Studies on self-differentiating and induction capacities of Hensen's node using intracoelomic grafting technique

By J. R. VISWANATH\(^1\) AND LEELA MULHERKAR\(^1\)

From the Department of Zoology, University of Poona

SUMMARY

Living Hensen’s node of the definitive primitive streak of chick embryo was prepared into ‘sandwiches’ with the competent ectoderm and the sandwich grafts were transplanted into the 2-5 day chick embryo using the intracoelomic grafting technique of Hamburger.

One hundred and twenty-four grafts were prepared and transplanted intracoelomically, 28 grafts were lost due to the death of the host embryos, 63 grafts did not differentiate at all, but 33 well-defined grafts were recovered, after cultivating the transplanted hosts for 12–14 days.

All kinds of tissues from feather germs to neural tissue were found to have differentiated in the grafts. The more frequently occurring tissues were feather germs, epidermal vesicle, neural tissue, kidney and muscle. Other differentiations were the cartilage notochord and gut. No definite combination pattern has emerged from the tissues. But when the tissues were traced to their germ-layer derivation, 22 of them belonged to the mesodermal complex, 11 to the ectodermal complex and 8 to the endodermal complex.

In the light of the above results, the probable existence of a mesodermal factor and an ectodermal factor independently responsible for the respective differentiations, as also the competence of the ectoderm, is discussed.

INTRODUCTION

The capacity of Hensen’s node for self-differentiation and induction has been investigated using different techniques by various investigators. Wetzel (1929), Hunt (1931), Willier & Rawles (1931) and Rudnick (1948) claimed that Hensen’s node alone has a capacity to form axial structures and is therefore an organizer. It was not until 1932 that Waddington, using intrablastodermal grafting technique, showed that the anterior third of the primitive streak possesses organizing capacity in chick embryo cultivated in vitro. Spratt (1942, 1948, 1952), using in vitro techniques, has also reported the differentiating capacity of the parts of the early chick blastoderm, including Hensen’s node. However, no work has so far been reported on the potentialities of Hensen’s node using intracoelomic grafting technique; although the studies on killed node using the same technique have been reported (Viswanath, Leikola & Rostedt, 1968). The present investi-

\(^1\) Authors’ address: Department of Zoology, University of Poona, Poona 7, India.
gation has been undertaken to study self-differentiation and induction capacities of living Hensen's node using an intracoelomic grafting technique and to compare them with those of the killed node where the same technique has been employed (Viswanath et al. 1968).

**MATERIAL AND METHODS**

Freshly laid White Leghorn eggs supplied by a local hatchery were incubated at 37.5 °C. Eggs used as donors were incubated to definitive primitive streak stage (Hamburger & Hamilton (1951), stage 4) and those that were used as hosts were incubated to 60–70 h embryos (Hamburger & Hamilton (1951), stage 15–16). The blastoderms from the donor eggs were separated from the underlying yolk mass and spread in Locke solution in the Petri dish with agar base. Competent ectodermal pieces antero-lateral to the Hensen's node were excised by carefully stripping off the endo- and mesodermal layers by means of sharp tungsten needles. Simultaneously the region of Hensen's node was excised, isolated and pipetted into a separate dish with Locke solution, Yamamoto (1949). Inner side of the already prepared competent ectoderm piece was brought in intimate contact with the inner surface of the Hensen's node to make a 'sandwich' which was kept for 30 min at room temperature to ensure proper attachment and then incubated at 37.5 °C for 3–6 h in order to facilitate contact between the reactive ectoderm and the mesoderm of the node. This was absolutely essential, since the contact time required is at least 6 h to produce a neuriod reaction in the ectoderm of area opaca (Gallera, 1965). In the area pellucida ectoderm typical neural inductions were obtained after 3–4 hours of contact (Gallera, 1970; Hara, 1961; Leikola & McCallion, 1967; Viswanath et al. 1968).

*Preparation of hosts*

Eggs incubated to 2.5 days, by which time the amniotic fold has reached the omphalomesenteric blood vessels, were used as hosts. That would be stage 16 of the Hamburger & Hamilton series. The intracoelomic grafting technique of Hamburger (1960) has been used throughout and guidelines envisaged by Hara (1961) and Rao (1968) have been mainly followed. The air chamber was punctured, a window was made on the shell and Locke solution was pipetted in. Part of the vitelline membrane covering the lateral somatopleure on either side of the embryo in the vicinity of the omphalomesenteric blood vessels was removed with the aid of tungsten needles. The cultured sandwich was gently pipetted close to the slit made in the embryo and inserted into the coelom. The hosts were cultivated generally for 12–14 days, but sometimes for 8–9 days in the incubator depending upon their condition, then they were sacrificed and opened. The grafts found inside the coelomic cavity were excised, fixed with Bouin, sectioned, stained with haematoxylin/eosin and examined histologically.
Table 1. Showing the number of instances of the induced or differentiated tissues and their combination pattern in grafts 1–33

<table>
<thead>
<tr>
<th>Tissues induced or differentiated</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neural tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Notochord</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3. Muscle</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. Cartilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5. Kidney</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. Blood vessels</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7. Gut</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Respiratory tubules</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. Epidermal vesicles</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Feather germs</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neural tissue</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>2. Notochord</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>3. Muscle</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>4. Cartilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>5. Kidney</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>6. Blood vessels</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td>7. Gut</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>8. Respiratory tubules</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>9. Epidermal vesicles</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>25</td>
</tr>
<tr>
<td>10. Feather germs</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10</td>
</tr>
</tbody>
</table>

+ = Tissues induced or differentiated.
In control operations only the competent ectoderm without the inductor kept for 3–6 h in a minimal amount of Locke solution at 37.5 °C was inserted into the host coelom.

RESULTS

Of a total of 124 grafts prepared and transplanted intracoelomically 28 grafts were lost due to the death of the host embryos, 63 grafts did not differentiate at all, but 33 well-defined grafts were recovered mostly on the posterior aspect of the coelomic cavity. Usually they were found attached to the body wall of the host embryo or to the mesentery. The grafts could be distinguished clearly from the host tissue by its spherical or bulbous structure with distinct feather germs noticed on the surface. All the identified tissues observed in the 33 grafts are detailed in Table 1. Out of 33 hosts showing grafts, 22 were alive till the 14th day, 5 up to the 10th day, while the rest lived less than 10 days.

All kinds of tissues from neural to feather germs were found to have differentiated in the grafts as categorized in Table 1. The more frequently occurring tissues were epidermal vesicle (Figs. 6, 9), muscle (Figs. 1, 2, 6, 8), neural tissue (Figs. 1, 4), kidney (Fig. 9) and feather germs (Fig. 1). As can be made out from Table 1, no definite combination pattern has emerged from the tissues. Virtually all kinds of tissues occurred as such, but when these tissue patterns were traced to their germ-layer derivation, 22 of them belonged to the mesodermal complex, 11 to the ectodermal complex and 8 to the endodermal complex (Table 1).

Of the 21 control experiments only 3 cases showed small epidermal vesicles and the remaining 18 were probably absorbed in the host tissue, or another possible explanation would be the failure of the control grafts to adhere to the body wall of the hosts.

DISCUSSION

It can be seen from the results that a number of structures from neural tissue to feather germs have been differentiated in the sandwiches (of Hensen's node and competent ectoderm) grown intracoelomically. The difficulty of distinguishing between the node (inductor) and the reacting ectoderm makes it impossible to state with certainty which tissues have differentiated as a result of the self-differentiating capacity of the node and those which are formed as a

ABBREVIATIONS ON FIGURES

Ct = Notochord, Ct = cartilage, Ev = epidermal vesicle, FG = feather germs, G = gut, K = Kidney tubules, M = muscle, N = neural tissue.

Fig. 1. Almost the entire graft in section, showing neural tissue, cartilage, muscle, gut and feather germs. × 48.

Fig. 2. Graft showing cartilage, muscle and gut. × 48.

Fig. 3. Part of the cartilage from Fig. 2 magnified. × 135.
Fig. 4. Part of the graft showing neural tube. ×165.
Fig. 5. Part of the neural tube from Fig. 4 magnified. ×750.
Capacities of Hensen’s node

Fig. 6. Graft showing notochord, muscle and epidermal vesicle. × 54.
Fig. 7. Part of the notochord from Fig. 6 magnified. × 155.
Fig. 8. Part of the graft showing notochord and muscle. × 54.
Fig. 9. Graft showing kidney tubules with blood vessels, and also an epidermal vesicle on the side. ×135.

Fig. 10. Part of the graft showing the gut. ×135.
result of induction by the node. It is probable that the reactive ectoderm in contact with Hensen's node is induced into neural tissue (Figs. 1, 4, 5). Existence of mesodermal competence in the epiblast as shown by Waddington & Taylor (1937) might have resulted in the formation and differentiation of muscles and cartilage (Figs. 3, 6, 8). It is equally possible that the presence of notochord and somite centres in the node (Spratt, 1955, 1957a, b, 1958) have attributed the self-differentiation of these structures—somites differentiating in course of time into dermatome giving rise to dermis, sclerotome to cartilage and myotome to muscles. According to Waddington (1952, p. 87), feather germs can originate from ectoderm independent of inductive stimulus from the axial mesoderm. However, some mesodermal cells might migrate out of the dermatome to form feather germs (Fig. 1) as the dermis has been shown to be the inductor of feather differentiation (Sengel, 1958). Endodermal derivatives such as gut (Fig. 10) or respiratory tubes in the present case may either be due to the inductive interaction between the competent ectoderm and the axial mesoderm of the node (Waddington, 1952, p. 87; Rudnick & Rawles, 1937) or to the presence and differentiation of endoblast in the node at stage 4 (Nicolet, 1970, 1971, p. 246). Bellairs (1953) and Nicolet (1970), using short-term culturing technique, have found the differentiation of foregut from the node. However, Rudnick & Rawles (1937) got the differentiation of small or large intestine from the fragments of node cultivated for 8–10 days on chorio allantois. In the present work also gut resembling small intestine (Fig. 10) was formed probably by the differentiation of the grafted node. The difference in the differentiation of the level of gut (pharynx or intestine) may be attributed to the use of different techniques as suggested by Bellairs (1953).

As the grafted sandwich is grown in the coelomic environment of the host, the question arises whether the environment has influenced the differentiation or induction of these structures. Waddington (1935) as well as Holtfreter (1934) suggested the possibility of blood or chick embryonic extract possessing the factor for neuralization in amphibians. No occurrence, however, of neuralizing factor in the intracoelomic environment was reported by Hara (1961), although he does not completely rule out such a possibility. Moreover, the reacting ectodermal pieces without the inductor in the control series grown in the same environment do not undergo any differentiation, indicating the neutrality of the environment.

It will be interesting to compare at this stage the potentialities of the living node with those of the killed node. The grafts of the killed node were unable to induce neuralization in the reacting ectoderm (Pasternak & McCallion, 1962) and therefore they concluded that the inductor is probably a component derived from the mesoderm of the living nodes. Gallera, Nicolet & Baumann (1968) and Gallera (1971) also failed to get neural reaction in the ectoderm of area opaca with killed node. Waddington (1933, 1934) got only two cases of neuralization with heat-coagulated chick organizer while Leikola & McCallion (1968) reported
neuralization in 17 out of 24 cases with alcohol-treated nodes. The property of induction by killed nodes has been shown to be similar to that shown by heat-coagulated dorsal lip of blastopore in Amphibia (Bautzman, Holtfreter, Spemann & Mangold, 1932). The self-differentiated capacity of the killed node has not been reported by any of these investigators. In fact, according to Waddington (1933, 1934), the dead implant is soon covered by mesenchyme cells of the host. All the investigators used short-term culture techniques, and therefore differentiation, if any, into cartilage or muscles which arise late in development could not be studied. A variety of structures exactly as shown by the living node in the present work has been shown to be differentiated or induced by the ethanol-killed node using intracoelomic grafting technique (Viswanath et al. 1968). If it is so it means there is no qualitative difference in the differentiation and induction by the living and killed node, or Hensen's node, which was immersed in 70% ethanol only for 3–5 min, was not completely killed and that the cells which were still alive evoked induction in competent ectoderm or self-differentiated into various structures as seen in the living node. A similar case has been reported by Gallera (1971, p. 172), in which the Hensen's node was incompletely killed by freezing. The problem will have to be reconsidered before the comparison is made. It will also be interesting to study the potentialities of the regressing node and the last third of the primitive streak using an intracoelomic grafting technique.

The authors wish to thank Professor Sulo Toivonen, Head, Laboratory of Experimental Embryology, University of Helsinki, Helsinki, Finland, for reading the manuscript and constructive criticism. One of the authors (J.R. V.) also wishes to thank the U.G.C., New Delhi, for the award of a Junior Research Fellowship and the Government of Mysore for granting study leave. Thanks are also due to Mr N. K. Naik for his photographic assistance.

REFERENCES

Capacities of Hensen's node


(Manuscript received 1 November 1971, revised 7 July 1972)