Effects of Radiation on Growth of Caudal Vertebrae of Mouse Fetus

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ABSTRACT

A total of 333 pregnant mice (ddY strain) and their 2759 fetuses were used. When the radiation-induced delay of ossification was used as an indicator of radiation effects on fetal growth, the gestation ages 8 and 16 days were found to be two radiosensitive stages. The degree of the delay of ossification was enhanced with increasing the radiation dose. However, the radiation-induced delay, estimated from the number of ossificated caudal vertebrae on day, was only slightly dependent on the dose rate (1 R/min to 100 R/min). The number of ossificated caudal vertebrae in response to fetal irradiation was paralleled to the body weight of fetus.

INTRODUCTION

The teratogenic effects of embryonic and fetal irradiation are worth noting from the point of view of biological indicators of radiation effect. In previous reports1-2 with regard to digital malformations of mice, authors described the fact that the appearance of ectrodactyly, brachydactyly, polydactyly and syndactyly depend upon dose-rate of γ-irradiation.

Retardation of growth is a frequent sequela of embryonic and fetal irradiation. Authors have taken an interest in the decrease of body weight and the delay of ossification which are sequelae of the retardation of embryonic and fetal growth. The experiments reported in this paper were designed to know whether or not the delay of ossification in the caudal vertebrae is of use as an indicator of radiation effect.

MATERIALS AND METHODS

The animals used were ddY-strain mice obtained from Nippon Bio-Supp. Co., Ltd. A total of 333 pregnant mice were provided for this study, and 2759 fetuses were

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observed. Virgin females of 10 weeks (70±2 days) of age were exposed from 5 p.m. till 9 a.m. of the next day with mature male of the same strain, and those with vaginal plugs at the end of the period were segregated. The day, when vaginal plugs were found, was considered to be day 0 in gestation ages.

Whole-body irradiation of the pregnant mice was carried out with the cesium-137 gamma ray source of Environmental-Controlled Irradiation Facility at the University of Tokyo. The exposure time was one hour in all cases except experiments on the dose-rate effect, so that the dose and dose-rate were regulated by a distance from the radiation source.

The experiments consisted of four parts. The first experiment was designed to find out the most radiosensitive period in regard to the delay of ossification. The pregnant mice were irradiated at times ranging from day 7 to day 17. The mice were grouped with respect to exposure day, and sacrificed on day 18 to observe their fetuses. The mice irradiated on day 11 were divided according to exposure hour into five groups, i.e. 4:00 a.m., 9:00 a.m., 2:00 p.m., 7:00 p.m. and 0:00 a.m. groups.

On the second experiment the growth of the caudal vertebrae of the fetuses irradiated on the susceptible period was observed. The pregnant mice irradiated on day 11 were sacrificed between day 13 and 19 to observe their fetuses.

The purpose of the third experiment was to know whether or not the number

| Gestation age (day) | Exposure starting time | No. of mice treated | Total No. of fetuses | Mean litter size | Fetal deaths | Fetuses examined | Fetus weight (g) | Ossification number |
|---------------------|------------------------|---------------------|----------------------|-----------------|--------------|-----------------|-----------------|------------------|
| 7                   | 2:00 p.m.              | 8                   | 71                   | 8.88±1.64       | 7            | 64              | 1.22±0.11       | 9.23±1.26        |
| 8                   | 2:00 p.m.              | 5                   | 29                   | 5.80±1.92       | 1            | 28              | 1.10±0.13       | 7.07±1.09        |
| 9                   | 2:00 p.m.              | 5                   | 42                   | 8.40±1.52       | 4            | 38              | 1.00±0.09       | 7.13±0.96        |
| 10                  | 2:00 p.m.              | 11                  | 90                   | 8.18±2.14       | 4            | 86              | 0.94±0.10       | 6.58±1.08        |
| 4:00 a.m.           | 13                     | 125                 | 9.62±2.90            | 12              | 113           | 0.87±0.11       | 6.67±0.89        |
| 9:00 a.m.           | 9                      | 85                  | 9.44±1.88            | 2               | 83            | 0.93±0.08       | 7.29±0.71        |
| 11                  | 2:00 p.m.              | 15                  | 129                  | 8.60±1.68       | 5            | 124             | 0.97±0.11       | 7.44±1.00        |
| 7:00 p.m.           | 13                     | 125                 | 9.61±1.76            | 6               | 119           | 0.95±0.11       | 7.24±1.16        |
| 0:00 a.m.           | 11                     | 97                  | 8.82±1.78            | 10              | 87            | 1.06±0.09       | 7.95±1.16        |
| 12                  | 2:00 p.m.              | 12                  | 105                  | 8.75±1.82       | 2            | 103             | 1.03±0.11       | 7.34±1.45        |
| 13                  | 2:00 p.m.              | 10                  | 89                   | 8.90±1.91       | 4            | 85              | 1.10±0.10       | 9.53±1.62        |
| 14                  | 2:00 p.m.              | 7                   | 71                   | 10.14±4.49      | 3            | 68              | 1.08±0.11       | 8.97±1.28        |
| 15                  | 2:00 p.m.              | 5                   | 57                   | 11.40±2.19      | 3            | 54              | 1.16±0.08       | 8.47±1.01        |
| 16                  | 2:00 p.m.              | 7                   | 76                   | 10.86±3.13      | 4            | 72              | 1.18±0.10       | 8.15±1.43        |
| 17                  | 2:00 p.m.              | 7                   | 78                   | 11.14±1.77      | 4            | 74              | 1.24±0.13       | 8.54±1.32        |
| 18                  | 2:00 p.m.              | 20                  | 167                  | 8.35±2.13       | 9            | 158             | 1.39±0.13       | 10.21±1.45       |

Table 1. The relation between irradiated ages of gestation and the number of ossificated caudal vertebrae (ossification number) as well as the body weight of fetuses.
of ossificated caudal vertebrae depend upon dose-rate. Six groups of the pregnant mice were irradiated with the dose of 200 R at the dose-rates of 1.0, 3.3, 6.7, 20, 40 and 100 R/min respectively. On day 18 the mice were sacrificed to observe their fetuses.

The fourth experiment was carried out to investigate the relation between the number of ossificated caudal vertebrae and the exposure dose. The mice used in this experiment were divided according to the dose into 75, 100, 150, 200, 250 and 300 R groups.

The fetuses removed from the pregnant mice were weighted, then stained for bone by the Dawson's method. By this method the centers of ossification appear deep red and can easily be distinguished from the cartilage. The specimens were kept in glycerin in dish and examined under a low-power binocular microscope.

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### Table 2. The dose-rate dependence of the number of ossificated caudal vertebrae and the body weight of fetuses irradiated on 11th day of pregnancy

| Dose rate (R/min) | Exposure time (min) | No. of mice treated | Total No. of fetuses | Mean litter size | Fetal deaths | Fetuses examined | Body weight (g) | Ossification number |
|-------------------|--------------------|---------------------|----------------------|-----------------|--------------|----------------|-----------------|-------------------|
| 1.0               | 200                | 13                  | 117                  | 9.00 ± 2.20     | 3 (2.5)      | 114            | 0.98 ± 0.11     | 6.87 ± 0.99       |
| 3.3               | 60                 | 15                  | 129                  | 8.60 ± 1.68     | 5 (3.9)      | 124            | 0.97 ± 0.11     | 7.44 ± 1.00       |
| 6.7               | 30                 | 5                   | 41                   | 8.20 ± 2.17     | 0            | 41             | 0.98 ± 0.11     | 7.37 ± 1.36       |
| 20                | 10                 | 10                  | 91                   | 9.10 ± 2.02     | 6 (5.6)      | 85             | 0.91 ± 0.09     | 6.56 ± 0.98       |
| 40                | 5                  | 6                   | 54                   | 9.00 ± 2.00     | 2 (3.7)      | 52             | 0.87 ± 0.10     | 6.00 ± 0.95       |
| 100               | 2                  | 8                   | 67                   | 8.38 ± 1.85     | 3 (4.5)      | 64             | 0.86 ± 0.12     | 5.94 ± 1.23       |
| Total             | —                  | 57                  | —                    | —               | —            | 480            | —               | —                 |

### Table 3. The radiation dose dependence of the number of ossificated caudal vertebrae and the body weight of fetuses.

| Exposure dose (R) | Dose rate (R/min.) | No. of mice treated | Total No. of fetuses | Mean litter size | Fetal deaths | Fetuses examined | Body weight (g) | Ossification number |
|-------------------|--------------------|---------------------|----------------------|-----------------|--------------|----------------|-----------------|-------------------|
| 0                 | —                  | 20                  | 167                  | 8.35 ± 2.13     | 9 (5.4)      | 158            | 1.39 ± 0.13     | 10.21 ± 1.45      |
| 75                | 1.25               | 13                  | 103                  | 7.92 ± 1.19     | 2 (1.9)      | 101            | 1.24 ± 0.14     | 8.87 ± 1.65       |
| 100               | 1.67               | 10                  | 77                   | 7.70 ± 2.26     | 0            | 77             | 1.11 ± 0.12     | 8.65 ± 1.36       |
| 150               | 2.50               | 18                  | 134                  | 7.44 ± 1.58     | 1 (0.8)      | 133            | 1.14 ± 0.11     | 8.62 ± 1.35       |
| 200               | 3.33               | 15                  | 129                  | 8.60 ± 1.68     | 5 (3.9)      | 124            | 0.97 ± 0.11     | 7.44 ± 1.00       |
| 250               | 4.17               | 10                  | 85                   | 8.50 ± 1.90     | 7 (8.2)      | 78             | 0.89 ± 0.09     | 6.45 ± 0.75       |
| 300               | 5.00               | 7                   | 56                   | 8.00 ± 1.91     | 3 (5.4)      | 53             | 0.71 ± 0.08     | 4.70 ± 0.61       |
| Total             | —                  | 93                  | —                    | —               | —            | 724            | —               | —                 |
The measurement of the number of ossificated caudal vertebrae (i.e. ossification number) was made with the vertebra on a line connecting the right and left illial processes as its starting-point. When the illial process was indistinct for the reason of the retardation of ossification, the measurement started at nineth distal vertebra from the first lumbar vertebra. It was ascertained from observations of about 3000 fetuses that the ossification of the caudal vertebrae progresses from the proximal to the distal.

RESULTS

The relation between the ossification number and the gestation age of irradiation is illustrated as a solid line in Figure 1 and in Table 1. The line includes two concavities which have the minimum of ossification number on day 8 to 11 and day 16 respectively. Figure 1 gives as a broken line the relation between the body weight of fetuses and the gestation age of irradiation. It is shown that the maximum of decrease in the body weight occurs in the group irradiated at 4:00 a.m. on day 11. On the assumption that ovulation of mice might be performed at 0:00 a.m.\(^4\)\(^5\), this exposure hour is estimated as 268 hours after the ovulation. The intra-uterine growth of the fetuses irradiated on day 11 were investigated together with the non-irradiated fetuses. As indicated in Figure 2, there is a
difference of body weight between the irradiated and the non-irradiated groups. The difference have a tendency to increase with the gestation ages. The ossificated caudal vertebrae of non-irradiated fetuses appeared on day 16 and the ossification number reached 12 on day 19. On the other hand, the ossification in the irradiated group could not be observed till day 17 and the ossification number became 10 on day 19.

![Diagram 2](image2.png)

**Fig. 2.** The progress of ossification in the caudal vertebrae of fetuses irradiated on 11th day of pregnancy.

![Diagram 3](image3.png)

**Fig. 3.** The dose-rate dependence of the number of ossificated caudal vertebrae and the body weight of fetuses irradiated on 11th day of pregnancy.
The number of ossificated caudal vertebrae as well as the body weight have been plotted against dose-rate in Figure 3 and in Table 2. There is a slight difference of the number between low dose-rate (1.0, 3.3 and 6.7 R/min) and high dose-rate (20, 40 and 100 R/min) groups.

In Figure 4 the solid line shows the dependence of ossification number on exposure dose (Table 3). It is obvious that the delay of ossification in the caudal vertebrae is enhanced with an increase of exposure dose. The same relation is also true for the case of the body weight.

**DISCUSSION**

During previous experiments on digital malformations induced by fetal irradiation of mice, authors faced the fact that a retardation of growth is observable even in fetuses without manifesting congenital anomaly.

It is possible to express quantitatively the extent of ossification of the digital bones and the caudal vertebrae. For this reason, Murakami\(^6\) stated that the digital bones and the caudal vertebrae are able to play a role as indicators of the progress of ossification in fetus.

Hoshino\(^7\) called attention to the fact that the cartilage formation in the spinal column begins about the same time in any part and any position of the vertebrae, while the time of the beginning of ossification is dependent on the position of the vertebrae.

Many embryological studies have been done about skeletal development in the fetus. In these studies, interest has been towards species-, strain-, and sex-differences of skeletal development in addition to the progress of chondrification and ossification\(^8, 9, 10-12, 13\). It has been pointed out in many reports that environ-
mental temperature\textsuperscript{14}, oxygen\textsuperscript{15}, antibiotics\textsuperscript{16-17}, ionizing radiation\textsuperscript{13, 15, 18, 19, 20} and maternal hypervitaminosis-A\textsuperscript{20} act as extrinsic factors which have effects upon the skeletal development in fetuses.

The abnormalities in skeletal development of the fetus appear as congenital malformation or stunting. Retardation of skeletal development is a sequela of stunting of the fetus.

From the results in this study, it became clear that the number of ossificated caudal vertebrae shows the degree of fetal growth and is of use as an indicator of radiation effect on fetuses.

As indicated in Figure 1, it was definitely shown that two gestation ages were radiosensitive on the delay of ossification. It is satisfactory to consider that the first sensitive age, day 8 to 11, is caused by the inhibition to the development of the somite and the bud of cartilage and second sensitive age, day 16, is resulted from the damage to the ossification in the cartilage.

As shown in the results of this study, the body weight of the fetus was similar to the number of ossificated caudal vertebrae in response to the fetal irradiation. The decrease of the body weight after the irradiation is a fetal change related to the stunting. In the radiosensitive gestation age, i.e. the critical period of radiation effect, the decrease of the body weight was more prominent than the retardation of ossification. The decrease in fetal body weight as a token of stunting have many problems remaining unsolved in its mechanism, but it may be able to play an important role as a biological indicator of radiation effects.

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