The effect of transection of the duck blastoderm on the orientation of the embryo

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Von Baer (1828) formulated a rule according to which the cephalo-caudal axis is perpendicular to the long axis of the shell, and the head of the embryo is directed forwards when the egg is placed with the blunt end to the left. This law has been checked in several species of bird: hen, duck, pigeon, goose, turkey (see Clavert, 1960, for references) and quail (Fargeix, 1963, 1964). Embryos obeying von Baer’s rule were found to constitute the most numerous class, but considerable interspecific as well as intraspecific differences were observed.

The studies by Lutz (1949) supply extensive information on the regulative capacities of the duck blastoderm, but do not provide any data concerning the determination of the presumptive axis of the blastoderm. The control material of this author revealed a large percentage of embryos obeying von Baer’s rule. In consequence Lutz defines transections perpendicular to the long axis of the shell as ‘parallel to the presumptive axis’, and transections parallel to the long axis as ‘perpendicular to the presumptive axis’. Vakaet (1962), however, has already drawn attention to the fact that transection of the duck blastoderm does to a certain extent affect the orientation of twin embryos. The experiments presented below further investigate this observation, the direction of transection affecting the orientation of the embryonic axis even in cases in which only one embryo developed.

RESULTS

Duck eggs of the Pekin strain were used for the experiments. The unincubated blastoderms were transected with a glass needle using the method described by Wolff & Lutz (1947) and Lutz (1949). After the operation the window was closed with a sheet of mica or a coverslip. The eggs were incubated for 48–98 h at 37-8 °C without turning. Control eggs, without windows, were incubated for the same period of time. Transection was performed on 329 blastoderms, 120 of which were cut in a direction perpendicular to the long axis of the shell, and 209 in a direction parallel to the long axis of the shell. In 97 cases the blastoderms

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failed to develop any further. In 37 cases it proved impossible to determine the position of the embryos: this group includes very degenerate or twisted embryos and cases in which only certain embryonic structures were observed (most often only the heart). After both types of transection single and twin embryos occurred with almost equal frequency. A total number of 195 experimental eggs and 198 control eggs were examined and the axis of the embryos defined in relation to that of the shells in terms of a hypothetical clock face. The results of the experiments are given in Table 1.

Table 1. *Results of transection of unincubated duck blastoderm*

<table>
<thead>
<tr>
<th>Type of transection</th>
<th>Perpendicular</th>
<th>Parallel</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of exps.</td>
<td>120</td>
<td>209</td>
<td>329</td>
</tr>
<tr>
<td>Single embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twin embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Types of alignment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>32</td>
<td>23</td>
<td>55</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>67</td>
<td>110</td>
</tr>
<tr>
<td>No embryo</td>
<td>33</td>
<td>64</td>
<td>97</td>
</tr>
<tr>
<td>Abnormal development</td>
<td>13</td>
<td>24</td>
<td>37</td>
</tr>
</tbody>
</table>

* I, Embryos aligned parallel or almost parallel to each other; II, embryos aligned head to head; III, embryos aligned head to tail; IV, embryos aligned at varying angles.

1. *Orientation of control embryos*

The use of von Baer's rule renders it necessary to distinguish blunt and narrow ends and to orient the shell accordingly. In the material examined eggs were not infrequently found in which both ends were equally rounded, in which case the blunt end was taken as that at which the air space was situated. Cases have however been described of the air space occurring at the narrow end (Romanoff & Romanoff, 1949). I therefore paid special attention to the shape of the egg and the position of the air space in a selected group of eighty-one eggs. In ten of these (12%) the two ends were equally rounded. Among eggs of an asymmetrical shape the occurrence of the air space at the narrow end was observed in two cases only (3%). There is, therefore, a high though not complete correlation between the shape of the shell and the position of the air space. It will therefore be assumed that the remaining eggs, oriented according to the position of the air space, are correctly oriented. This is also borne out by the similar distribution of orientations of embryos in shells oriented according to
Text-fig. 1. Alignment of embryos in control and transected blastoderms. Numbers above columns denote number of blastoderms. 1–7, Alignment of embryos along twelve axes of hypothetical clock face; 8–12, alignment of embryos along six axes. 1, 2, 3, 8, Control embryos (for explanation see text). 4, 9, Single embryos; 5, 10, twin embryos; after transection perpendicular to the long axis of the shell. 6, 11, Single embryos; 7, 12, twin embryos; after transection parallel to the long axis of the shell.
the position of the blunt end (Text-fig. 1 (1)) and according to the position of the air space (Text-fig. 1 (2)). Data from these two series of observations were therefore combined (Text-fig. 1 (3) and (8)) and in the further discussion reference will be made to these combined data. As can be seen from them, the material examined provides only slight confirmation of von Baer's law. Only 19% of the embryos had their heads directed towards 12 on a hypothetical clock face; those most similarly aligned, i.e. towards 11 and towards 1, amounted to 15% and 9% respectively. The distribution of the frequencies of the various alignments reveals that there is a slight peak at 12, a second (smaller) peak at 6, and a fairly regular decrease on both sides of the 12–6 axis. A slight increase of the frequencies at the 3–9 axis may also be significant. As the tendency for the embryo to be oriented in accordance with von Baer's rule is very weakly expressed, we will not refer below to transection parallel or perpendicular to the presumptive axis of the blastoderm, but only to transection parallel or perpendicular to the long axis of the shell.

2. Orientation of embryos after transection of the blastoderm

The twin embryos obtained as a result of successful operations were divided into four groups (Table 1). The axis of the blastoderm as well as its direction in relation to a hypothetical clock face was defined for each pair of embryos. Attention was also paid to whether the axis of the blastoderm coincided with the line of transection.

When the embryos lay parallel or nearly parallel to each other (group I; Plate 1, figs. A–E) the axis of the blastoderm was taken as a line parallel to both embryos or as the resultant of their individual axes. The direction of the heads of the embryos defines the direction of this axis. In this group the axis of the blastoderm is almost always identical with the line of transection.

EXPLANATION OF PLATES

Orientation of each figure conforms to the orientation of the blastoderm in the egg placed with its blunt end to the left. The blastoderms were stained in toto with Ehrlich's haematoxylin and rendered transparent by clearing in benzene. Figures A, B, F, G, H, I show alignments of embryos following transection parallel to the long axis of the shell. Figures C, D, E, J show alignments of embryos after transection perpendicular to the long axis of the shell. With the exception of the blastoderm shown in figure D, the blastoderm axis always coincides with the line of transection. The basis of classifying the blastoderms into one of the four groups (I–IV) is explained in the text.

**PLATE 1**

Fig. A. Six and nine somites, 53 h of incubation, group I. ×12
Fig. B. Seventeen and nineteen somites, 66 h of incubation, group I. ×12
Fig. C. Thirty-five and thirty-six somites, 98 h of incubation, group I. ×1.5
Fig. D. Twenty-six and twenty-seven somites, 93 h of incubation, group I. ×1.5
Fig. E. Four and nine somites, 56 h of incubation, group I. ×12
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Blastoderm orientation

If the embryos were aligned head to head (group II; Plate 2, figs. F, G) or head to tail (group III; Plate 2, fig. H) it was taken that the axis of the blastoderm was common to both embryos and that the direction of this axis was determined by the direction of the more advanced of the two embryos, or of the posterior one. If the embryos were arranged head to head and were of the same size, the direction of the blastoderm axis could not be defined further than with respect to the six axes traversing the clock face. Consequently, the six blastoderms falling into this category could be included only in the right-hand side of the figure (Text-fig. 1 (12)).

In group IV the embryos lie almost at right angles to each other (Plate 2, figs. I, J), and most often differ considerably in their degree of development. The direction of the more advanced embryo was taken as that defining the direction of the blastoderm axis. This is a mixed group in which the blastoderm axis may be parallel to the line of transection or form varying angles with it.

The alignment of twin embryos in relation to the axes of the shell proved to be completely different from that of the control embryos. Transection perpendicular to the long axis of the shell most frequently (65%) results in embryos with their axis directed towards 12 (Text-fig. 1 (5)). Almost all the remaining embryos are directed towards 6. It must be emphasized that in this case the axis 12–6 coincides with the line of transection, and Text-fig 1 (10) shows the very marked predominance of alignments along this axis (86%). At the same time, the percentages for the two adjacent axes (11–5 and 1–4) are markedly lower than in the control material. It seems that the variability in orientation is reduced as a result of the operation.

Very similar results were obtained from examination of the alignment of single embryos formed as a result of unsuccessful transection of the blastoderm, the only difference being that an equal number of embryos were directed towards 12 and 6 (Text-fig. 1 (4)). No difficulty is encountered in defining the axes of these blastoderms and the agreement of the results justifies the conclusion that the axes of blastoderms forming two embryos were defined correctly. Transection of the blastoderm therefore has a definite effect on the orientation of the embryo, and the operation itself determines the position of single embryos in the same way as that of twin embryos.

After transection parallel to the long axis of the shell the effect of the operation

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Plate 2

Fig. F. Sixteen and sixteen somites, 66 h of incubation, group II. × 12
Fig. G. Eleven and twenty-one somites, 73 h of incubation, group II. × 12
Fig. G. Five and five somites, 70 h of incubation, group III. × 12
Fig. I. Three and four somites, 56 h of incubation, group IV. × 12
Fig. J. Thirty and thirty-five somites, 96 h of incubation, group IV. (In this case it was taken that the blastoderm axis is defined by the right-hand embryo, the alignment of which had probably been towards 12 in the earlier stages.) × 1.5
is also evident, although less distinct. Embryos lying on axis 3–9, i.e. along the
line of transection, amount to 40% of the cases, whereas in control blastoderms
the percentage of alignments along this axis is only 12% (Text-fig. 1 (8) and
1 (12)). Again, the percentages for the two adjacent axes (2–8 and 10–4) are
distinctly lower. What is more striking, however, is that the percentage for the
axis perpendicular to the line of transection, i.e. the 12–6 axis, is not lower
than in the control material, as might have been expected, but actually some-
what higher. This is again accompanied by a lowering of the percentages for
the two adjacent axes. This surprising finding seems difficult to explain at present.

The fact that similar results were obtained with blastoderms that despite the
transection formed single embryos again emphasizes the important role of the
operation itself (Text-fig. 1 (11)). In blastoderms which gave rise to two embryos
the embryos are directed more often towards 9 than towards 3 (Text-fig. 1 (7));
this is not clearly the case with single embryos (Text-fig. 1 (6)). This difference
between single and twin embryos is similar to that observed after perpendicular
transection, in that the twin embryos show a more pronounced predominance
of orientation towards one of the ends of the line of transection (9 and 12
respectively) than do the single embryos. The explanation for this fact is obscure;
it must also be borne in mind that owing to the limited number of cases these
differences may not be significant.

Table 2. Relationship between axis of blastoderm and line of transection

<table>
<thead>
<tr>
<th></th>
<th>Axis 12-6 (a)</th>
<th>Axis 3-9 (b)</th>
<th>a+b</th>
<th>a/b</th>
</tr>
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<tbody>
<tr>
<td>Controls</td>
<td>33%</td>
<td>12%</td>
<td>45%</td>
<td>2.7</td>
</tr>
<tr>
<td>Perpendicular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transection</td>
<td>1 embryo</td>
<td>71%</td>
<td>81%</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>2 embryos</td>
<td>86%</td>
<td>93%</td>
<td>12.2</td>
</tr>
<tr>
<td>Parallel transection</td>
<td>1 embryo</td>
<td>44%</td>
<td>84%</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2 embryos</td>
<td>45%</td>
<td>85%</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The percentages of alignments along axis 12–6 and axis 3–9 are given in
Table 2. These are the axes that coincide with the lines of transection of the
blastoderm. Axis 12–6 is the strongest in all the control and operated blasto-
derms. Transection along its course strengthens it considerably. The more
effective the operation, the greater the degree of strengthening: 71% of single
and 86% of twin embryos were found to show this alignment. Axis 3–9 is also
very slightly stronger in control blastoderms. (A certain increase in the number
of embryos aligned along axis 3–9 can also be seen in diagrams illustrating the
orientation of normal chick, duck and quail embryos (Clavert & Vintemberger,
1957; Fargeix, 1964). Transection along this axis strengthens it to the extent of
making it almost equal to the 12–6 axis, which, however, is strong enough not
to be completely suppressed. When the percentages of alignments along the
two axes are summed, it is found that transection of the blastoderm considerably reduces the number of embryos oriented along axes other than these two. The alignment of embryos, whether single or twin, is therefore significantly affected by the transection.

DISCUSSION

The orientation of duck embryos has been examined by Féré (1896), Lutz (1949), Vintemberger & Clavert (1954), Miček (1955), Clavert & Vintemberger (1957) and Vakaet (1962), but the results obtained differ greatly. By accepting not only embryos aligned towards 12, but also those directed towards 11 and 1 as confirming von Baer's law, I obtained a figure of 43% in control material; Lutz found a figure of 65%, and Vakaet of 84%.

The symmetry of the blastoderm, which we define on the basis of the later position of the embryo, is determined much earlier. As shown by Clavert (1960), the embryo's orientation is determined before the egg is laid, the decisive factors being the rotations of the egg in the uterus and its position in relation to the long axis of the uterus. These rotations, particularly in the hen, are such that embryos arranged towards 12 occur most frequently afterwards. The appearance of the area pellucida denotes, according to Clavert, the moment of final establishment of bilateral symmetry. It appears that a correlation exists between the degree of differentiation of the blastoderm at the moment of egg-laying and the percentage of embryos aligned in accordance with von Baer's rule. If the area pellucida forms early, as in the hen, then a high percentage of alignments in accordance with von Baer's rule is observed. Quail eggs are not uniformly developed at the time of egg-laying, and the quail provides slightly fewer cases confirming von Baer's law. The smallest number of embryos exhibiting this alignment is usually found in the duck, where the area pellucida does not form until after several hours of incubation, which is later than in the other species.

Vakaet (1962) observed that deviation of one of a pair of twin embryos from von Baer's rule often occurs if a 'pull' of the needle was applied during operation. My first series of 250 experiments includes both conventional transections and transections with a 'pull', to which no attention was paid at first. The final series of eighty-one transections, however, was made without a 'pull', and the alignment of embryos did not differ from that of the previous series.

It may be mentioned that when in control material a large percentage of embryos is observed to be aligned in accordance with von Baer's rule, the operation itself has almost no effect on the direction of the blastoderm axis. This seems true for the results of Lutz (1949) and partially for those of Vakaet (1962), but even from the tables of Vakaet's paper it can be inferred that transection parallel to the long axis of the shell resulted in an increase of the number of embryos aligned along the line of transection.

The effect of transection on the orientation of twin embryos was probably so distinct in my experiments because I worked with material in which any align-
ment of the embryos was almost equally probable. The fact that the orientation of single embryos resulting from closure of the fissure conforms to that of twin embryos strongly emphasizes the importance of the operation itself. These observations justify the statement that the embryonic axis in the unincubated duck blastoderm is not yet irrevocably determined, and that its course can be experimentally altered.

**SUMMARY**

1. The proportion of embryos aligned exactly in accordance with von Baer's rule (with the head towards 12 on a hypothetical clock face) was 19% in material consisting of 198 Pekin duck eggs. A second but smaller peak was observed for alignment towards 6. As departure from the 12–6 axis increases, the frequency of alignments of embryos gradually decreases, exhibiting only a slight increase at the 3–9 axis.

2. The number of embryos oriented exactly in accordance with von Baer's rule is considerably increased by transection perpendicular to the long axis of the shell, irrespective of whether the operation results in twin embryos or in one embryo only, as a result of closure of the fissure; the axes of these blastoderms often coincide with the line of transection.

3. After transection parallel to the long axis of the shell the number of embryos aligned along axis 3–9 is considerably higher than in control material, and almost equals the number of embryos oriented along axis 12–6. Similar results were obtained both for blastoderms yielding twin embryos and for those from which only one embryo developed.

4. The embryonic axis in the unincubated duck blastoderm is not irrevocably determined. Transection of the blastoderm affects the orientation of the embryos, which tend to lie parallel to the line of transection.

**RÉSUMÉ**

*L'effet de transection du blastoderme de canard sur l'orientation de l'embryon*

1. La distribution de l'orientation des embryons a été étudiée chez 198 œufs de canard de Pékin. 19% sont orientés exactement selon la règle de von Baer (la tête vers 12 h sur l'horloge hypothétique). Un deuxième pic moins important a été observé pour l'orientation vers 6 h. Au fur et à mesure de l'augmentation de l'écart de l'axe 12–6, la fréquence d'orientation des embryons décroît, ne montrant que peu d'augmentation à l'axe 3–9.

2. Le nombre d'embryons qui s'orientent exactement selon la règle de von Baer montre une augmentation considérable lorsque la transection se fait perpendiculaire à l'axe longue de la coquille, aussi bien s'il en résulte des embryons jumeaux ou un seul embryon, suite à la fermeture de la fente; les axes de ces blastoderms coïncident souvent avec la ligne de transection.

3. Après une transection parallèle à l'axe longue de la coquille, le nombre d'embryons orientés sur l'axe 3–9 est beaucoup plus important que chez le
matériel témoin, il est presqu’égal au nombre d’embryons orientés sur l’axe 12–6. Les résultats sont analogues, que les blastoderms fournissent des embryons jumeaux ou un seul embryon.

4. L’axe embryonnaire du blastoderme non-incubé de canard n’est pas déterminé définitivement. La transsection du blastoderme influence l’orientation des embryons, qui ont tendance à se mettre parallèle à la ligne de transection.

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