Regional developmental capacities of the rat embryonic endoderm at the head-fold stage

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SUMMARY

Three areas, composed of all three germ layers, were isolated from Fischer strain rat embryonic shields at the head-fold stage, and grafted separately under the kidney capsule of adult male rats of the same strain. The areas were from the neural plate, Hensen’s node and the primitive streak. The resulting teratomas were examined histologically for the presence of derivatives of the primitive gut.

The grafts differed strikingly in their capacity to develop into different segments of the gut. Endoderm underlying the neural plate developed into derivatives of the foregut, while endoderm underlying the primitive streak developed mainly into derivatives of the mid- and hindgut.

It was concluded that, at the head-fold stage, the capacities to develop into different segments of the definitive gut are already roughly limited to particular areas of the endoderm.

INTRODUCTION

Exact knowledge of the organ-forming areas of the embryonic shield is essential for any detailed experimental work on early embryos. This is particularly relevant to the endoderm because of the great variety of its numerous derivatives.

It has been shown that in the early amphibian gastrula the endoderm already shows the regionally specific capacity to differentiate into various sections of the definitive gut (Holtfreter, 1938a, b). Using the method of grafting on the chorioallantoic membrane, Rawles (1936) determined the organ-forming areas in the chick blastoderm at the head-process stage. Through the use of radioactively labelled grafts Rosenquist (1970, 1971a, b) succeeded inlocalizing the prospective areas of different gut segments and their derivatives in the chick epiblast at the primitive streak stage.

Mammalian embryos have not yet been subjected to this type of experiment. Only recently a paper appeared dealing with the localization of the presumptive gastric area in the endoderm of the rabbit embryo (David, 1971).

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The present paper deals with the regionally restricted developmental capacities of the rat embryonic endoderm at the head-fold stage.

**MATERIAL AND METHODS**

The inbred Fischer strain of albino rats was used in the experiment. Egg-cylinders belonging to the head-fold stage (stage 15 of Nicholas, 1962, fig. 1) were isolated from uteri of females in the morning of the 10th day of pregnancy. The ectoplacental cone and the Reichert’s membrane were removed. The embryonic shield was separated from the extra-embryonic part by an oblique cut below the insertion of the amnion. Three areas, composed of all three germ layers, were isolated from the embryonic shield, as demonstrated in Fig. 2. These areas contained the neural plate (graft I), Hensen’s node (graft II) and the primitive streak (graft III) respectively. An intermediate region of the shield was discarded. Each of the three areas was grafted separately under the kidney capsule of a syngeneic adult male rat. After 15 days the recipient animals were
Fig. 2. Schema of the dissection of the 9-day rat egg-cylinder in order to isolate the three areas of the embryonic shield. The orientation of the egg-cylinder as in Fig. 1.

RESULTS

The general characteristics of resulting teratomas were the same as of those originating from grafted whole embryonic shields (Škreb, Švajger & Levak-Svajger, 1971) or separated germ layers (Levak-Svajger & Švajger, 1974). The teratomas derived from grafted Hensen’s node area were remarkably smaller than those derived from the other two areas. The results of the histological differentiation within the teratomas are summarized in Table 1 and partly illustrated in Figs. 3–6.

In Table 1 it can be seen that the three isolated areas of the embryonic shield do not differ essentially in their capacity to differentiate into ectodermal and mesodermal tissues. The only differences were: the poor development of the skin in the Hensen’s node area grafts, of the brown adipose tissue grafts from the area of the neural plate, and of the smooth muscle for both of these areas.

On the contrary, the particular isolated areas of the embryonic shield differ strikingly in their capacity to give rise to various derivatives of the primitive gut. These derivatives did not develop in grafts of isolated Hensen’s node area. In teratomas derived from the area of the neural plate, only derivatives of the anterior part of the foregut have developed (Fig. 3). Respiratory tube (ciliated columnar epithelium + cartilage + glands) was the most prominent structure in these teratomas. On the contrary, the grafted area of the primitive streak gave rise predominantly to derivatives of the mid- and hindgut. Tubes and/or cysts lined with a histologically well-differentiated mucosa of the small and large
Figs. 3-6. Details of the histological structure of teratomas obtained from the areas of the neural plate (Fig. 3) and of the primitive streak (Figs. 4-6). B, Bone; Br, brain; C, cartilage; G1, glands; Int, intestine; M, skeletal muscle; Pr, epithelial buds of the prostate; RT, respiratory tube; Sk, skin; St, stomach; Thy, thymus; UGS, urogenital sinus.
Regional capacities of rat endoderm

Table 1. Differentiation of mature tissues in homografts of isolated areas of the rat embryonic shield at the head-fold stage

<table>
<thead>
<tr>
<th>Area of the embryonic shield</th>
<th>Graft series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I Neural plate</td>
</tr>
<tr>
<td>No. of successful grafts</td>
<td>10</td>
</tr>
<tr>
<td>Ectodermal tissues</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>10</td>
</tr>
<tr>
<td>Neural tissue</td>
<td>10</td>
</tr>
<tr>
<td>Mesodermal tissues</td>
<td></td>
</tr>
<tr>
<td>White adipose tissue</td>
<td>9</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>1†</td>
</tr>
<tr>
<td>Cartilage</td>
<td>10</td>
</tr>
<tr>
<td>Bone</td>
<td>10</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>0</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>10</td>
</tr>
<tr>
<td>Endodermal tissues</td>
<td></td>
</tr>
<tr>
<td>Oral cavity,* pharynx, oesophagus</td>
<td>9</td>
</tr>
<tr>
<td>Respiratory tube</td>
<td>10</td>
</tr>
<tr>
<td>Glands</td>
<td>8</td>
</tr>
<tr>
<td>Thymus</td>
<td>4</td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
</tr>
<tr>
<td>Intestine</td>
<td>0</td>
</tr>
<tr>
<td>Urogenital sinus</td>
<td>0</td>
</tr>
</tbody>
</table>

* Partly of ectodermal origin (tongue as endodermal derivative).
† Rudimentary.
‡ The only oesophagus in all three series.

intestine, and surrounded by a typical two-layered muscularis, were regularly found in these teratomas (Fig. 4). Derivatives of the urogenital sinus: the urinary bladder (or urethra) and epithelial cords resembling rudiments of the male genital glands, differentiated in close association with the large intestine (Fig. 5). The stomach was found in only 3 out of 11 grafts of this series (Fig. 6).

DISCUSSION

The advantages of this method of grafting early rat embryonic material under the kidney capsule and the reliability of the obtained results have been discussed elsewhere (Škreb et al. 1971; Levak-Švajger & Švajger, 1974).

The fact that each of the isolated and grafted areas of the embryonic shield was composed of all three germ layers explains the presence of tissue derivatives of all three germ layers in the resulting teratomas. The failure of Hensen's node area to give rise to skin may be attributed to the fact that it included only a small part of the ectoderm, which was already determined to form neural
structures. The absence of smooth muscle in teratomas derived from both the neural plate and Hensen’s node areas is concomitant with the lack of the stomach and the intestine in these teratomas. As yet we cannot explain the absence of brown adipose tissue in teratomas derived from the area of the neural plate. It is surprising that hardly any endodermal derivatives have developed in grafts of Hensen’s node area. This may result from the small number of endodermal cells in this area, and their continuity with cells migrating through Hensen’s node.

The clear-cut difference in the type of endodermal tissues present in teratomas derived from different areas of the embryonic shield suggest that at the head-fold stage the capacities to develop into particular segments of the definitive gut are already limited to particular areas of the endoderm. The endodermal area underlying the neural plate seems to be determined to form the anterior part of the foregut and its derivatives, while the area underlying the primitive streak gives rise to the mid- and hind-gut derivatives. The area of the endoderm below Hensen’s node and the anterior end of the primitive streak might be supposed to correspond to the posterior part of the foregut (oesophagus and stomach).

From our previous experiments (Levak-Švajger & Švajger, 1971, 1974) it seems very probable that at the pre-primitive-streak and the primitive-streak stages all presumptive endodermal cells are localized within the primitive ectoderm. At the head-fold stage, however, the capacity to differentiate into derivatives of the primitive gut is restricted to the endoderm. At this developmental stage, the anterior part of the endoderm (underlying the neural plate) isolated and grafted together with the adjacent mesoderm (but without the overlying ectoderm), gave rise only to the derivatives of the foregut (Levak-Švajger & Švajger, 1974). This is consistent with the results of the present experiment, and, moreover, rules out the influence of the ectoderm on the differentiation of the primitive gut at this stage.

On the other hand, the presence of the mesoderm seems to be essential for the differentiation of the endoderm (Levak-Švajger et al. 1969; Levak-Švajger & Švajger, 1974). It is therefore only reasonable to speak of the regionally specific interactions between endoderm and mesoderm rather than of the regional developmental capacities of the endoderm alone. The same statement seems to be valid for the amphibian embryo (Nieuwkoop, 1973).

It must be mentioned that in the present experiment the ectoderm also displayed some regionally specific histogenetic capacities. Some ectodermal structures belonging to the head (choroid plexus, vibrissae, tooth germs) developed only in grafts of the anterior area of the embryonic shield.
ZUSAMMENFASSUNG

Regionale Entwicklungspotenzen des embryonalen Endoderms der Ratte auf dem Stadium des Kopffortsatzes


Die Transplantate einzelner Regionen unterschieden sich wesentlich je nach ihrer Potenz sich in die verschiedenen Segmente des Primitivdarms zu entwickeln. Das der Neuralplatte unterliegende Endoderm hat sich in die Derivate des Vorderdarms differenziert. Das dem Primitivstreifen unterliegende Endoderm differenzierte sich größtenteils in die Derivate des Mittel- und Enddarms.

Diese Befunde führten zum Schluss, dass die regionale Determinierung verschiederener Segmente des Darms schon auf dem Stadium des Kopffortsatzes besteht.

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REFERENCES


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