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 HH 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 4.1i-a) 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 4.1j-b-4.1j-f) 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 4.1k-g,4.1k-h) 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 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 : 80.0% (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 | NO. OF CASES | % | NO. OF CASES | % | NO. OF CASES | % | NO. OF CASES | % | NO. OF CASES | % | NO. OF CASES | % | FREQUENCY OF NEURAL PLATE INDUCTION % |
|-------|---------------|--------------------------|-----------|-------------------|--------------|---|--------------|---|--------------|---|--------------|---|--------------|---|-------------------------------------|
| 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  | 1            | 20.0  | 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 4.1.1b 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 4.1.1d  Photomicrograph of chick embryo with complete embryonic axis indicated by arrow, induced by graft of donor embryo, Stage 5, having all germ layers (×300).
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 4.1.1f  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).
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 difficult when compared to stage 4. The endoderm was separated in Ca²⁺-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.
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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 4.1.2b 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).
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 Ca²⁺-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 embryonic axis was not 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 5D 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.

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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 4.1.3b 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).
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 germ 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
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 germ 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
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 germ 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
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 germ 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
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 resected 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).
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).
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 of Hensen's node

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 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 \( \mu m \) to 15 \( \mu m \), width 4.5 \( \mu m \) to 7 \( \mu m \) and their ND was 3 \( \mu m \).

**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 42 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 \( \mu m \) to 16.5 \( \mu m \), width 6 \( \mu m \) to 9 \( \mu m \) and their ND 3 \( \mu m \) to 4.5 \( \mu m \).

**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).
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 \( \mu m \) to 16.5 \( \mu m \), width 6 \( \mu m \) to 9 \( \mu m \) and their ND 3 \( \mu m \) to 4.5 \( \mu m \).

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 \( \mu m \) to 14 \( \mu m \), width 6 \( \mu m \) to 7 \( \mu m \) and their ND 3 \( \mu m \) to 4.5 \( \mu m \).

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 \( \mu m \) to 16.5 \( \mu m \), width 6 \( \mu m \) to 7 \( \mu m \) and their average ND was 3 \( \mu m \).
C. Experiments with grafts 6 EcMEn

Since the pilot experiments performed with grafts 6 EcMEn did not elicit any neurofil 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 neurofil 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.

<table>
<thead>
<tr>
<th>TIME INTERVAL (MINUTES)</th>
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<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
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<td>ND</td>
<td>L</td>
<td>W</td>
<td>ND</td>
</tr>
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<td>8</td>
<td>3</td>
<td>14</td>
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<td></td>
<td>I</td>
<td>15</td>
<td>6</td>
<td>2</td>
<td>15</td>
<td>8</td>
</tr>
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<td>7</td>
<td>4</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>12</td>
<td>8</td>
</tr>
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<td>4</td>
<td>3</td>
<td>18</td>
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<td></td>
<td>I</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>ROUNDED CELLS</td>
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<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
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</tr>
<tr>
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<td>I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>6</td>
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<td>TOTAL NO. OF CELLS</td>
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<td>NEUROID RESPONSE</td>
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</table>

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.

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<th>15</th>
<th>20</th>
<th>25</th>
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<td>C</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>12</td>
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<td>I</td>
<td>12</td>
<td>6</td>
<td>4.5</td>
<td>14</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
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<td>7</td>
<td>4</td>
<td>8</td>
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<td>I</td>
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<td>6</td>
<td>4.5</td>
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<tr>
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<td>4.5</td>
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<td>3</td>
</tr>
<tr>
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<td>7</td>
<td>3</td>
<td>-</td>
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<td>I</td>
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<td>6</td>
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<td>6</td>
<td>4.5</td>
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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

<table>
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<tr>
<th>TYPES OF CELLS</th>
<th>DIMENSIONS ($\mu m$)</th>
<th>TIME INTERVAL</th>
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<th>2 hrs.</th>
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<td>ND</td>
<td>L</td>
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<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>12</td>
<td>6</td>
<td>3</td>
</tr>
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<td>CUBOIDAL CELLS</td>
<td>C</td>
<td>14</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>15</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>TALL COLUMNAR CELLS</td>
<td>C</td>
<td>16</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ROUNDED CELLS</td>
<td>C</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>IRREGULAR SHAPED CELLS</td>
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<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>I</td>
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<td>4.5</td>
<td>3</td>
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<tr>
<td>TOTAL NO. OF CELLS</td>
<td>I</td>
<td>18</td>
<td>-</td>
<td>-</td>
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</table>

PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT

<table>
<thead>
<tr>
<th>NEUROID RESPONSE</th>
<th>(-)</th>
<th>(+)</th>
</tr>
</thead>
</table>

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

○--○ Length in the host induced neurectoderm.

○--○ Length in the host normal neurectoderm.
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 (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
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).
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 Hønsen'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 cells 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 neural ectodermal 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 \( \mu m \) to 15 \( \mu m \), width 6 \( \mu m \) to 8 \( \mu m \) and their ND 1.5 \( \mu m \) to 3.0 \( \mu m \).

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 \( \mu m \) to 15 \( \mu m \), their width was 6 \( \mu m \) to 8 \( \mu m \) and ND varied from 3 \( \mu m \) to 4.5 \( \mu m \).

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 ranged stratified. The length of cells from 9 \( \mu m \) to 18 \( \mu m \), width 6 \( \mu m \) to 7 \( \mu m \) and ND 1.5 \( \mu m \) to 4 \( \mu m \).
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 at stage 6 ECM

Since pilot experiments with grafts of Hensen's node without endoderm at stage 6 showed very feeble neuroepithelial response even after 2 hours of contact, present experiment was conducted with 2 hours 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 \( \mu m \) to 13 \( \mu m \), width 6 \( \mu m \) to 9 \( \mu m \) and their ND 1.5 \( \mu m \) to 4.5 \( \mu m \).

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 \mu m to 15 \mu m, width 6 \mu m to 8.0 \mu m and their ND 3 \mu m to 4.5 \mu m
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.

<table>
<thead>
<tr>
<th>TIME INTERVAL (MINUTES)</th>
<th>TYPE OF CELLS</th>
<th>DIMENSIONS (µm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>BOTTLE-SHAPED CELLS</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>CUBOIDAL CELLS</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>TALL COLUMNAR CELLS</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>ROUNDED CELLS</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>IRREGULAR SHAPED CELLS</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>TOTAL NO. OF CELLS PRESENT IN THE HOST ECTODERM AT THE AREA OF CONTACT WITH THE GRAFT</td>
<td>I</td>
<td>6</td>
</tr>
</tbody>
</table>

NEUROID RESPONSE
(-) Absence of neuroid response; (+) Presence of 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 4.2.2

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

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<th>25</th>
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<td>W</td>
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<td>6</td>
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<td>CUBOIDAL CELLS</td>
<td>C</td>
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<td>6</td>
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<td>I</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>12</td>
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<tr>
<td>TALL COLUMNAR CELLS</td>
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<td>9</td>
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<td>10</td>
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<tr>
<td>I</td>
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</tr>
<tr>
<td>I</td>
<td>12</td>
<td>10.5</td>
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<td>9</td>
<td>6</td>
</tr>
<tr>
<td>IRREGULAR SHAPED CELLS</td>
<td>C</td>
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<td>6</td>
<td>3</td>
<td>-</td>
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<td>9</td>
<td>6</td>
<td>3</td>
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<td>-</td>
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</tbody>
</table>

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 neurulation response induced by grafts of the Hensen’s node of stage 6 embryo without endoderm at different time interval of contact with the graft

<table>
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<tr>
<th>TIME INTERVAL</th>
<th>L (µm)</th>
<th>W (µm)</th>
<th>ND (µm)</th>
</tr>
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<td>2 hrs 30 mins</td>
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<td>6</td>
</tr>
<tr>
<td>I 13, 6</td>
<td>15</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>CUBOIDAL CELLS</td>
<td>C 10, 6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>I 12, 8</td>
<td>12</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>TALL COLUMNAR CELLS</td>
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<td>12</td>
<td>6</td>
</tr>
<tr>
<td>I 12, 6</td>
<td>12</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>ROUNDED CELLS</td>
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<td>9</td>
<td>7</td>
</tr>
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<td>I 10, 9</td>
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</tr>
<tr>
<td>IRREGULAR SHAPED CELLS</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I -</td>
<td>-</td>
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</tbody>
</table>

**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 response; (-) - 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).

○—○ Length in the host induced neurectoderm.

●——● Length in the host normal neurectoderm.
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 neur ectoderm at stages 3, 4, 5 and 6 reveals the following changes:

1. The sections of neur ectoderm 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 one another with less intercellular spaces. The neur ectodermal 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 neur ectoderm and their dimensions at stages 3, 4, 5 and 6

<table>
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<tr>
<th>TYPES OF CELLS</th>
<th>STAGE 3</th>
<th></th>
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<th>STAGE 4</th>
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<th>STAGE 5</th>
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<tbody>
<tr>
<td></td>
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<td>DIMENSIONS (μm)</td>
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</tbody>
</table>
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
Plate 4.3a Photomicrograph of a section of chick embryo at stage 3 sectioned in the normal neur ectoderm 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 neur ectoderm showing presence of different cell types (x400).
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 4.3e  Photomicrograph of a section of chick embryo at stage 5 sectioned in the normal neur ectoderm showing presence of different cell types (x 250).

Abbreviations:

BSc - Bottle-shaped cells
CC - Cuboidal cells
RC - Rounded cells
TCC - Tall columnar cells
Plate 4.3f 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 4.3c Photomicrograph of a section of chick embryo at stage 6 sectioned in the neural fold showing presence of different cell types (x400).
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
of cells: (1) deeply staining elongated cells
(2) lightly staining irregular shaped mesenchymal
cells each with a number of pseudopod-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 neuroectoderm and interspersed
with irregularly shaped mesenchymal cells in the
groups of three or four ectodermal cells. Towards
the ventral or lower surface of the neuroectoderm
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 prospective normal neuroectodermal 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 4.4c  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 neur ectoderm at stage 4 (x 4000).
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 4.4g TEM Photomicrograph showing a tall columnar cell (x 4000).

Abbreviations:

m - mitochondria
n - nucleus
R - Ribosomes
Y - Yolk granules
droplets or yolk granules (Plate 4.4a). This indicates some ongoing 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 4.4b, 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 degree 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 4.4j 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 4.4k  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
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 4.4j), 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 neur ectoderm. 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 4.4l 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 endoplasmic 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 4.4n

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

Abbreviations:

G - Golgi
m - mitochondria
TCC - Tall columnar cells
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 endoplasmic 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 4.4q 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).
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 4.4u 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).