morph. 70 : 189-196.

Veini, M. and Hara, K. 1975. Changes in the differentiation tendencies of the hypoblast free Hensen's node during gastrulation in the chick embryo. Roux's Archives 177 : 89-100.

Viswanath, J.R., Leikola, A. and Rostedt, I. 1968. Induction by killed Hensen's node in the chick embryo ectoderm. Annales Zool. Fennice 5 : 384-388.

Waddington, C.H. 1930. Developmental mechanics of chick and duck embryos. Nature 125 : 924-925.

Waddington, C.H. 1932. Experiments on the development of chick and duck embryos, cultivated *in vitro* Phil. Trans. B 221 : 179-230.

_____ 1933. Induction by the primitive streak and its derivatives in the chick. J. exp. Biol. 10 : 38-46.

_____ 1952. The Epigenetics of Birds Cambridge University Press.

Waddington, C.H. and Schmidt, G.A. 1933. Induction by hetero plastic grafts of the primitive streak in birds. Roux' Arch. Entw.Mech. Org. 128 : 522-563.

Waddington, C.H. and Taylor, J. 1937. Conversion of presumptive ectoderm to mesoderm in the chick. J. exp. Biol. 14 : 335-339.

Wakely, J. and England, M.A. 1977. Scanning Electron Microscopy of the chick embryo primitive streak. Differentiation. 7 : 181-186.

_____ 1979. The chick embryo late primitive streak and head process studied by scanning electron microscopy. J. Anat. 129(3). pp 615-622.

Weinberger, C. and Brick, I. 1981. Primary hypoblast development in the chick 1. Scanning electron microscopy of normal development. Wilhelm Roux's Arch. Dev. Biol 191(2) : 119-126.

*Wetzel, R. 1929. Untersuchungen am Huhnchen, Die Entwicklung des keims Wahrend der ersten beiden Bruttage. Roux Arch. EntwMech. Org. 119 : 188-321.

Wolk, M. and Eyal-Giladi, H. 1977. The dynamics of antigenic changes in the epiblast and hypoblast of the chick during the process of hypoblast, primitive streak and head process formation as revealed by immuno-fluorescence. Devl. Biol. 55 : 33-45.

Woodside, G.L. 1937. The influence of host age on induction in the chick blastoderm. J. exp. Zool. 75(2) : 259-281.

Yamada, T. 1967. Factors of embryonic induction comprehensive Biochemistry. 28 : 113-143.

Zagris, N. and Eyal-Giladi, H. 1982. 5-Bromodeoxyuridine Inhibition of the epiblast competence for primitive streak formation in the young chick blastoderm. Dev. Biol. 91 : 208-214.

*Not seen in original.