Influence of extrinsic factors on the development of the bulboventricular loop of the chick embryo

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SUMMARY

Of several chick culture methods investigated, New's technique produced the greatest percentage of normal bulboventricular loops.

In embryos with two left cardiogenic areas or with two right cardiogenic areas or with the areas exchanged, the bulboventricular loop develops to the right in the cases in which there is integration. When each of the areas was permitted to be expressed without the other and there was closure of the gut, both areas were expressed according to their intrinsic quality. We believe that in stage 5, the type of loop is not determined.

INTRODUCTION

There are numerous investigations concerning the origin of the heart in mammals (Goss, 1952), birds (Rawles, 1943; DeHaan 1963; Rosenquist & DeHaan, 1966) and amphibians (Copenhaver, 1926). These investigations demonstrate that the heart is formed by fusion of two primordia lateral to the primitive streak in birds and mammals. In birds, the heart-forming areas become developmentally determined (that is, the cardiogenic areas cannot change their prospective fate and in every medium they can develop as cardiac tissue) during Hamburger & Hamilton stage 5 (Rawles, 1943). The primitive heart tube formed by fusion of the primordia immediately develops a loop, the convexity of which is directed to the right and the concavity of which is directed to the left side of the embryo. Various investigators (Stalsberg, 1970; Castro-Quezada, Nadal-Ginard & de la Cruz, 1972; Nadal-Ginard & García, 1972) have tried to explain the mechanisms which control the formation of the bulbo-ventricular loop convex toward the right. They suggest that the curvature of the bulboventricular loop is determined by a delicate balance involving an interplay between factors intrinsic to the two heart primordia and extrinsic factors. The basic problem is the relative importance of the intrinsic and extrinsic factors.

The different developmental potentials of the two cardiogenic areas within the context of the embryo have been shown by means of the studies of cardia bifida in amphibians (Bacon, 1945) and birds (Nadal-Ginard & García, 1972).

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In contrast, outside the context of the embryo, by means of experiments developed by DeHaan (1964) in which isolated cardiogenic areas of stage-5 chick embryos were cultivated in different media, it was observed that occasionally each of these areas developed a tubular shape in which the atrium, ventricle and conus could be identified. In these experiments, each cardiogenic area has a different morphologic expression. It is thought that these differences could be among the factors which determine the formation of the normal bulboventricular loop. This led us to investigate whether the right and left halves of the blastoderm (extrinsic factor) have a primary influence on the direction of the curvature or whether the cardiogenic areas themselves present right or left characteristics (intrinsic factor) with expression independent from the medium (right hemiblastoderm or left hemiblastoderm) in which they are placed. To study this problem, two basic types of experiments were performed:

1. Replacing the left cardiogenic area from one blastoderm by the right area of another and vice versa at stage 5.
2. Exchanging the right and left cardiogenic areas of a blastoderm at stage 5.

MATERIAL AND METHODS

Stage-5 embryos (Hamburger & Hamilton, 1951, stages) were obtained from White Leghorn hen eggs incubated under normal temperature and humidity conditions (37-6 °C and 86% respectively). The investigation was developed in three series: (1) control of culture techniques, (2) sham operations, (3) experiments.

Series 1

Several culture techniques were tried: (a) paper ring technique, (b) Spratt’s (1947) technique, (c) New’s (1955) technique. In addition, observations were made in ovo on embryos with the same incubation time as those in vitro (see Table 1). After observing the results, New’s technique was chosen because it provided the greatest percentage of normal loops (94%). The embryos were explanted according to New’s technique, incubated for 1-2 h and then operated. After being operated, the embryos were reincubated in a Hot Pack incubator at a temperature of 37-5 °C, CO₂ pressure 0-8% and 100% humidity. The type of bulboventricular loop developed by the embryos could be determined after 29-30 h of incubation (Hamburger & Hamilton, stage 12). Later the embryos were fixed and stained with Light Green and photographed.

Series 2 (sham operation)

In 52 embryos the left and right cardiogenic areas (endoderm + mesoderm) were cut out with a fine glass needle, following the limits indicated by Rosenquist & DeHaan (1966) which correspond to squares 3–12. After the cardiogenic areas were separated from the ectoderm, they were replaced in their respective places.
Fig. 1. (A) Blastoderm at stage 5 immediately after receiving graft of left cardiogenic area of another embryo to the right hemiblastoderm. (B) The same embryo at stage 12. (C) Blastoderm at stage 5 immediately after operation to exchange cardiogenic areas. (D) The same embryo at stage 12.
Table 1. Results of different culture techniques

<table>
<thead>
<tr>
<th>Type of bulboventricular loop</th>
<th>Loop to the right</th>
<th>Loop to the left</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper ring technique</td>
<td>70 (70 %)</td>
<td>30 (30 %)</td>
<td>100</td>
</tr>
<tr>
<td>Spratt's technique</td>
<td>82 (82 %)</td>
<td>18 (18 %)</td>
<td>100</td>
</tr>
<tr>
<td>New's technique</td>
<td>47 (94 %)</td>
<td>3 (6 %)</td>
<td>50</td>
</tr>
<tr>
<td>Development in ovo</td>
<td>82 (100 %)</td>
<td></td>
<td>82</td>
</tr>
</tbody>
</table>

Table 2. Sham operation: removal and replacement of one cardiogenic area in embryos cultured by New's technique

<table>
<thead>
<tr>
<th>Ventral view</th>
<th>Type of bulboventricular loop</th>
<th>Right</th>
<th>Left</th>
<th>Cardia bifida</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right area</td>
<td>46 (88.5 %)</td>
<td>4 (7.7 %)</td>
<td>2 (3.8 %)</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Left area</td>
<td>47 (90.4 %)</td>
<td>3 (5.8 %)</td>
<td>2 (3.8 %)</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

Series 3 (experiments)

(a) Embryos with two left cardiogenic areas. In 39 embryos the right cardiogenic area was removed and in its place the left cardiogenic area of another embryo of the same stage was placed so that the embryo had two left cardiogenic areas (Fig. 1A). The grafted area kept its cephalocaudal orientation.

(b) Embryos with two right cardiogenic areas. In 46 embryos the left cardiogenic area was removed and in its place the right cardiogenic area of another embryo of the same stage was placed so that the embryo had two right cardiogenic areas.

(c) Exchange of right and left cardiogenic areas. In 23 embryos the right and left cardiogenic areas were exchanged, keeping their cephalocaudal axis so that the left cardiogenic area was placed in the right hemiblastoderm and the right cardiogenic area in the left hemiblastoderm (Fig. 1C).

(d) Removal of the left cardiogenic area. In 16 embryos the left cardiogenic area was removed.

(e) Removal of the right cardiogenic area. In ten embryos the right cardiogenic area was removed.

RESULTS

Series 1

Table 1 shows that, of the different culture techniques investigated, New's technique allowed development of the greatest percentage of normal bulboventricular loops (94% to the right). There were no inverted loops in in ovo development.
Table 3. Results of experiments

<table>
<thead>
<tr>
<th>Exp. (a)</th>
<th>Type of bulboventricular loop</th>
<th>Right</th>
<th>Left</th>
<th>Cardia bifida</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Embryos with two left cardiogenic areas</td>
<td>34 (87.2%)</td>
<td>—</td>
<td>5 (12.8%)</td>
<td>39</td>
</tr>
<tr>
<td>Exp. (b)</td>
<td>Embryos with two right cardiogenic areas</td>
<td>31 (67.4%)</td>
<td>9 (17.4%)</td>
<td>7 (15.2%)</td>
<td>46</td>
</tr>
<tr>
<td>Exp. (c)</td>
<td>Exchange of two areas</td>
<td>12 (52.2%)</td>
<td>1 (4.4%)</td>
<td>10 (43.4%)</td>
<td>23</td>
</tr>
<tr>
<td>Exp. (d)</td>
<td>Embryos without left cardiogenic area</td>
<td>3 (18.2%)</td>
<td>13 (81.8%)</td>
<td>—</td>
<td>16</td>
</tr>
<tr>
<td>Exp. (e)</td>
<td>Embryos without right cardiogenic area</td>
<td>10 (100%)</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
</tbody>
</table>

Series 2

Of 52 embryos in which the right cardiogenic area was dissected and replaced, 46 (88.5%) developed a normal bulboventricular loop (toward the right), 4 (7.7%) developed an inverted loop (toward the left) and 2 (3.8%) developed a double heart (cardia bifida) (Table 2).

Of 52 embryos in which the left cardiogenic area was dissected and replaced, 47 (90.4%) developed a bulboventricular loop convex to the right (normal), 3 (5.8%) developed an inverted bulboventricular loop (convex to the left) and 2 (3.8%) developed a double heart (cardia bifida) (Table 2).

Series 3

(a) Embryos with two left cardiogenic areas. Of the 39 embryos with two left cardiogenic areas, 34 (87.2%) developed a bulboventricular loop to the right (normal) (Fig. 1B) and 5 (12.8%) developed cardia bifida. No cases of inversion of the bulboventricular loop were observed (Table 3).

(b) Embryos with two right cardiogenic areas. Of the 46 embryos with 2 right cardiogenic areas, 31 (67.4%) developed a right bulboventricular loop, 8 (17.4%) developed a bulboventricular loop to the left and 7 (15.2%) developed cardia bifida (Table 3).

(c) Exchange of the left and right cardiogenic areas in the same embryo. Of 23 embryos operated, 12 (52.2%) developed a bulboventricular loop to the right (Fig. 1D), 1 (4.4%) developed a left bulboventricular loop and 10 (43.4%) developed cardia bifida (Table 3).

(d) Removal of the left cardiogenic area. Of 16 embryos without a left cardiogenic area, 3 (18%) developed a loop to the right and 13 (81.8%) developed a left bulboventricular loop (Table 3).

(e) Removal of the right cardiogenic area. Of 10 embryos without a right
cardiogenic area, all 10 (100%) developed a right bulboventricular loop (Fig. 2) (Table 3).

Healing of the cardiogenic areas in all the experiments (series 2 and 3a, b and c) came about in from 2 to $2\frac{1}{2}$ h with a little more rapid healing in experiments of series 2.

**DISCUSSION**

The study of different culture techniques led us to choose New's technique as the best for studying the bulboventricular loop, because it provided the least number of inverted loops. This is probably due to the fact that in this technique, the embryos are in a liquid medium, with their own vitelline membrane, and they experience conditions nearer to those of the *in ovo* embryo. On the other hand, the embryos incubated *in ovo* showed no bulboventricular inversion, which confirms that explantation itself alters the normal conditions of development, as
expressed in a percentage of bulboventricular inversions not present in normal development.

The act of removing the cardiogenic area and immediately replacing it in its own bed does not increase the number of inversions of the bulboventricular loop, since the percentage of inversions obtained was equal to that observed in control embryos using the same culture technique.

When the cardiogenic area of one side developed after removal of the cardiogenic area of the other side, it developed according to its situation. That is, the left area without the presence of the right area forms a normal loop (to the right), the closure of the primitive gut being more or less normal; conversely, when the right area developed without the presence of the left area, the curvature of the loop was inverted in the majority of cases (to the left) and also the closure of the gut was almost normal. These results agree with those obtained by Nadal-Ginard & García (1972) in more advanced stages of development.

The formation of cardia bifida, which was more frequent in those cases in which both cardiogenic areas were operated, was due to alteration in the closure of the anterio portal of the intestine following operatory trauma. In this case, both loops were always convex outward, that is, the morphologic expression was opposite to that obtained when both areas developed in association with normal closure of the intestine (Nadal-Ginard & García, 1972).

In the embryos with two left or two right cardiogenic areas or with exchanged areas, the number of bulboventricular loops to the right (normal loop) was greater than the number of inverted loops. In the cases in which there was complete integration of the grafted area, a loop was formed to the right (normal). When there was no integration of the grafted area, the area which was expressed was the non-operated one. For example, in the case of the embryos with two right cardiogenic areas, the number of observed inversions was greater than that obtained in the rest of the experiments because if the grafted area was not integrated and the non-operated (right) area was expressed, an inverted bulboventricular loop to the left was formed, which is the normal expression of the right area.

In conclusion, when there is integration of the areas to the hemiblastoderm in which they are placed, in most of the cases a bulboventricular loop is formed to the right. This fact suggests that in stage 5 of the chick embryo, the areas can be integrated into the contralateral hemiblastoderm and that their intrinsic properties for forming one type of loop are not determined in this stage.

The recombination technique does not permit recognition of the stage at which the type of bulboventricular loops is determined, because cardia bifida is always produced.
REFERENCES


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