

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

ABSTRACT

Rupa Nylla Hooroo

DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES



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ABSTRACT

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

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

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

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

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

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

The following is a summary of morphological and histological analysis :

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

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

(b) In the second set of experiments, a total of 95 grafts of the primitive streak without endoderm of stages 3, 4 and 5 were implanted of which 42 (44%) died. Complete embryonic axis was not induced by any of the grafts. Incomplete embryonic axis was induced by A and B grafts, and by C grafts only at stage 4. D grafts of all the three stages did not show any induction. Neural plate was induced ^{by} all grafts except graft D. In the absence of endoderm the formation of complete embryonic axis does not occur, thereby indicating that the presence of endoderm might be essential for the induction of other axial structures other than the neural plate.

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

was induced by A, B, C grafts and by D grafts at stage 4 while other grafts are capable of inducing only the neural plate.

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

(a) Grafts of Hensen's node of stages 4, 5 and 6 with all the germ layers, designated as 4 EdMEh, 5 EdMEh and 6 EdMEh. At stage 4 and 5 grafts were implanted for periods ranging from 5 to 30 minutes while stage 6 grafts were left in contact for 50 minutes and also for 2 hours since no neuroid response was observed when the contact period was for 50 minutes.

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

The result of the comparative study of the difference in the timing of induction under experimental condition by grafts of Hensen's node at stages 4, 5 and 6 with all the germ layers and without endoderm:

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

(b) When endoderm was removed from the grafts, they could evoke only a slow and weak response. There was not much difference in the timing of neural induction at stage 4 but at stage 5, it took ~~twice~~ as long for the formation of a thickened neural plate. At stage 6, there was a weak response in the responding tissue even at 2 hours of contact. Thus, it is clear that at stage 4, Hensen's node has prospective embryonic endoderm and chordal mesoderm cells. At stage 5, chordal mesoderm cells have emigrated anteriorly from Hensen's node to give rise to head process, ^{and} the endoderm cells have emigrated to the base of Hensen's node to give rise to embryonic endoderm. Thus, grafts with all germ layers intact are able to bring about changes in the reacting ectoderm faster than those which did not have endoderm. At stage 6, all endoderm cells have emigrated to the base of the Hensen's node. Instructive investigations in this field has been carried out by Gallera (1964, 1965, 1966).

(3) In the third part of the investigation, histological

changes in the normal neurectoderm at stages 3, 4, 5 and 6 have been studied. The embryos were excised, fixed, processed and embedded in epoxy resin and sectioned in the normal neurectoderm for histological analysis. An assessment of histological changes was made by recording the different cell types present and their dimensions. The sections of neurectoderm at stage 3 appeared as a thin strip of cells composed of different cell types with intercellular spaces. At stage 4, the neurectoderm showed a slight increase in the degree of stratification with intercellular spaces between the different cell types. At stage 5, the entire layer appeared as a thickened strip with less intercellular spaces. Similarly, at stage 6 the neurectoderm appeared thickened with cells closely packed except in a section at neural fold region where some intercellular spaces appeared. The different cell types also show an increase in their length as development proceeds.

(4) In the fourth part of the investigation, the ultra-structural changes of the normal neurectoderm at stages 4, 5 and 6 were studied. The embryos were excised and processed for TEM, thin sections were observed (thickness 600 \AA to 900 \AA). At stage 4, the sections revealed that normal neurectoderm consists of two types of cells (a) elongated and deeply staining cells and (b) irregular shaped and lightly staining cells, mesenchymal in character with a number of pseudopod-like processes. The deeply staining cells were firmly attached to the upper or dorsal surface of the neurectoderm,

and had prominent nucleus, and a dense concentration of yolk granules and mitochondria. At stage 5, the cells are nicely aligned, elongated and densely packed throughout the thickness of the neurectoderm. The mesenchymal cells present in the upper or dorsal surface of the neurectoderm are very few in number. Similarly, at stage 6, the cells were distinctly elongated and densely packed, closely apposed to neighbouring ectodermal cells. Mesenchymal cells were rarely seen.

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

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