Initial stimulus and subsequent interactions in embryonic induction

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WITH ONE PLATE

An artificial environmental stimulus of short duration (10–15 min.) leads to either mesodermal or neural differentiation of competent amphibian ectoderm in certain experimental conditions (Johnen, 1956a; Yamada, 1962). Subsequent to this stimulus development into well organized structures is autonomous, independent of environmental ‘inductive’ factors. Hence, two alternatives for the control mechanism have to be considered; either the whole pathway to the complex end-result is triggered and determined during the short-term initial pulse (primary induction), or only the first step of a long chain reaction has been activated, and this is followed by secondary interactions between the tissue components. Recently Grobstein (1963) discussed these alternatives in the mechanism of embryonic induction and concluded that a single step process is most unlikely in many known examples of induction (kidney tubule induction, induction of cartilage etc.). Seen from the viewpoint of primary induction, there seems to be good reason to agree with him, and we have in previous discussions often stressed the obvious significance of a neural/mesodermal interaction subsequent to the primary inductive stimulus (Saxén & Toivonen, 1961, 1962). The following experiment was planned to gain further evidence with this view in mind.

METHOD

Sandwich-type explants were prepared from competent Triturus vulgaris ectoderm. Either alcohol treated guinea-pig bone marrow or liver tissue of the same species treated for 30 min. at 70°C. was used as inductor. The former is known as a ‘mesodermal’ inductor with weak neuralizing capacity (Toivonen,

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1954), and the latter as a ‘neuralizing’ inductor leading to the formation of forebrain structures only (Toivonen & Kuusi, 1948).

The explants were cultivated for 24 hr. at 18°C. whereafter the inductor was mechanically removed. According to earlier experience, this time is, in our experimental conditions, sufficient to complete the induction (Toivonen, 1958) and to abolish the competence of the ectoderm to respond to primary inductive stimuli (Leikola, 1963). Subsequently, the explants were disaggregated following the method of Jones & Elsdale (1963). EDTA treatment in Ca and Mg free medium for 10–15 min. led to a complete disaggregation of the ectodermal pieces, and this cell suspension was then pipetted to small hollows in an agar-layed culture dish containing normal culture medium (Tris-buffered Holtfreter solution). The material of two explants (four ectoderms) was always combined and thoroughly mixed to become one coherent re-aggregate. The following experiments were made: Liver induced explant + liver induced explant (thirty-nine specimens); Bone marrow induced explant + bone marrow induced explant (forty-six specimens); Liver induced explant + bone marrow induced explant (eighty-two specimens).

After re-aggregation the tissue was transferred to fresh culture medium and cultivated for 12 days in an incubator at 18°C. The samples were then fixed, sectioned in paraffin and analysed under a microscope.

In addition to these series, twenty-two empty sandwiches (without any kind of inductor tissue) were prepared and identically treated.

RESULTS

The distribution of structures in the explants of the different series is illustrated in Text-fig. 1, and some typical cases in the Plate.

This kind of pseudo-quantitative presentation of the differentiations cannot, however, give an adequate idea of the differences between the different series. Hence, some additional, descriptive comments are warranted.

The differentiation of the explants induced with heat-treated liver tissue was poor, and in only one-third of the cases the neural structures could be classified as forebrain vesicles. In the other cases the neuralization was evident but the

PLATE

Figs. 1 & 2. Two specimens of re-aggregates originally induced by heat-treated liver tissue. Definite forebrain structures, neuroids and atypical epidermis are to be seen.

Figs. 3 & 4. Two examples of recombinations from one liver-induced and one bone marrow-induced, and subsequently disaggregated explant. In addition to structures seen in Figs. 1, 2, 5 and 6, definite hindbrain parts and ear vesicles can be seen.

Figs. 5 & 6. Two specimens induced by bone marrow, and subsequently treated similarly to the one in Figs. 1 and 2. The sections show differentiated notochord and muscle tissue, pronephric tubules and mesenchyme. In addition, small spinal cord-like structures can be seen.
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structures were lacking regional characteristics (except in one case showing a hindbrain-like structure and a short spinal cord?). In re-aggregates of the bone marrow induced explants, neural structures were noted in 50 per cent. of the cases.

![Text-fig. 1. The percentage distribution structures in the experimental series.](image)

In addition to short spinal cord-like tubes, hindbrain vesicles were noted in four cases. When cell suspensions of one liver-induced and one bone marrow-induced explant were combined, the neural structures were more prominent, and, in addition, the regional pattern of the central nervous system was different from that noted in the other series (Table 1). Large, often symmetrical and well-differentiated hindbrain vesicles were the most frequently found in this series.

A slight neuralization without any regional characteristics was noted in three of the control explants without any inductor tissue. The other cases showed only a proliferation of undifferentiated ectodermal cells generally referred to as ‘atypical epidermis’.

### Table 1

The percentage distribution of structures belonging to the different regions of the central nervous system in the experimental series

<table>
<thead>
<tr>
<th>Source of Explants</th>
<th>Forebrain</th>
<th>Hindbrain</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver induced explants</td>
<td>30</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Combined explants</td>
<td>39</td>
<td>71</td>
<td>69</td>
</tr>
<tr>
<td>Bone marrow induced explants</td>
<td>0</td>
<td>8</td>
<td>52</td>
</tr>
</tbody>
</table>
The above results show that all the different regions of the central nervous system can be obtained as a consequence of a short-term primary inductive stimulus. The length of this exposure required for subsequent autonomous differentiation is not exactly known and is obviously dependent on experimental conditions (c.f. Saxén, 1963) but it is certainly shorter than 24 hr. which was employed in the present study (Johnen, 1956a, b; Toivonen, 1958; Englännder, 1962; Yamada, 1962; Katoh, 1962). Subsequent to this 24-hr. period the differentiation of the ectoderm cannot be affected any more by similar inductive stimuli as shown by several authors (Nieuwkoop, 1958; Leikola, 1963). The results presented above indicate, however, that the differentiation during this latent period prior to detectable morphogenesis is still labile in certain respect. The comparison of the frequency of different structures of the CNS in our experimental series (Table 1) show that the regional segregation of the neural tube can still be altered subsequent to the competent period of the ectoderm.

The mechanisms involved in these different steps of morphogenetic interactions are not known. It is tempting, of course, to compare the primary, abrupt and obviously irreversible neuralization to known examples of changes induced through genetic material in micro-organisms. All evidence for the existence of such mechanisms in primary induction is lacking, however, and some findings like the 'induction' through unspecific environmental conditions are difficult to explain in the light of this theory (e.g. Masui, 1960; Barth & Barth, 1963). Subsequent interactions obviously occur between the cells neuralized during the first step and the similarly mesodermalized tissue components. There is ample evidence of the importance of the rôle of the underlying mesoderm to the growth and differentiation of the neural plate (cf. Saxén & Toivonen, 1962, pp. 221–6). The nature of this interaction is not known but it is obviously different from the first steps, and, for instance, direct effects on the proliferation of the neural plate have been shown (Takaya, 1959; Takaya & Watanabe, 1961).

SUMMARY

An experiment was planned to study the possible rôle of late tissue interactions in the segregation of the central nervous system. Ectodermal explants were induced to either 'archencephalic' or 'spinocaudal' direction by the employment of known heterogenous inductors. Twenty-four hours later, when the induction was completed and the competence of the ectoderm was lost, the explants were disaggregated and the inductors removed. The subsequent differentiation of the reaggregated cells was followed in three types of recombinations: (1) Archencephalic induced explants + archencephalic induced explants; (2) Spinocaudally induced explants + spinocaudally induced explants; (3) Archencephalic induced explants + spinocaudally induced explants.
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Microscopical analysis of these series showed that deuterencephalic inductions (hindbrain structures) were very rare in series 1 and 2 but frequent in series 3. Hence, it is concluded that the regional pattern of the CNS is determined by late interactions and not during the short-term primary inductive stimulus.

RÉSUMÉ

Effet initial et interactions secondaires dans l’induction

Des expériences ont été instituées pour étudier le rôle possible d’interactions tardives entre les tissus au moment de la ségrégation des parties du système nerveux central. On soumet des explants ectodermiques à l’induction ‘archencephalique’ ou ‘spinocaudale’, en employant des inducteurs d’origine connue. Vingt quatre heures plus tard quand l’induction s’est produite et que l’ectoderme a perdu sa compétence, les explants sont désagrégés et les inducteurs enlevés. La différenciation ultérieure des cellules réagrégées a été la suivante, dans trois types de recombinations: (1) Explant soumis à l’inducteur archencephalique + explant soumis à l’inducteur archencephalique; (2) Explant soumis à l’inducteur spinocaudal + explant soumis à l’inducteur spinocaudal; (3) Explant soumis à l’inducteur archencephalique + explant soumis à l’inducteur spinocaudal.

L’analyse microscopique de ces séries montre que les inductions deutérencephaliques (structures rhombencéphaliques) sont rares dans les séries 1 et 2 mais fréquentes dans la série 3.

On peut conclure de ces résultats que la mosaïque régionale du système nerveux central est déterminée par des interactions tardives et non par le stimulus inducteur primaire dans une première phase de courte durée.

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