EFFECT OF RADIATION ON CHICK EMBRYO

BY

Tadashi ADACHI and Shūichi MIKURIYA*

INTRODUCTION

This is one of the serial studies on effect of radiation on hard tissues, which have been performed in our institute1).

In the former reports, the objects of irradiation were growing tissues of young animals or those of the newborn, but in this experiment radiation was given in embryonic developmental stages.

As the experimental material chick embryo was used, which is very efficient in excluding maternal influences. In three stages of embryonic development, irradiations were done and several effects as mentioned below were studied thereafter.

MATERIAL AND METHOD

I. Material.

Fertilized eggs of the single crest white leghorn, qualified by the National Laboratory, were used with special care of uniformities of weight, shape and thickness of eggshell etc. A flat type incubator (for 50 eggs) was used, temperature being kept 100~108°F and relative humidity at 60~70%. Eggs were turned twice daily2) and their life and death were examined by "candling"2).

II. Radiation.

1) Condition of radiation: 170 kV, 4 mA, HVL 1 mm Cu, 30 cm, 30 r/min. Each irradiation was done for one group of five eggs, placed horizontally and embedded in rice phantom for homogeneous irradiation.

2) Time of irradiation.

In this experiment, chick embryos were named according to days after incubation, for example, n-day chick embryo, and single irradiation was given for 4-day chick embryo, 11-day chick embryo and 18-day chick embryo. Twice irradiations, i.e. 4- and 11-day or 11- and 18-day were made also.

* 足立 忠, 御厨修一: Dept. of Radiology (Chief: Prof. T. ADACHI), School of Medicine. Received for Publication, Oct. 5, 1962.
3) Dose

Doses in this paper were expressed in “air dose” and each group was given single dose of 200 r, 400 r, 600 r and 800 r at the surface of rice-phantom. With the scattered radiation, eggs were really irradiated by 1.36 (upper surface), to 0.89 (bottom) times of the air dose.

RESULTS

I. Lethal effect.

Following the irradiation in the embryonic stages, death of chick embryo was observed. There were two kinds of death, that is, acute death and delayed death\(^\text{2,8,4,5}\). Acute death means death within 24 hours after irrdadiation. Number of acute death was marked in groups irradiated in the late stages (18-day and 11-day chick embryo) and in groups of early stage irradiation (4-day chick embryo) acute death was seen only in groups of the large dose (800 r) (Figs. 1, 2, 3, Tables 1, 2, 3).

II. Hatch (survival) rate.

After 21 days of incubation chick embryo, breaking the eggshell, became chicken\(^9\). In Table 4 and Fig. 4, hatch rate and hatch (survival)
Fig. 2. Survival of 11-Day Chick Embryo Following Irradiation

Table 1. Death of Chick Embryo (irradiated at 4 Day Chick Embryo)

<table>
<thead>
<tr>
<th>Type of death</th>
<th>Acute death</th>
<th>Delayed death</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 r</td>
<td>0</td>
<td>2 ( 2)</td>
</tr>
<tr>
<td>400 r</td>
<td>0</td>
<td>7 ( 6)</td>
</tr>
<tr>
<td>600 r</td>
<td>0</td>
<td>19 (12)</td>
</tr>
<tr>
<td>800 r</td>
<td>12</td>
<td>8 ( 3)</td>
</tr>
</tbody>
</table>

( ) numbers of dead-in-shell chicks.

Table 2. Death of Chick Embryo (Irradiated at 11-Day Chick Embryos)

<table>
<thead>
<tr>
<th>Type of death</th>
<th>Acute death</th>
<th>Delayed death</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 r</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>400 r</td>
<td>1</td>
<td>4 ( 2)</td>
</tr>
<tr>
<td>600 r</td>
<td>16</td>
<td>13 ( 1)</td>
</tr>
<tr>
<td>800 r</td>
<td>18</td>
<td>1 ( 1)</td>
</tr>
</tbody>
</table>

( ) numbers of dead-in-shell chicks.
curves are given. Earlier the stage of irradiation, lower the hatch-rate. As shown in Fig. 4, LD_{50} are 360 r for 4-day chick embryo, 420 r for 11-day chick embryo and 540 r for 18-day chick embryo.

III. Prolongation of hatching (or incubation) period.

As shown in Table 5, when dose of radiation was not heavy, large numbers of chick embryo were hatched, however compared with the control group, prolongation of the hatching time especially in 4-day and 11-day chick embryo was observed. Almost no difference was observed in 18-day chick embryo (Table 6).
Table 4. Hatch (Survival) Rate

<table>
<thead>
<tr>
<th>Exposure dose</th>
<th>4-day chick embryo</th>
<th>11-day chick embryo</th>
<th>18-day chick embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 r</td>
<td>90% (80–96)*</td>
<td>95% (86–96)</td>
<td>100% (96–100)</td>
</tr>
<tr>
<td>400 r</td>
<td>65% (51–77)</td>
<td>75% (62–85)</td>
<td>100% (96–100)</td>
</tr>
<tr>
<td>600 r</td>
<td>5% (1–16)</td>
<td>15% (7–28)</td>
<td>35% (23–50)</td>
</tr>
<tr>
<td>800 r</td>
<td>0 (0–8)</td>
<td>5% (1–16)</td>
<td>5% (1–16)</td>
</tr>
</tbody>
</table>

* Figures in brackets show 60% confidence interval.

Fig. 4. Dosage Survival Curve

IV. Body weight of chicken after hatching.

Body weight of chickens were traced up to 21-day after hatching. As shown in Fig. 5, in 200 r groups no deteriorating effect on increase of body weight was seen, but in 400 r group, obviously in 4-day chick embryo and to some degree in 11- and 18-day chick embryo, suppression of increase of body weight was observed.

Some of chickens died during this period. Number of the death was large in 4-day chick embryo as well as in 18-day chick embryo and very small in 11-day chick embryo. Death of chickens seemed to be related to diarrhoe (Table 7) and limbs anomalies (Table 8) and none to feedings state or closure of umbilicus (Table 9).

V. Morphological study of acute death of chick embryo.

Haemorrhages were chief findings on cases of the acute death. Macroscopically, they were noticed in and around the chick embryo. On 4-day
Table 3. Hatching Time and Numbers of Hatching

<table>
<thead>
<tr>
<th>Hatching Time</th>
<th>Hatching before 21 day</th>
<th>21 day–21.5 day</th>
<th>21.5 day–22 day</th>
<th>Hatching after 22 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 r</td>
<td>0</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>0</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 day chick embryo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 r</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>400 r</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>600 r</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>800 r</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 day chick embryo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 r</td>
<td>6</td>
<td>15</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>400 r</td>
<td>2</td>
<td>16</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>600 r</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>800 r</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18 day chick embryo</td>
<td></td>
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</table>

Table 6. Stage of Development at Irradiation and Times up to Hatching

200 r irradiation

<table>
<thead>
<tr>
<th>Hatching before 21.5 day</th>
<th>Control</th>
<th>4-day chick embryos</th>
<th>11-day chick embryos</th>
<th>10-day chick embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>7</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Hatching before 21.5 day</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

\[ x^2 = 5.9 \quad x^2 = 5.0 \quad x^2 = 0.02 \]

400 r irradiation

<table>
<thead>
<tr>
<th>Hatching before 21.5 day</th>
<th>Control</th>
<th>4-day chick embryos</th>
<th>11-day chick embryos</th>
<th>18-day chick embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Hatching before 21.5 day</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

\[ P = 0.000016 \quad x^2 = 4.7 \quad x^2 = 1.1 \]
EFFECT OF RADIATION ON CHICK EMBRYO

Fig. 5. Body weight change in chicken

Table 7. Diarrhoe

<table>
<thead>
<tr>
<th>Diarrhoe</th>
<th>+</th>
<th>±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 8. Limbs Anomalies

<table>
<thead>
<tr>
<th>Limbs anomalies</th>
<th>+</th>
<th>—</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 9. Closure of Umbilicus

<table>
<thead>
<tr>
<th>Closure of umbilicus</th>
<th>Normal</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>

chick embryo irradiated, narrowing and disappearing of the yolk vessels, a blood stagnation in sinus terminalis and moderate haemorrhage in area pellucida especially in the cranial part of the embryo were seen. These were marked in 11-day chick embryo, in which subcutaneous bleedings were seen in the occipital region as well as around the eyes (Fig. 6).
Table 10. Number of Malformation Following Irradiation

<table>
<thead>
<tr>
<th>Exposure dose</th>
<th>Numbers used</th>
<th>Dead-in-shell chicken</th>
<th>Numbers hatched</th>
<th>Dead chicken</th>
<th>Survived chicken</th>
<th>Dead chick embryo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group irradiated at 4-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 r</td>
<td>20</td>
<td>2(0)</td>
<td>18</td>
<td>1(1)</td>
<td>17(0)</td>
<td>0</td>
<td>1(1)</td>
</tr>
<tr>
<td>400 r</td>
<td>20</td>
<td>6(4)</td>
<td>13</td>
<td>5(3)</td>
<td>8(0)</td>
<td>1</td>
<td>7(7)</td>
</tr>
<tr>
<td>600 r</td>
<td>20</td>
<td>12(10)</td>
<td>1</td>
<td>1(1)</td>
<td>0</td>
<td>7</td>
<td>11(11)</td>
</tr>
<tr>
<td>800 r</td>
<td>20</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>3(3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>80</td>
<td>23(17)</td>
<td>32</td>
<td>7(5)</td>
<td>25(10)</td>
<td>25</td>
<td>22(22)</td>
</tr>
<tr>
<td><strong>Group irradiated at 11-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 r</td>
<td>20</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>19(0)</td>
<td>1</td>
<td>0(0)</td>
</tr>
<tr>
<td>400 r</td>
<td>20</td>
<td>2(1)</td>
<td>15</td>
<td>1(1)</td>
<td>14(0)</td>
<td>3</td>
<td>2(2)</td>
</tr>
<tr>
<td>600 r</td>
<td>20</td>
<td>1(0)</td>
<td>3</td>
<td>1(1)</td>
<td>2(0)</td>
<td>16</td>
<td>1(1)</td>
</tr>
<tr>
<td>800 r</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1(0)</td>
<td>18</td>
<td>0(0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>80</td>
<td>4(1)</td>
<td>38</td>
<td>2(2)</td>
<td>36(0)</td>
<td>38</td>
<td>3(3)</td>
</tr>
<tr>
<td><strong>Group irradiated at 18-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 r</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>3(2)</td>
<td>27(1)</td>
<td>0</td>
<td>3(3)</td>
</tr>
<tr>
<td>400 r</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>2(1)</td>
<td>28(1)</td>
<td>0</td>
<td>2(2)</td>
</tr>
<tr>
<td>600 r</td>
<td>20</td>
<td>0</td>
<td>7</td>
<td>2(2)</td>
<td>5(0)</td>
<td>13</td>
<td>2(2)</td>
</tr>
<tr>
<td>800 r</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1(0)</td>
<td>19</td>
<td>0(0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>68</td>
<td>7(5)</td>
<td>61(2)</td>
<td>32</td>
<td></td>
<td>7(7)</td>
</tr>
</tbody>
</table>

**Twice Irradiation Groups.**

<table>
<thead>
<tr>
<th>Exposure dose</th>
<th>Numbers used</th>
<th>Dead-in-shell chicken</th>
<th>Numbers hatched</th>
<th>Dead chicken</th>
<th>Survived chicken</th>
<th>Dead chick embryo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group irradiated at 4 and 11-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 r×2</td>
<td>20</td>
<td>14(10)</td>
<td>5</td>
<td>3(2)</td>
<td>2(0)</td>
<td>1</td>
<td>12(12)</td>
</tr>
<tr>
<td>400 r×2</td>
<td>20</td>
<td>15(15)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>15(15)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>29(25)</td>
<td>5</td>
<td>3(2)</td>
<td>2(0)</td>
<td>6</td>
<td>27(27)</td>
</tr>
<tr>
<td><strong>Group irradiated at 11 and 18-day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 r×2</td>
<td>20</td>
<td>7(5)</td>
<td>11</td>
<td>4(1)</td>
<td>7(1)</td>
<td>2</td>
<td>7(7)</td>
</tr>
<tr>
<td>400 r×2</td>
<td>5</td>
<td>1(1)</td>
<td>3</td>
<td>2(0)</td>
<td>1(0)</td>
<td>1</td>
<td>1(1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25</td>
<td>8(16)</td>
<td>14</td>
<td>6(1)</td>
<td>8(1)</td>
<td>3</td>
<td>8(8)</td>
</tr>
</tbody>
</table>

Figures in brackets show numbers of malformation.

The acute death cases of 18-day chick embryo showed the marked subcutaneous haemorrhages of whole body and also spotted bleedings in the liver.

Histological studies of 11-day and 18-day chick embryo reveal subcutaneous haemorrhages and bleedings in many internal organs (liver, lung, heart, etc.). No haemorrhages were noticed in the bone tissue.

It may be said that one of the important causes of the acute death is related to these haemorrhages.
Fig. 6. Acute death of 11-day chick embryo and control (right).
↓: Subcutaneous bleeding.

Fig. 7. Normal chicken.

Fig. 8. Club foot.

Fig. 9. Extension Contracture.

Fig. 10. Defect of toe.
VI. Malformations.

Malformations were detected on the survived and also dead-in-shell chickens. As shown in Table 10, malformations were most frequent in groups irradiated at 4-day, next was 18-day and only few in 11-day groups. In this case, malformations were noticed in limbs, beak, and feathers. Incomplete closure of umbilicus was adopted as such. Malformations of the cranium and the eye reported in many papers were not observed in this experiment. It is known that malformation is dependent on the time of irradiation related to the time of organogenesis. From this it may be suggested that for malformation of the eye and the cranium irradiation in more earlier stage may be necessary.
1) Malformation of limbs.

As the malformation of limbs, club foot\(^9\text{-}^{12}\) (Fig. 8), extension contracture (Fig. 9), defect of toe (Fig. 10) and its dorsal deflection (Fig. 11) and medial deviation (Fig. 12) were noticed. Normal chicken is shown in Fig. 7.

![Fig. 14. Malformation of feather](image1)

**Calamus root**

Lost swelling of calamus root, curving and making a mass of barb and barbule.

![Fig. 15. Malformation of feather](image2)

Miliar and patch distribution of down feather.

![Fig. 16. Short upper beak](image3)

![Fig. 17. Partial defect of beak and crossed beak](image4)
2) Malformation of feathers.

Lost swelling of calamus root (Fig. 14), irregular linings of calamus of barb and barbule, curving and making a mass (Fig. 14), miliar and patch distribution of down feather (Fig. 15) were noticed. Normal feather is shown in Fig. 13.

3) Malformation of beaks.

Short upper beak (Fig. 16), partial defect of beak (Fig. 17), crossed beak (Fig. 17) and parrot beak (Fig. 10) were seen. The partial defect accompanied by the cleft palate (Fig. 18) was also observed.

4) Incomplete closure of umbilicus.

As a rule, closure of umbilicus is seen on 19-day chick embryo, but in some cases of embryonic disturbance, the closure is incomplete and a part of yolk sack remains outside of body. That is the incomplete closure of umbilicus.

In general, malformation was more frequent in cases of dead-in-shell chickens than the survived (Table 10) and as for the irradiated they are large in numbers in cases of the twice irradiation than the single irradiation. In the single irradiation, malformations of limbs were predominant and in the twice irradiation, that of feathers were marked (Tables 11, 12).

VII. Histological findings of hard tissues, especially those of the bone tissues, are summarized as follows (Table 13).

1) Almost no histological changes were observed in each 200 r group and changes are initiated in 400 r or more. Fig. 19~21 are histological findings of knee joint of hind-limb in normal 7-day chicken.

2) On the growth of cartilage cells, most marked injuries were
Table 11. Types and Numbers of Malformation in Dead-in-Shell Chickens.

<table>
<thead>
<tr>
<th>Exposure dose</th>
<th>Malformation of limbs</th>
<th>Malformation of beaks</th>
<th>Malformation of feathers</th>
<th>Incomplete closure of umbilicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>400 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>600 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>800 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Group irradiated at 4-day</td>
<td>200 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
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<tr>
<td>Total</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group irradiated at 11-day</td>
<td>200 r</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group irradiated at 18-day</td>
<td>200 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

| Group irradiated at 4 and 11-day | 300 r×2         | 6                     | 2                      | 8                       | 2 |
|                                | 400 r×2         | 10                    | 6                      | 14                      | 10 |
| Total                          | 16                    | 8                     | 22                     | 12                       |
| Group irradiated at 11 and 18-day | 300 r×2         | 3                     | 1                      | 3                       | 1 |
|                                | 400 r×2         | -                     | -                      | 1                       | - |
| Total                          | 3                     | 1                     | 4                      | 1                       |
| Total                          | 19                    | 9                     | 26                     | 13                       |
| Sum Total (Single+Twice)       | 36                    | 16                    | 26                     | 16                       |

observed in 4-day chick embryo of 400 r groups, but in 11-day and 18-day chick embryo only slight injuries were seen. For 600 r groups, similars but to some extent severers could be seen (Figs. 22~30).

3) On the resorptions process of cartilage, injuries were marked in cases of 4-day chick embryo (Figs. 22~24), next in 18-day chick embryo (Figs. 28~30) and compared with nonirradiated control almost no changes
Table 12. Types and Numbers of Malformation in Chickens.

<table>
<thead>
<tr>
<th>Single Irradiation Groups.</th>
<th>Exposure dose</th>
<th>Malformation of limbs</th>
<th>Malformation of beaks</th>
<th>Malformation of feathers</th>
<th>Incomplete closure of umbilicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group irradiated at 4-day</td>
<td>200 r</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group irradiated at 11-day</td>
<td>200 r</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Group irradiated at 18-day</td>
<td>200 r</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td></td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Twice Fractionated Irradiation Groups.

<table>
<thead>
<tr>
<th>Group irradiated at 4 and 11-day</th>
<th>300 r×2</th>
<th>2</th>
<th>—</th>
<th>1</th>
<th>—</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 r×2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group irradiated at 11 and 18-day</th>
<th>300 r×2</th>
<th>2</th>
<th>—</th>
<th>2</th>
<th>—</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 r×2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total                            | 4       | 3 |   |   |   |

| Sum Total (Single+Twice)         | 15      | 4 | 2 |   |   |
Table 13. Histological Changes Following Irradiation to Bone and Cartilage Tissues.

<table>
<thead>
<tr>
<th>Single Irradiation Groups.</th>
<th>Exposure dose</th>
<th>Materials</th>
<th>Injury on growth of cartilage tissue</th>
<th>Injury on resorption of cartilage tissue</th>
<th>Injury on removal of cartilage tissue</th>
<th>Injury on marrow tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group irradiated at 4-day</td>
<td>200 r</td>
<td>7-day chicken</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>7-day chicken</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>7-day chicken</td>
<td>+</td>
<td>#</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>Dead-in-shell</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
<td>(±)</td>
</tr>
<tr>
<td>Group irradiated at 11-day</td>
<td>200 r</td>
<td>7-day chicken</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>7-day chicken</td>
<td>±</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>7-day chicken</td>
<td>±</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>7-day chicken</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group irradiated at 18-day</td>
<td>200 r</td>
<td>7-day chicken</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r</td>
<td>7-day chicken</td>
<td>±</td>
<td>±</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>600 r</td>
<td>7-day chicken</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>800 r</td>
<td>Acute death chick embryo</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
<td>(±)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Twice Irradiation Groups.</th>
<th>Exposure dose</th>
<th>Materials</th>
<th>Injury on growth of cartilage tissue</th>
<th>Injury on resorption of cartilage tissue</th>
<th>Injury on removal of cartilage tissue</th>
<th>Injury on marrow tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group irradiated at 4 and 11-day</td>
<td>300 r×2</td>
<td>7-day chicken</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>(±)</td>
</tr>
<tr>
<td></td>
<td>400 r×2</td>
<td>Dead-in-shell</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
<td>(±)</td>
</tr>
<tr>
<td>Group irradiated at 11 and 18-day</td>
<td>300 r×2</td>
<td>7-day chicken</td>
<td>±</td>
<td>±</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>400 r×2</td>
<td>Dead-in-shell</td>
<td>(+)</td>
<td>(++)</td>
<td>(++)</td>
<td>(±)</td>
</tr>
</tbody>
</table>

5) Slight changes were observed in the marrow tissue of 4-day (Fig. 24) and 18-day chick embryo (Fig. 30) and here also 11-day chick embryo (Fig. 27) showed almost no injury.

6) In 800 r group, of which most chickens were dead-in-shell or in a state of acute death, changes of the bone and the marrow tissue were very marked, but similar phenomena were observed in the non-irradiated dead-in-shell. The survived chickens in 800 r group of 11-day chick embryo showed similar injuries as 4-day or 18-day chick embryo of 600 r group.

7) In cases of the twice irradiation group of 300 r×2 showed smaller changes of bone tissues than single dose of 600 r in 4-day or in 18-day chick embryo and in twice irradiation, 4-day and 11-day chick embryo showed more marked injuries of the bone tissue than 11-day and 18-day chick embryo. Groups of 400 r×2 were all dead-in-shell or in state of acute death.
Fig. 19. Normal bone tissue in 7-day chicken. (Knee joint of hind limb)

Fig. 20. Normal cartilage tissue.

Fig. 21. Normal marrow tissue.

Fig. 22. Bone tissue (600r group of 4-day chick embryo).
Zone of proliferation is thinner than control. Growth and resorptions process of cartilage, and process of removal, are injured.

Fig. 23. Cartilage tissue (600r group of 4-day chick embryo).

Fig. 24. Marrow tissue (600r group of 4-day chick embryo)
Slight changes are seen.
9) As a whole, the most marked changes of the bone tissue were seen in single irradiation of 4-day chick embryo and next was that of 18-day chick embryo. 11-day chick embryo showed only slight degree of changes.

10) Almost no histological changes were observed in other hard tissues, i.e. the beak and the nagels.
Summary

1. Chick embryos were irradiated in their three stages of embryonal development, i.e. 4th day (4-day chick embryo), 11th day (11-day chick embryo) and 18th day (18-day chick embryo).

2) As dose of radiation increased, increasing death of chick embryo was observed. There were two kinds of death of chick embryo, that was, acute death and delayed one. Acute death meant the death within 24 hours after irradiation and one of its causes was suggested as haemorrhages appearing subcutaneous as well as in many internal organs. Acute death was seen in the groups irradiated in the later embryonic stage (18-day and 11-day chick embryo).

3) From the survival (hatch) rate LD₉₀ are 360 r for 4-day chick embryo, 420 r for 11-day chick embryo and 540 r for 18-day chick embryo.

4) Among the survived chick embryos, prolongation of hatching time was proved.

5) As for the hatched chickens, the irradiated groups showed some deteriorating effects on growth of body. Body weight curves lowered compared with the non-irradiated control. In this experiment 4-day chick embryo was affected mostly, to this followed 18-day chick embryo and 11-day chick embryo showed the least damages.

6) Death of chickens, obvious also in groups irradiated at 4-day and 18-day chick embryo, seemed to be related to the symptoms of diarrhoea and limb anomalies.

7) Malformations were seen in the survived and also in the dead-in-shell chickens. Malformations could be detected almost in the hard tissue, namely limbs, claws, feathers and beaks, and incomplete closure of umbilicus was included as such.

8) As for the number of malformation occurred, most frequent in 4-day chick embryo, next 18-day chick embryo and only a few in 11-day chick embryo. In the single irradiation, limbs anomalies were most prominent and in the two time irradiation anomalies of the feathers were marked.

9) Malformations of the eye and the cranium by irradiation, which were said very frequent, were not observed in this experiment. It is known that malformation is dependent on the time of irradiation related to the time of organogenesis. From this it may be suggested that for the malformation of the eye and the cranium earlier stage of irradiation may be necessary.

10) Histological studies on the bone tissue of the hatched chickens, which seemed to be apparently normal, revealed also considerable injuries on the bone growth. In this subject too, 4-day chick embryo was more
markedly affected, next came 18-day chick embryo and 11-day chick embryo showed relatively slight changes.

REFERENCES