Chapter 2

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

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 Vakeff, 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 area pellucida. 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 asymmetrical 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 structures 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 amniibian 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 possesses 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
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 \( \text{H}^2 \) 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 $[^{3}H]$-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 crescent 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 proceeded 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 suggest 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.
Ishiziva - 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 - ³H 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 (Nicole, 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 Nicole (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 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 Leikola (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 dorso-ventral 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 CNS 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 Sømhilt, 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 Pritch (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 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 Mulharkar (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 (1965b) 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-bromodeoxyuridine (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 information transfer during embryonic induction in amphibians. Neural induction and differentiation has been studied using Concanavalin A, Cyclic AMP, tunicamycin and calcium ionophore 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 (\(\alpha\) - D-mannoside and \(\alpha\) - 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 prerequisite 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 ionophore A 23187 does not elicit neural inductions. Calcium ionophore 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.

Duorat, 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.