Abnormalities in Liver Morphogenesis attributed to the
Bh (black at hatch) Lethal Gene in the Japanese Quail

TAMAO ONO and NOBoru WAKASUGI

Laboratory of Animal Genetics, School of Agriculture,
Nagoya University, Nagoya, 464

Introduction

The first lethal mutant discovered in the Japanese quail was micromelia\(^1\). Subsequently, four embryonic lethals were found, \textit{i.e.}, crooked neck dwarf\(^2\), yellow-feather\(^3\), chondrodystrophy\(^4\) and black at hatch\(^5\).

MINEZAWA and WAKASUGI\(^5\) reported that the \textit{Bh} (black at hatch) character was controlled by an autosomal dominant gene with homozygous lethality and that the homozygous embryos died at 4–6 days of incubation, showing whole body subcutaneous haemorrhage and degeneration of the liver tissue. Through the routine examination of the dead embryos from the BH (black at hatch) strain, it was suspected that some homozygous embryos might survive beyond 6 days of incubation, for which influence of genetic background was suggested. In addition, it was supposed that the \textit{Bh} gene might have some effects on the development of the liver also in the heterozygous condition as in the case of \textit{Cp} (Creeper) gene in the chicken\(^6\), \textit{i.e.}, it gives rise to micromelia in the homozygotes and short legs in the heterozygotes.

The present study is concerned with (1) the re-examination of the developmental stages at which the \textit{Bh} homozygous embryos die under two different mating systems, (2) effects of the \textit{Bh} gene on the development of the liver in the heterozygous condition and (3) culture of the embryonic liver fragments of the quail on the chorioallantoic membrane of the chick.

Materials and Methods

(1) Matings

Three stocks of the Japanese quail are maintained at our laboratory. Each stock comprises three sister strains and each strain is maintained by the rotational crosses with 3–4 pairs per generation. Occasionally inter-strain crosses within a stock is performed to prevent inbreeding depression. Stock 1 consists of SW (sex-linked white), REB (red-eyed brown) and B (brown) strains, stock 2 of WP (wild type plumage), DB (dominant black) and S (silver) strains and stock 3 of Y (yellow), BH (black at hatch) and W (white) strains. Dominant black character is thought to be the same as the incomplete dominant brown character in the report of TRUAX and JOHNSON\(^7\). The plumage character of other strains and general care of the quail have been described by WAKASUGI and KONDO\(^8\) and immunological characteristics of these strains was studied by KATOH and WAKASUGI\(^9\).

Embryos used in this study were taken from the two types of matings, \textit{Bh/\(+ \times Bh/\)} (experimental matings) and \textit{Bh/\(+ \times +/\)} (control matings: includes reciprocal matings).

Received January 13, 1983
These matings were arranged either in the BH strain or with F₁ hybrids between BH strain and the individuals with wild type plumage from stock 2.

(2) Observations of Embryos

Eggs within 10 days of storage were used for experiment. They were incubated at 37.5°C with 60-70% relative humidity in the standard fashion. Embryos were taken out at desired period of incubation, weighed and classified according to the developmental stages described by ZACCHEI. Embryos showing heart-beat were regarded as living. Some embryos were transferred for histological observation. They were fixed in Bouin's fluid, embedded in paraffin through the routine procedure, sectioned at 5-10 μm, stained with Mayer's hemalum and eosin, and observed under microscope.

(3) Graftings of Embryonic Liver to the Chorioallantoic Membrane (CAM)

Fertile White Leghorn eggs obtained from the Laboratory of Animal Nutrition, the School of Agriculture, Nagoya University were used as host embryos. The fragments of the liver taken from the quail embryos at 6 days of incubation were grafted to the bifurcation of the blood vessel on the CAM of chick embryos at 9-11 days of incubation according to the technique described by COULOMBRE. After 3 or 5 days' culture, the grafts were fixed in Bouin's fluid for histological observation.

Results

(1) Observations of Embryos

Table 1 shows the difference in embryo mortality between the experimental and control matings at each period of incubation in the intra BH strain matings. At 4-6 days of incubation, there were little differences in embryo mortality between the two types of matings. The difference was 10-15% at 7 and 8 days of incubation and about 30% at 9 and 10 days. The results obtained with F₁×F₁ matings are shown in Table 2. The difference in embryo mortality between the two types of matings was 12.0% at 6 days of incubation and exceeded 25% at 10 days. From these results the lethal period of the Bh homozygous embryos is roughly estimated to be 6-9 days of incubation, although there is a discrepancy between the two experiments, i.e., the difference in embryo mortality at 6 and 7 days of incubation was considerably large in the F₁×F₁ matings.

Figures 1 and 2 show frequency distribution of dead embryos expressed as percentage according to the developmental stages described by ZACCHEI. Incidence of the embryos judged as dead before Stage 16, corresponding to 3 days of development (early death), was almost the same at all periods investigated. In the intra BH strain matings, the differences in embryo mortality excluding early death between the two types of matings increased from 4.9% to 27.2% in the period of 6-9 days of incubation (Fig. 1). These differences and distribution pattern of the dead embryos indicate that the homozygous embryos for Bh gene die at 6-9 days of incubation (Stages 18-26). The results from F₁×F₁ matings (Fig. 2) indicate that the homozygous embryos die at 5-9 days of incubation (Stages 18-26). These findings show that the homozygous embryos from the latter matings begin to die slightly earlier than those from the former as judged with the period of incubation. However, no difference was seen when judged with the standardized developmental stages. This discrepancy may be explained by the observation that the embryos from the latter matings showed slightly
Table 1. Mortality of embryos from the experimental ($Bh/+ \times Bh/+\)$ and control ($Bh/+ \times +/+\)$ matings arranged in the BH strain

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Types of matings</th>
<th>No. of embryos observed</th>
<th>Embryonic mortality (%)</th>
<th>Difference in mortality between two types of matings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Exp.</td>
<td>67</td>
<td>3.0</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>43</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Exp.</td>
<td>103</td>
<td>2.9</td>
<td>-1.6</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>67</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Exp.</td>
<td>173</td>
<td>8.7</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>135</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Exp.</td>
<td>190</td>
<td>22.6</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>89</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Exp.</td>
<td>145</td>
<td>22.1</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>63</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Exp.</td>
<td>81</td>
<td>34.6</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>42</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Exp.</td>
<td>135</td>
<td>37.8</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>195</td>
<td>6.2</td>
<td></td>
</tr>
</tbody>
</table>

Control matings include the reciprocal matings between $Bh$ heterozygous and wild type quail.

Table 2. Mortality of embryos from the experimental ($Bh/+ \times +/+\)$ and control ($Bh/+ \times +/+\)$ matings arranged with the $F_1$ hybrids

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Types of matings</th>
<th>No. of embryos observed</th>
<th>Embryonic mortality (%)</th>
<th>Difference in mortality between two types of matings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Exp.</td>
<td>52</td>
<td>5.8</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>42</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Exp.</td>
<td>35</td>
<td>8.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>57</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Exp.</td>
<td>41</td>
<td>17.1</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>39</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Exp.</td>
<td>70</td>
<td>28.6</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>46</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Exp.</td>
<td>94</td>
<td>22.3</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>80</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Exp.</td>
<td>120</td>
<td>26.7</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>62</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Exp.</td>
<td>389</td>
<td>33.7</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>327</td>
<td>7.3</td>
<td></td>
</tr>
</tbody>
</table>

Explanation of the control mating is given in Table 1 and that of the $F_1$ hybrids in Materials and Methods.
faster development than those from the former.

Almost all dead embryos from the experimental matings at 5-9 days of incubation showed heavy whole body subcutaneous haemorrhage (Fig. 3). A few live embryos from the experimental matings showed slight haemorrhage in the eyes and other part of the body as shown in Fig. 4, which were regarded as the living homozygous embryos, since such embryos were not observed in the control matings at any developmental stages.

(2) Homozygous Embryos for Bh Gene

Some of the dead embryos that showed whole body subcutaneous haemorrhage were histologically observed. Subcutaneous haemorrhage at cranial, cervical and abdominal regions was histologically confirmed as a common feature of the Bh homozygous embryos. In the liver, hepatic cords were disintegrated in all regions. Dispersion of cytoplasm and pyknosis or karyolysis were observed in the degenerated cells. Disintegration of tissue and infiltration of red blood cells were also observed in the mesonephros and gonad.

Histological observation of the moribund homozygous embryos disclosed partial degeneration of the liver (Fig. 5). The degenerated region was observed in both left and right lobes.
Weak eosinophilia of cytoplasm, pyknosis and karyolysis were the characteristics of degenerated cells. In the mesonephros and gonad, no sign of abnormalities were observed except for infiltration of a few red blood cells into the tissue space. Therefore, degeneration of mesonephros and gonad in the dead homozygous embryos is thought to be postmortem changes.

(3) Heterozygotes for Bh gene

On histological examination of the liver from normal embryos at 4-9 days of incubation, the degenerative changes was observed at the posterior tip of the left lobe (focal degeneration). Table 3 shows incidence of this abnormality in live embryos from the experimental and control matings. At 7 and 8 days of incubation, the abnormality was found in three embryos from the experimental matings. Determination of their genotypes was impossible although they did not show subcutaneous haemorrhage. However, the embryos with this abnormality at 9 days of incubation were not regarded as the homozygotes because of the absence of subcutaneous haemorrhage and small degenerated area in the liver.

After 10 days of incubation, it was possible to distinguish between the Bh and wild type embryos by the pigmentation pattern of down feathers. Segregation ratio of the Bh and
Table 3. Incidence of focal degeneration of the liver tissue in normal embryos from the experimental \((Bh/+ \times Bh/+}\) and control \((Bh/+ \times +/+}\) matings at 4-9 days of incubation

<table>
<thead>
<tr>
<th>Types of matings</th>
<th>Incubation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Exp.</td>
<td>0/19*</td>
</tr>
<tr>
<td>Cont.</td>
<td>0/20</td>
</tr>
</tbody>
</table>

Explanation of focal degeneration of the liver tissue is given in the text.
* No. of embryos showing focal degeneration at the posterior tip of the left lobe per no. of embryos examined. Embryos from both intra BH strain and F1×F1 matings were used for observation.

Table 4. Incidence of focal degeneration of the liver tissue in live embryos at 10, 12 and 15 days of incubation

<table>
<thead>
<tr>
<th>Genotypes*</th>
<th>Degeneration at</th>
<th>Incubation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>[Bh/+]</td>
<td>L. 144/386(37.3%)**</td>
<td>61/186(32.8%)</td>
</tr>
<tr>
<td></td>
<td>R. 1/386 (0.3%)</td>
<td>0/186 (0.0%)</td>
</tr>
<tr>
<td>[+/+]</td>
<td>L. 25/311 (8.0%)</td>
<td>9/137 (6.6%)</td>
</tr>
<tr>
<td></td>
<td>R. 1/311 (0.3%)</td>
<td>0/137 (0.0%)</td>
</tr>
</tbody>
</table>

* Embryos from both intra BH strain and F1×F1 matings were used for observation.
** Incidence of embryos showing focal degeneration.

wild type embryos at 10 days of incubation was approximately 2:1 and 1:1 in the experimental and control matings, respectively. Accordingly, all the Bh type embryos were regarded as the heterozygotes after this stage. On examination of the liver from the embryos after 10 days of incubation, it was found that the liver of some embryos had locally degenerated region at the posterior tip of the left lobe as shown in Figs. 6 and 7 (focal degeneration). Its incidence in the heterozygous and wild type embryos at 10, 12 and 15 days of incubation is shown in Table 4. The abnormality was observed in about one-third of the heterozygous and in about one-thirteenth of the wild type embryos at 10 and 12 days of incubation. At 15 days of incubation, the incidence decreased to about half in the heterozygotes, whereas, no significant decrease was seen in the wild type embryos. Three embryos showed the abnormality in the right lobe. One heterozygote and a wild type embryo at 10 days showed the abnormality at both left and right lobes. One heterozygote at 15 days showed the abnormality only at the right lobe. The region at the focal degeneration was pale yellow and the cells showed weak eosinophilia of cytoplasm and pyknosis or karyolysis on histological observation (Fig. 8). This cytological feature was the same as that observed in the degenerated liver tissue of the homozygous embryos. The abnormal region was limited at the small area and smaller in 15 days’ embryos than in the 10-12 days’. This region is thought to be replaced by the neighboring normal tissue with the progress of development. It does not appear that the focal degeneration of the liver affect the viability or development of embryos, since no signifi-
Explanation of plates

Fig. 3. Dead embryo with whole body subcutaneous haemorrhage from the experimental mating at 7 days of incubation. (×2.5)

Fig. 4. Abnormal embryo from the experimental mating at 7 days of incubation. Note subcutaneous haemorrhage around the eye. (×2.5)

Fig. 5. Histological structure of the liver of the live embryo showing subcutaneous haemorrhage and haemorrhage in the eyes from the experimental mating at 8 days of incubation. Note focal degeneration both in the left and right lobes. (×20)
cant difference in body weight was seen between Bh heterozygous and wild type embryos at 10-15 days of incubation. Breeding data of the BH strain also showed no significant differences in hatchability and viability between the Bh heterozygotes and wild type.

4) Graftings of Embryonic Liver to the Chorioallantoic Membrane (CAM)

In order to investigate the developmental abilities of the liver of the homozygous embryos, the fragments of the organ taken from the embryos at 6 days of incubation were grafted to the CAM for culture. In this experiment the embryos from the intra BH strain matings were used, because proportion of the Bh homozygous embryos that begin to die before 6 days of incubation was smaller in the intra BH strain matings than in F₁ × F₁ matings (Figs. 1 and 2). After 3 or 5 days, histological structure of the cultured liver was observed and the following four kinds of the grafts were found. (1) Well developed: showing well developed hepatic cords in all regions of the graft (Fig. 9). (2) Partially developed: having well developed hepatic cords at the peripheral region but showing degeneration at the center (Fig. 10). (3) Degenerate: showing no histologically integrated structure. (4) Dislocated: the grafts which moved away from the bifurcation of the blood vessel on the CAM and dried up.

The summary of the culture experiment of the liver is shown in Table 5. No significant differences were unfortunately seen in the development of the grafts taken from the experi-

<table>
<thead>
<tr>
<th>Culture period</th>
<th>Types of matings</th>
<th>No. of embryos set</th>
<th>No. of hosts died</th>
<th>No. of grafts</th>
<th>well developed</th>
<th>partially developed</th>
<th>degenerate</th>
<th>dislocated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>Exp.</td>
<td>33</td>
<td>3(9.1%)</td>
<td>0 (0.0%)</td>
<td>21 (63.6%)</td>
<td>6 (18.2%)</td>
<td>3 (9.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>34</td>
<td>0 (0.0%)</td>
<td>1 (2.9%)</td>
<td>18 (52.9%)</td>
<td>6 (17.6%)</td>
<td>9 (26.5%)</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>Exp.</td>
<td>43</td>
<td>2 (4.7%)</td>
<td>8 (18.6%)</td>
<td>9 (20.9%)</td>
<td>9 (20.9%)</td>
<td>15 (34.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cont.</td>
<td>61</td>
<td>1 (1.6%)</td>
<td>14 (23.0%)</td>
<td>17 (27.9%)</td>
<td>13 (21.3%)</td>
<td>16 (26.2%)</td>
<td></td>
</tr>
</tbody>
</table>

* Liver tissue was taken from the quail embryo at 6 days of incubation from the experimental (Bh/+ × Bh/+) and control (Bh/+ × +/+ ) matings arranged in the BH strain. Classification of the grafts is explained in the text.
mental and control matings, although the total incidence of the well developed and partially
developed grafts in the 5 days' culture was about 10% lower than in the experimental group.
Incidence of the partially developed grafts was higher in the 3 days' culture, whereas, that
of the well developed grafts was higher in the 5 days' culture. This difference may be due
partly to the technical problems and partly to the size of the grafts, because a series of the
3 days' culture was performed prior to the 5 days' culture and the size of the grafts was
larger in the former.

Discussion

The present study clarified that the homozygous embryos for Bh gene died at 6-9 days of
incubation in the intra BH strain matings and at 5-9 days of incubation in the F1 × F1 matings.
Slight difference in the lethal period between these two experiments is thought to be due to
the difference in the progress of embryonic development. This view may be supported by
the fact that the Bh homozygotes died between Stages 18 and 26 in both experiments when
judged by the standardized developmental stages of ZACCHETI10). However, Minezawa and
Wakasugi5) previously reported that the Bh homozygotes died at 4-6 days of incubation (Stages
18-22 of ZACCHETI's standard). It is inferred that the discrepancy between the two reports
is due to the difference in genetic background. Changes in the expressivity of lethality due
to the genetic background was shown for the Cp (Creeper) gene of the chicken. When the
Cp gene from the Jitokko breed was introduced into WL–G (White Leghorn) and NG–A
(Nagoya) strains, its expressivity, i.e., the lethal stage became different4). The selection
toward the uniformity of blood group genes was applied to the BH and other strains3).
This may be the major factor causing the change in genetic background. Therefore, it is
pertinent to state collectively that lethal period of the Bh homozygous embryos is 4–9 days of
incubation.

Characteristics of the Bh homozygous embryos were subcutaneous haemorrhage and
degeneration of the liver tissue. There are two possibilities concerning the lethality of the
Bh homozygous embryos. The first is that the primary defect attributed to the Bh gene
might be the abnormality in liver morphogenesis. In this case, subcutaneous haemorrhage
is thought to be secondary effects derived from the weakening of the capillaries caused by
dysfunction of the liver. Higher incidence of the focal degeneration at the posterior tip of
the left lobe in the Bh heterozygous embryos than in the wild type at 10 and 12 days of
incubation may indicate that the Bh gene has some effect on the liver morphogenesis also in
the heterozygous condition. In the chick embryos, the first indication of the liver is observed
as a small endodermal evagination at the margin of the anterior intestinal portal12,13). Sub-
sequently, the liver develops from the endoderm of the anterior intestinal portal and the
adjacent mesenchyme and their interaction is essential for development of the liver14).
Fukuda15) reported that the early development of the three major tissue components related
to the differentiation of the liver, i.e., the hepatic endoderm, the hepatic mesenchyme and
the embryonic endothelium was basically similar between the quail and chick embryos and
that the hepatic mesenchyme developed at the 20–22 somite stage in both quail and chick
embryos. In the chick embryos, the formation of hepatic cords is already seen at 3 days of
incubation18,19). Although, degeneration of the liver tissue in the Bh homozygotes occurs
after formation of the hepatic cords, it might be caused by some developmental defects residing in the above mentioned tissue components or failure in their mutual interaction.

Second possibility is that the primary defect caused by the Bh gene might be the disintegration or dysfunction of the capillaries, which is thought to be the cause of haemorrhage in the eyes, whole body subcutaneous haemorrhage and degeneration of the liver. With regard to the last characteristics, there might be a possibility that the cell degeneration is caused by the deficiency in the oxygen supply, since the clear separation between degenerated region and adjacent normal hepatic cords was a characteristic feature at the initial stage of development. Robbins has stated that when the cell is deprived of oxygen, the glycolytic mechanism continues to produce lactic acids, accumulation of which causes the intracellular pH to drop and then lowered pH activates lysosomal enzymes and the proteolytic digestion of the cell follows. This view may be supported by the study on the graftings of embryonic liver to the chorioallantoic membrane of the chick embryo. The large liver fragments showed healthy development at the peripheral region but tended to show degeneration at the center. In the case of the small fragments, however, the incidence of the grafts that showed well developed structures in the central region was considerably higher. These results may indicate that vascularization at the central region is difficult in the large fragments, and degeneration of tissue follows because of insufficiency of oxygen.

The focal degeneration of the liver in the Bh heterozygous and wild type embryos is interpreted as follows. Deficiency in blood supply or oxygen deficiency may easily occur at the posterior tip of the left lobe of the liver around 10-12 days of incubation and the liver tissue itself or the capillaries at this region may be more susceptible in the Bh heterozygous embryos than in the wild type.

Summary

Homozygous Japanese quail embryos for Bh (black at hatch) gene died at 6-9 days of incubation in the intra BH (black at hatch) strain matings and at 5-9 days of incubation in the matings arranged with F1 hybrids between BH strain and alien colony. In both experiment, however, the lethal period was Stages 18-26 of Zacchei's standard. The lethal period of the Bh homozygous embryos is thought to be 4-9 days of incubation incorporating the previously reported results by Minezawa and Wakasugi. Characteristics of the Bh homozygous embryos were degeneration of the liver tissue and whole body subcutaneous haemorrhage. Two possibilities were presented concerning the lethality of the homozygous embryos. The first was that degeneration of the liver tissue might be the major factor. The second was that disintegration or dysfunction of the capillaries might be the major factor. These possibilities were discussed in relation to the normal process of the liver morphogenesis and the results obtained through the culture of embryonic liver grafts on the chorioallantoic membrane of the chick.

In the liver of some embryos after 9 days of incubation, degenerated region was found at the posterior tip of the left lobe (local degeneration). This abnormality was observed in about one-third of the Bh heterozygous and in about one-thirteenth of the wild type embryos at 10-12 days of incubation. At 15 days of incubation, the incidence decreased in heterozygotes. The abnormal tissue at this region seems to be replaced by the neighboring
normal tissue with progress of development.

Acknowledgements

The authors wish to thank Professor K. Kondo for his encouragement and criticism during the course of the study. They are also grateful to Mrs. T. Hayakawa for taking care of animals. This study was supported by Grant No. 81-04-06 from National Center for Nervous, Mental and Muscular Disorders (NCNMMD) of the Ministry of Health and Welfare, Japan.

References

ニホンウズラの Bh（black at hatch）致死遺伝子による肝形成異常

小野珠乙・若杉昇

名古屋大学農学部 名古屋市 464

Bh（black at hatch：黒色初毛）形質は羽撃に関しては優性、致死性に関しては劣性の二面効果をもつ常染色体性の遺伝子により支配されていること、ならびにホモ型致死胚は孵卵4～9日ににおいて全身出血および肝臓組織の変性を示して死亡することが認められ、報告されている（遺伝誌，52，183，1977）。しかし著者らの調査によるとホモ型胚のなかには孵卵6日以上生存する個体が観察され、遺伝的背景の関与が示唆された。また、ニワトトリの Cp（Creeper）遺伝子のホモ型個体はmicro-melia，ヘテロ型は短脚であることより，Bh遺伝子のヘテロ型胚も肝臓形成過程において何らかの異常を示す可能性が推定された。本研究においては（1）二種類の異なる交配におけるBhホモ型胚（Bh/Bh）の死亡時期の推定，（2）ヘテロ型胚（Bh/+）の肝臓の器官形成過程における異常の有無の調査および（3）ウズラ胚の肝臓のニワトトリ胚肌膜上における器官培養を行なった。

Bhホモ型胚はBh系統内交配では孵卵6～9日，F1同志の交配では孵卵5～9日に死亡した。しかし肝臓の発生ステージは両交配群ともZacchei（Archo，Ital．Anat．，66，36，1961）のステージ18～26（孵卵4～9日の胚発生に相当する）であった。したがって従来と若杉（1977）の結果を考慮し，総合的に考えればBhホモ型胚の死亡時期は孵卵4～9日にみつかるのが妥当であろう。Bhホモ型胚は全身皮下出血あるいは眼に出血を示し，肝臓には左右両葉ともに変異部位が認められた。Bhホモ型胚の致死性に関しては肝臓組織の変性が主因であるとする仮説と毛細血管の変性が主因であるとする仮説の二つが考えられる。前報によると皮下出血は肝臓機能障害による毛細血管凝集のためにひきおこされた二次的性状であると考えられる。後報によると毛細血管形成異常による皮下出血および肝臓組織変性がひきおこされること考えられる。

孵卵9日以後，肝臓左葉先端部に変性（巢状変性）を示す胚が認められた。孵卵10日においてBhヘテロ型と野生型とは軽毛色パターンによる識別が可能であり，実験交配群では両者の比は2：1，対照交配群では約1：1であった。肝臓の局部変性は孵卵10～12日においてBhヘテロ型胚の約1/3，野生型胚の約1/13に認められた。孵卵15日のヘテロ型胚においてはその出現率は減少した。また孵卵10日のBhヘテロ型および野生型胚においてそれぞれ1例，肝臓左右両葉の先端部に変性の認められた胚が出現した（386例および311例中それぞれ1例）。孵卵15日のBhヘテロ型胚において肝臓右葉先端部のみに変性を示す胚が1例認められた（155例中1例）。この変性は胚胎的部位に限られていたが，肉眼的にも観察できた。この組織学的特徴はホモ型胚のそれと同一であったが胚発生進行とともにまわりの正常組織によっておきかえられ，胚発生進行にはほとんど影響ないと考えられる。この解釈は孵卵10～15日におけるBhヘテロ型および野生型胚の体重はほとんど同じであったことおよび両者の孵化率，育成率には差は認められなかったことからも支持される。ウズラ胚の肝臓組織片のニワトトリ胚肌膜上における培養実験において，大きな移植片では中心部において上述のような組織の変性所見が観察され，これは酸素供給の不足によって生じたと解釈される。したがって孵卵10～12日のウズラ胚の肝臓左葉先端部は酸素供給不足がおきやすい部位であり，Bhヘテロ型胚では感受性が高くその影響が強く現れると考えられる。

（家禽会誌 20，158—169，1983）