Development of left/right handedness in the chick heart

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Summary

The chick heart tube develops from the fusion of the right and left areas of precardiac mesoderm and in almost all cases loops to the embryo’s right-hand side. We have investigated whether any intrinsic difference exists in the right and left areas of precardiac mesoderm, that influences the direction of looping of the heart tube. Chick embryos incubated to stages 4, 5 and 6 were cultured by the New method. Areas of precardiac mesoderm were exchanged between donor and host embryos of the same stage and different stages to form control, double-right and double-left sided embryos. Overall, double-right sided embryos formed many more left-hand loops than double-left sided embryos. At stages 4 and 5 a small percentage of double-right embryos formed left-hand loops (13%) whereas at stage 6 almost 50% of hearts had left-hand loops. Control embryos formed right-hand loops in 97% of cases. The stability of right-hand heart looping by double-left sided embryos, may be related to the process of ‘conversion’, whereas the direction of looping by double-right sided embryos has become randomised. There is some indication that an intrinsic change occurred in the precardiac mesoderm between stages 5 and 6 that later influenced the direction of looping of the heart tube. The direction of body turning is suggested to be linked to the direction of heart looping.

Key words: heart tube, precardiac mesoderm, asymmetry, chick embryo.

Introduction

Within a few hours of development of the chick embryo, the three body axes have been determined: dorso-ventral, antero-posterior and right/left. The dorso-ventral axis is the first to be determined by the orientation of the blastoderm in relation to the yolk. The first indication of the antero-posterior axis is seen in the very early two-layered blastoderm (stage XI, Eyal-Giladi and Kochav, 1976), with the formation of Koller’s sickle at the future posterior side. Out of this, the hypoblast grows anteriorly and later the axis is more clearly defined by the primitive streak. Once the first two axes are in place, so, by definition is the third, the left/right axis. However the time at which handedness, that is when the two sides, right and left become different, is not known.

The first clear indication of the differences between left and right sides is seen during the looping of the early heart. However, there have been reports of visible handed asymmetries as early as stage 4 and 5. Wetzel (1929) observed that in the chick at the primitive streak stage, the node is asymmetric. The node can be seen to consist of two parts, with the right-hand part being bigger than the left. Stalsberg (1969) measured the axial deviations in early chick embryos and found that in the majority of those at stage 5, the primitive streak curved to the right-hand side. Lepori (1966) found a similar shift of the primitive streak to the right-hand side and, that during the shortening process of the primitive streak, movements occurred faster on the left-hand side as compared to the right. In the early neurula rat embryo, Long and Burlingame (1938) observed asymmetry in the embryonic axis, with the head and tail ends of the neural fold inclined slightly towards the right. Stalsberg (1969) concluded that the heart primordia fused in a plane to the right of the body midplane, as measured through the notochord. Lepori (1969) thought this to be due to the asymmetric closing of the foregut caused by the faster movement of the left splanchnopleure as compared to the right.

The heart forms from the fusion of the left and right precardiac areas above the anterior intestinal portal. Two separate heart tubes develop which later fuse to form a single tube. This tube then undergoes the process of looping and twisting to the embryo’s right-hand side. This direction of looping is a feature which has been conserved within the vertebrate phyla, from and including the Elasmobranchs onwards (Patten, 1922). The incidence of left sided heart looping is rare in most species. In adult man the incidence of dextro-cardia is 0.01% (Torgersen, 1949), and is often
associated with congenital heart disease (Campbell and Deuchar, 1966). The development of left/right asymmetry has recently been reviewed by Brown and Wolpert (1990). They propose a mechanism for the conversion of asymmetries at a molecular level to the formation of right and left structures by specific tissues. This three-step mechanism involves the process of ‘conversion’ in which a molecular handedness is converted to cellular handedness. A mechanism is required by which random asymmetry can be generated. This random asymmetry allows the formation of either the left and right variation on a structure with equal probability, but can be biased by the handedness generated by the conversion process. Specific tissues would then use an ability to act upon the right and left bias and develop the appropriate form of the structure.

Central to the theory of Brown and Wolpert (1990) is the idea of randomization of left/right asymmetries. This was first proposed by Wilhelm (1921), based on the twinning experiments of Spemann and Falkenburg (1919). Support for the idea of randomization comes from the morphology of the mouse homozygous for the situs inversus viscerum mutation, (iv/iv). 50% of these mice show situs inversus. Brown and Wolpert (1990) explain this in terms of the right sides now being insensitive to ‘conversion’ and so handedness is random.

The mechanisms by which looping in the heart may occur have been studied by many authors, but no clear mechanism has emerged. The importance of the cytoskeleton, especially the actin bundles and myofibrils, was investigated by Manasek et al. (1972,1978), Icardo and Ojeda (1984), Icardo et al. (1982) and Itasaki et al. (1989,1991). The possibility that early differences between the right and left areas of precardiac mesoderm could effect the direction of looping was investigated by Salazar Del Rio (1974). He constructed embryos with either two right or two left areas of precardiac mesoderm at stage 5, and followed the development of the heart. He found that in the majority of cases, a right-sided heart loop was produced and concluded that at this stage the intrinsic properties of the areas of precardiac mesoderm which affected the direction of looping had not yet been determined. It is important to know when left and right differences are determined. The following experiments investigate the development of left/right handedness in the chick heart.

Materials and methods

Fertilized chick eggs were incubated at 38±1°C to stages 4,5 or 6 in accordance with Hamburger and Hamilton (1951). The embryos were prepared as for New cultures (New, 1955). The area of tissue used for grafting included that found by DeHaan (1963) to give rise to the heart (Fig.1A,B). The prospective heart mesoderm together with the underlying endoderm was dissected out using tungsten needles. These pieces of tissue were transferred between cultures using a small tungsten spatula. The donor tissue was orientated wherever possible within the host along the same antero-posterior axis as the donor. This could not be done with a 100% accuracy as the tissue from stages 4 and 5 often contracted immediately after removal from the donor, making it difficult to orientate. However, this is not thought to be critical as that the gradient of fibronectin, along which the precardiac cells migrate towards the head, is not effective until stage 7 (Linask and Lash, 1986,1988). For each operated embryo, the right or left area of the precardiac mesoderm was removed and replaced with a piece of precardiac mesoderm from another embryo. Control embryos had either the right or the left area of precardiac mesoderm removed and replaced with a piece of tissue of the same sidedness (see Fig.1A). Double-sided embryos were formed by removing one area of precardiac mesoderm and replacing it with tissue from the opposite side (see Fig.1B), giving either double-right or double-left sided embryos. Both control and double-sided embryos were made, using donor tissue of the same or a different stage to that of the host embryo.

Fig. 1. (A) Diagram of two stage-4 embryos to illustrate the right area of precardiac mesoderm (hatched). A right-sided control operation was performed by removing the right area of precardiac mesoderm from the host (straight arrow), and grafting into this space the right area of precardiac mesoderm from the donor (curved arrow). (B) Diagram of two stage-6 embryos to illustrate the formation of a double-right side embryo. The left area of precardiac mesoderm is removed from the host (straight arrow), and the right area of precardiac mesoderm from the donor is transferred to the left-hand side of the host.

The cultures were incubated for a further 24-36 hours at 37°C and 5% CO₂. Cultures were fixed in 1/2 strength Karnovsky’s (1965) solution for 4-12 hours, washed in distilled water, stained with Anthracene blue for whole mounts, photographed and assessed. The embryos were then dehydrated and embedded in araldite for transverse sectioning at 1 µm thickness. Sections were stained with Toluidine blue. The results were assessed for statistical significance using the χ² test corrected for small sample sizes: a probability of 1% or 5% was used as the level of significance.

Embryos of stage 4 and 5 were classed together as they are both developmentally pre-headfold, in contrast to stage 6. All statements of sidedness refer to those of the embryo.

To control for the possible non-inclusion of the transferred tissue into the host, an additional group of experiments were performed. Here the precardiac mesoderm was removed from either the right or left side of the embryo, but no tissue was grafted into the embryo to replace it.
Fig. 2. (A) Photograph of a stage-11 wholemount seen from the ventral view with a normal heart looped to the right. (B) Line drawing of above photograph, to enhance features of the heart. (C) Transverse section through the heart region of A. (D) Ventral view of stage-11+ wholemount with a left-hand looped heart. (E) Line drawing of wholemount photograph (D). (F) Transverse section through the heart region of D. (G) Ventral view of stage-12+ embryo showing cardia-bifida, with a larger tube on the right. (H) Line drawing of whole mount photograph (G). (I) Transverse section through the heart tubes of G. R, right hand side; L, left hand side; NT, neural tube; HT, heart tube. Bars, 500µm.
Results

Five different criteria of heart shape were recorded: (1) right-hand bulbo-ventricular loop, (2) a heart normal in structure but with a left-hand loop, (3) cardia-bifida, (4) one abnormally shaped tube, (5) no recognisable heart tube. The side to which the body turned was also recorded, as a right-hand side turn, a left-hand side turn or no turn. Hearts classified in the first two categories have a normal structure and differed only in the direction of looping, to the right or to the left. All such hearts are called normal in this paper.

Only 40% of the embryos that survived the operations formed normal hearts (87/218). In almost all of double-sided and control embryos the most common heart malformation was cardia-bifida (see Fig.2G). Cardia-bifida is the formation of two separate heart areas due to the disrupted migration of the precardiac mesoderm across the anterior intestinal portal. Where a tube formed, it often showed some degree of bending. The direction of bending was not recorded. From general observations the two heart tubes were seen to beat at different rates. The left-hand tube was usually observed to beat faster, as seen by other authors (DeHaan, 1959; Yada et al., 1985; Satin et al., 1988).

The results of control operations between embryos of the same stage show that only 1/17 normally developed hearts had left-hand loops (Table 1). Grafting tissue between embryos of different developmental stages shows that all 12 of the normally developed hearts looped to the right (Table 2, Fig.2A).

Heart shapes of double-sided embryos (two areas of precardiac mesoderm of the same sidedness) are presented in Tables 1 and 2. Table 1 shows grafts between embryos of the same stage. In double-right and left-sided embryos at stage 4 and 5, only 1/17 (6%) normally developed hearts looped to the left. This was formed by a double-right sided embryo. When the same grafts were done later at stage 6, 6/24 (25%) double-right sided embryos formed normal hearts but with left-hand loops (see Fig.2D). No left-hand loops were developed by double-left sided embryos. Thus at both stages, all 7 left-hand looped hearts were formed from double-right sided embryos. However the difference in the number of right- and left-hand looped hearts at stages 4-5 and 6 was not significant at the 5% probability level.

The results of double-sided grafts between host and donor embryos of different stages are shown in Table 2. Where the donor tissue was younger than the host, 4/7 normally developed hearts looped to the left. These 4 hearts were all seen in double-right sided embryos. When the donor tissue was older than the host embryo, 3/11 normal hearts had left-hand loops. Of these, 2 formed from double-left sided embryos in which the older tissue was on the right-hand side. A summary of the numbers of right-looped and left-looped hearts in control and double-sided embryos is presented in Fig.3. Between double-right and double-left sided embryos there is a significant difference at the 1% probability level, in the number of hearts that looped to the right or to the left. Double-sided embryos in 93% of cases developed right-hand loops whereas in double-right sided embryos the number of right-hand loops 16(57%), and left-hand loops 12(43%), was almost equal.

Table 3 presents the results of the additional group of control experiments. In this group one side of the precardiac mesoderm was removed and not replaced with a grafted piece of tissue. The heart therefore developed only from either the right or left precardiac mesoderm. Eleven out of the 32 embryos produced normally developed hearts that looped to the right, where the right precardiac mesoderm had been removed (Table 3). When the left precardiac mesoderm was removed, only 5/32 developed normal hearts and these looped to the right. These hearts were typically smaller than those of the other control groups but appeared to be similar in every other respect. Left-hand loops were not present in either of these control groups (Table 3). When excising one side of the precardiac mesoderm without regrafting, the formation of cardia-bifida was an indication that an insufficient amount of tissue had been removed (Table 3).

Table 1. Development of the heart in control and double-sided embryos. Grafts carried out between embryos of the same developmental stage

<table>
<thead>
<tr>
<th>Stage at operation</th>
<th>Side of donor tissue</th>
<th>Side of host tissue</th>
<th>Post-operated host sidedness of mesoderm Embryo</th>
<th>Number operated</th>
<th>Number survived/operated</th>
<th>Heart shape (% shape/survived)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RHS</td>
<td>RHS</td>
<td>RHS</td>
<td>15</td>
<td>10 (67)</td>
<td>Normal 5 (50)</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>RHS</td>
<td>8</td>
<td>7 (88)</td>
<td>Abnormal 3 (43)</td>
</tr>
<tr>
<td>4/5</td>
<td>RHS</td>
<td>RHS</td>
<td>RHS</td>
<td>10</td>
<td>9 (90)</td>
<td>Right-hand loop 4 (44)</td>
</tr>
<tr>
<td>6</td>
<td>RHS</td>
<td>RHS</td>
<td>RHS</td>
<td>8</td>
<td>8 (100)</td>
<td>Left-hand loop 4 (50)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>RHS</td>
<td>41</td>
<td>34</td>
<td>Cardia-bifida 0 (0)</td>
</tr>
<tr>
<td></td>
<td>RHS</td>
<td>LHS</td>
<td>RHS</td>
<td>8</td>
<td>8 (100)</td>
<td>Abnormal 4 (50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RHS</td>
<td>3</td>
<td>3 (11)</td>
<td>No heart tube 0 (0)</td>
</tr>
<tr>
<td>Double sided</td>
<td></td>
<td></td>
<td>RHS</td>
<td>29</td>
<td>20 (69)</td>
<td>Normal 7 (35)</td>
</tr>
<tr>
<td>4/5</td>
<td>RHS</td>
<td>LHS</td>
<td>RHS</td>
<td>27</td>
<td>21 (78)</td>
<td>Abnormal 9 (43)</td>
</tr>
<tr>
<td>6</td>
<td>RHS</td>
<td>LHS</td>
<td>RHS</td>
<td>30</td>
<td>25 (83)</td>
<td>Right-hand loop 7 (28)</td>
</tr>
<tr>
<td>6</td>
<td>LHS</td>
<td>RHS</td>
<td>RHS</td>
<td>34</td>
<td>30 (88)</td>
<td>Left-hand loop 11 (37)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>RHS</td>
<td>120</td>
<td>96</td>
<td>Cardia-bifida 12 (40)</td>
</tr>
</tbody>
</table>

RHS, right hand side; LHS, left hand side.
Directional development in chick heart

A small isolated area of beating tissue was seen on the side where the operation had been performed. The side of the host which receives the transplant appears to have some effect on the success of the integration of the precardiac mesoderm. Operations where tissue was transferred from left side to left side showed the highest percentage of survivors, compared to the number of embryos operated (95%). The success rate in the other groups was approximately the same, about 78%. The percentage of embryos with normal hearts compared to those that had sur-

Table 2. Development of the heart in double-sided and control embryos. Grafts carried out between embryos of different stages

<table>
<thead>
<tr>
<th>Stage and side of donor tissue</th>
<th>Stage and receiving side of host</th>
<th>Post-operated host sidedness of mesoderm embryo</th>
<th>Number operated</th>
<th>Number survived (% survived/operated)</th>
<th>Heart shape (% shape/survived)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RHS LHS</td>
<td>RHS LHS</td>
<td>RHS (30) 0 (0) 5 (50) 1 (10) 1 (10)</td>
<td>Normal RHS 0 (0) 5 (50) 1 (10)</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td>11</td>
<td>12 (100) 7 (78) 1 (10) 0 (0) 1 (14)</td>
<td>Abnormal RHS 0 (0) 5 (50) 1 (10)</td>
</tr>
<tr>
<td>4/5 RHS</td>
<td>6 RHS</td>
<td>RHS LHS</td>
<td>12</td>
<td>7 (78) 1 (10) 0 (0) 1 (14) 1 (14)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>6 LHS</td>
<td>4/5 LHS</td>
<td>RHS LHS</td>
<td>11</td>
<td>10 (90) 7 (78) 1 (10) 0 (0) 1 (14)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>43</td>
<td>12 (100) 7 (78) 1 (10) 0 (0) 1 (14)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>Double sided</td>
<td></td>
<td></td>
<td>14</td>
<td>12 (86) 0 (0) 4 (33) 6 (50) 2 (17)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>4/5 RHS</td>
<td>6 LHS</td>
<td>RHS RHS</td>
<td>17</td>
<td>11 (65) 3 (27) 0 (0) 6 (55) 2 (18)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>6 RHS</td>
<td>4/5 LHS</td>
<td>RHS RHS</td>
<td>18</td>
<td>11 (61) 2 (18) 1 (9) 2 (18) 6 (55)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>6 LHS</td>
<td>4/5 RHS</td>
<td>RHS RHS</td>
<td>15</td>
<td>15 (83) 6 (40) 2 (13) 2 (13) 4 (27)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>67</td>
<td>49 (86) 11 (16) 7 (11) 1 (16) 14 (21)</td>
<td>No heart LHS 1 (14) 1 (14) 1 (14)</td>
</tr>
</tbody>
</table>

RHS, right hand side; LHS, left hand side.

Fig. 3. Summary of the number of right- and left-looped hearts in control and double-sided embryos.
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vived the operation was highest in transfers from one right side to another right side at 47%, whereas transfers from one left side to another left side were the least successful at 30% (Table 4).

The direction of body turning, which is usually to the right, and the association with heart shape were recorded (Table 5). However in the majority of embryos the direction of turning was not seen. This is because embryos were fixed before stage 12 to allow easier identification of the heart shape, or they may have been mechanically prevented from turning by the cardia-bifida heart formation. 32 embryos were analysed, with the majority (23) turning as normal to the right. The remaining 9, turned to the left. Of these left-turning embryos, one had a normal heart which looped to the right, six developed normally hearts that looped to the left, one had an abnormal heart tube formation, and one embryo showed cardia-bifida. There is a significant difference at the 1% level, in the direction in which the heart looped, between the embryos that turned to the left and those that turned to the right. All the embryos that turned to the right with a normal heart, showed right-hand looping of the heart tube. Whereas of the nine embryos that turned to the left, seven had normally developed hearts and 6 of these looped to the left.

### Discussion

Precardiac mesoderm was grafted between embryos, changing the sidedness of the tissue from which the heart would develop, to see if this influenced the direction of looping of the heart tube.

The most striking observation was that overall, double-right sided embryos developed many more left-looped hearts than double-left sided embryos, 12/28 normal hearts compared to 2/31. This was significant at the 1% level of probability using the $\chi^2$ test. The two examples seen of double-left sided heart embryos forming left-looped hearts, are when the stages of the host and donor tissue are different and the oldest tissue is on the right-hand side of the embryo.

In double-right sided embryos with donor and host tissue of the same age (Table 1) there is a difference in the number of left-looped hearts formed between operations carried out at stage 4-5, compared to 6. At stage 4-5, 13% of hearts had left-hand loops (1/8) whereas when the operation was performed at stage 6, closer to 50% (6/13) of normal hearts had left-hand loops. Therefore, there is a suggestion that an intrinsic change has occurred in the precardiac mesoderm between stages 5 and 6, that later influences the direction

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**Table 3. Control operations, removing one side of the precardiac mesoderm at stages 4, 5 and 6**

<table>
<thead>
<tr>
<th>Side from which mesoderm was removed</th>
<th>Number operated</th>
<th>Number survived (% survived/operated)</th>
<th>Heart shape (% shape/survival)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHS</td>
<td>36</td>
<td>32 (89)</td>
<td></td>
</tr>
<tr>
<td>LHS</td>
<td>37</td>
<td>32 (87)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.** Success rates for transfers between sides, irrespective of stage

<table>
<thead>
<tr>
<th>Sidedness of donor piece</th>
<th>Sidedness of receiving host</th>
<th>Total number operated</th>
<th>Total number survived</th>
<th>Number of normal hearts (right or left loop)</th>
<th>% survived/operated</th>
<th>% normal hearts/survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHS</td>
<td>RHS</td>
<td>45</td>
<td>36</td>
<td>17</td>
<td>80</td>
<td>47</td>
</tr>
<tr>
<td>RHS</td>
<td>LHS</td>
<td>91</td>
<td>68</td>
<td>28</td>
<td>75</td>
<td>41</td>
</tr>
<tr>
<td>LHS</td>
<td>RHS</td>
<td>96</td>
<td>77</td>
<td>31</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>LHS</td>
<td>LHS</td>
<td>39</td>
<td>37</td>
<td>11</td>
<td>95</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 5.** Relationship between the direction of body turn and heart shape in embryos of stage 12 and over

<table>
<thead>
<tr>
<th>Direction of body turning</th>
<th>Number of embryos</th>
<th>Right-hand loop</th>
<th>Left-hand loop</th>
<th>Cardia-bifida</th>
<th>Abnormal tube</th>
<th>No heart tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHS</td>
<td>23</td>
<td>15</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>LHS</td>
<td>9</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

RHS, right hand side; LHS, left hand side.
of looping of the heart tube. However, these results are not significant at the 5% level of probability.

Control operations show that when a left and a right area of precardiac mesoderm were present in the correct left/right orientation, 97% of the normal hearts looped to the right. An important observation is that, this is true even when the stages of the precardiac mesoderm in the donor and the host are different. Therefore in these operations handedness is not due to one side developing slightly faster than the other. The ability to develop the correct handedness has not been destroyed by the operation.

The above results correlate well with those of Salazar Del Rio (1974). 90% of control operations resulted in right looped hearts. In double-sided embryos at stages 4-5, those with two left cardiogenic areas formed only right-looped hearts, whereas embryos with two right cardiogenic areas developed a small number of left-looped hearts. He concluded that at these stages, intrinsic differences are not present in the precardiac mesoderm which influence the sidedness of the loop.

The stability in the formation of right-looped hearts by the fusion of two left sides can be explained by the process of conversion, in the three step model by Brown and Wolpert (1990). If the conversion process occurs relatively early in development and results in some stable change on the right-hand side, bisection of the embryo after this process allows the left side to undergo conversion again, and to respecify the right and left sides; development is therefore normal. But, if the right sides are already stably converted, there is now nothing to bias the generation of random asymmetry. Asymmetry develops randomly, with a 50:50 chance of a right-looped or a left-looped heart forming, as seen in the double-right sided embryos at stage 6 compared to stages 4 and 5. Another example of this can be seen in the twinning experiments of Spemann and Falkenburg (1919) using the newt *Triturus*. They bisected the gastrula longitudinally, and found that half of the twins were normally orientated (left twins) but half showed situs inversus viscerum (right twins). This led Wilhelmi (1921) to suggest that the control of right and left structures had been lost in the right side, and was now due to chance.

Another theory of the development of left/right handedness is put forward by Corballis and Morgan (1978) and Morgan and Corballis (1978). They propose an asymmetric maturational left/right gradient with the left side leading. This is coupled with reciprocal inhibition, ‘a mechanism for amplifying initially small developmental asymmetries into the qualitative asymmetries seen’ (Corballis and Morgan, 1978). Asymmetry is thought not to be directly coded for by genes, but that genetic effects are indirect, acting on a basic asymmetry in the cytoplasm. Situs inversus could be caused by abolishing the gradient so that the cell cannot read it or by the reading mechanism of the cells being affected so that they read the gradient in the ‘wrong’ way (Morgan, 1991). There does indeed seem to be some sort of left/right gradient, as shown for example by the differences in rate of heart beat. It is possible to account for our results in terms of a left or right gradient by assuming, for example, that the graded differences in double-right embryos are too small or become dissipated, with the result that looping is random. However this gradient theory is unlikely because the handedness of twinned or triplet quail embryos is normal even when the embryos have opposite antero-posterior polarity (unpublished results).

The change in the behaviour of the right-hand side may be related to visual and also biochemical differences that have been found in the period of development between the primitive streak and the first somite. In the rat embryo, adrenergic agonists were found to disrupt the development of asymmetry (Fujinaga and Baden, 1991). Using methoxamine, a window of sensitivity to the agonist was found, beginning just before headfold formation and ending with the formation of the first somite (McCarthy et al., 1990). However using other situs-disrupting agents such as colchicine and lithium chloride, the window of sensitivity was found to begin as early as the late primitive streak stage (Brown et al., 1991).

Small right-hand looped hearts developed in almost a third of cases when either area of cardiac mesoderm was removed, leaving the other area intact. In terms of the model, one might have expected that hearts developing from the right area of precardiac mesoderm only, would show randomness in the direction of looping. This was not seen. However, Salazar Del Rio (1974) found that the majority of hearts that developed from the right-hand area of precardiac mesoderm had left-hand loops.

The morphological expression of each cardiac primordia has been of interest to many authors and investigated in amphibians (Spemann and Falkenburg, 1919; Copenhaver, 1926), birds (Grafer, 1907; DeHaan, 1958; Lepori, 1967) and in mammals (Goss, 1935; Dwinnell, 1939). Another approach to this problem has been through observing the direction of tube looping in cardia-bifida hearts. Cardia-bifida hearts can be produced experimentally, but with some damage to the foregut in birds. Nadal-Ginard and Paz Garcia (1972) confirmed that in both amphibians and birds both hemi-hearts developed a convex loop towards the midline. The left primordium has therefore been considered as the side that develops the right-handed loop and the right side a left-handed loop.

During development the chick embryo undergoes a process of torsion. From lying with its dorsal side to the vitelline membrane the embryo turns to lie on its right-hand side. The turning process is common to both mammalian and avian embryos and of evolutionary interest because both groups turn the same way i.e. to their right. 9 out of the 32 embryos that turned, turned to the left. The method of New culturing (1955) is reported to increase the number of embryos that form left-hand loops as compared to those in ovo (Salazar Del Rio, 1974). The same increase was found between unoperated cultured embryos which looped and turned to the left-hand side in 8.5% of cases, compared to 0.2% in ovo (authors’ unpublished data). Waddington (1937) also found an unexplainable increase in the number of embryos that turned to the left, when culturing on plasma clots.

There is some debate as to whether the direction of heart looping controls the direction of body torsion (Weber, 1902; Waddington, 1937; Itasaki et al., 1991) or if the two processes are independent (Icardo and Ojeda, 1984). The results presented here show a concordance in the direction...
of turning and looping of the heart, except for one example of an embryo with a right-hand looped heart that turned to the left. This suggests that normally the direction of heart looping and body turning are linked.

In conclusion, double-right sided embryos formed many more left-looped hearts than double-left sided embryos. This is linked to the expression of sidedness of each single primordium and can be explained by the process of conversion in the Brown and Wolpert theory (1990). There is a suggestion that in the chick changes occur in the precardiac mesoderm between stages 5 and 6 that may define the difference between right and left sides in the embryo. This change causes an effect 10-13 hours later, on the direction of looping of the heart tube. The direction in which the heart loops shows concordance with the direction of body torsion, and the two processes are therefore likely to be interlinked.

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References


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