

Title	Radiation Effects on the Bone Growth and Effects of Salivary Gland Hormone (parotin) on Irradiated Bone Tissues
Author(s)	岩崎, 善助
Citation	日本医学放射線学会雑誌. 1971, 31(1), p. 78-95
Version Type	VoR
URL	https://hdl.handle.net/11094/15259
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Radiation Effects on the Bone Growth and Effects of Salivary Gland Hormone (Parotin) on Irradiated Bone Tissues

by

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放射線の骨発育に及ぼす影響と被照射骨に対する 唾液腺ホルモン（パロチン）の効果

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（昭和45年10月30日受付）

多くの文献によれば、骨は必ずしも放射線感受性が低いとはいえず、放射線後期障害の問題は重要である。著者は骨の放射線感受性が年令とともにどう変化するか、どの線量までは障害をまぬがれるか、また骨の成長発育を促進させるといふ唾液腺ホルモンに果して放射線障害防護作用があるか否かについても検討した。

生後1カ月および3カ月の Wistar 系ラットの脛骨全体（WB群）、脛骨々端部（E群）および脛骨々幹部（D群）照射の3群に分けてそれぞれに5,000R、2,000R、1,000Rの一回照射を行なった。またパロチンは0.1mg/kgを週2回宛24週間に亘つて注射した（P群）。照射後経時的にX線写真による計測を行ない、また照射後1、3、6カ月目に屠殺した後肢骨を摘出して病理組織学的検索を行なった。

放射線骨障害は幼若なものほど、線量の多いほど強く現われた。また障害の程度はWB群がもつとも強くE群がこれにつぎD群がもつとも軽度であった。生後1カ月の幼若ラットについて見る

と、もつとも障害の軽度な1,000R照射群でも対照群に比べ照射後6カ月後における脛骨の成長率はWB群では86.4%、E群89.7%、D群95.5%となお明らかに成長遅延が認められた。したがって障害発現の限度は1,000Rより低線量であろうと推定された。

X線像では照射部位に一致して脛骨の彎曲像、横径の短縮、腓骨の細小化などが認められた。病理組織学的には線量に相当して、骨端部においては骨端軟骨細胞層の巾の狭小、柱状細胞配列の扁平化、細胞の膨化、核濃縮、軟骨基質の硝子化などが認められた。

一方P群では1,000R照射の場合、照射のみの群よりも骨端軟骨細胞の配列は規則的であり、軟骨細胞の核の消失壊死も少なく、予備石灰化層の伸びや造骨細胞の数も対照群と大差なくある程度の障害修復の作用が認められた。しかし2,000R以上の照射群では障害の方が強くパロチンの効果は期待できなかつた。

I. Introduction

Many reports have been done on the experimental and clinical studies of radiation injury of the bones¹⁾⁻⁶⁾.

Many authors have stated that the bone injuries induced by X-ray irradiation were intensified in the case of the younger bone and the larger dosage⁷⁾⁻¹²⁾.

The clinical observation indicated frequent occurrences of the growth retardation of the bones of juveniles after radiotherapy, and of the pathological fracture of ribs or femurs where no cancerous metastasis was detected in the adult patients of cancer of the breasts or uterus, who have been subjected to radiotherapy.

Adachi (1956)¹³⁾ has reported that the necrosis of epiphyseal cartilage cells and the reduction of osteoblasts, which are caused shortly after irradiation, are causative of the retardation or stoppage of the growth of irradiated bones.

Thus radiation damage to bones manifests itself in the form of disturbed growth at the comparatively early stage after the irradiation. The author attempted roentgenological and histopathological experiments, for a long period, on the indications of suppressed growth of bones due to irradiation imparted to the whole hind leg bone, the epiphysis of the tibia, and the diaphysis of the tibia, with the use of rats aged one month and three months. On the other hand, an examination was made to determine whether irradiation damage to the bones can be averted by the hormone of the salivary gland credited with a capacity of accelerating the growth of bones. As the result, several interesting findings were obtained as follows.

II. Material and Methods

1. Materials:

Seven hundred and twenty Wistar-strain male rats aged one month and three months were used for this experiment. After being fed with the "Oriental" solid yeast feed with sufficient drinking water for at least 10 days prior to the experiment, the rats were irradiated and reared afterward under the same conditions. The rats were divided into following 36 groups. One group consists of 8 rats.

2. Conditions of irradiation:

With the use of Toshiba KXC-18 Depth Therapy Units, the irradiation was carried out in the conditions of 170 kVp, 20 mA, Cu 0.5 mm + Al 0.5 mm (HVL 1.00 mm Cu), FSD 15 cm, 291.2 R/min.

During irradiation, a rat was introduced into a lead cylinder, with only its left hind leg dragged and



Left to right: whole bone, epiphyseal only and diaphyseal only.

Fig. 1. Sketch of each elected site of irradiation.

fixed by means of a cord through a hole on the side. The rats were classified into three groups by the irradiated regions, namely, the whole hind leg bone, only the epiphysis of the tibia, and only the diaphysis of the tibia (hereinafter called "Group WB, Group E, and Group D" respectively). Each group was subjected to single dose irradiation of 5,000 R, 2,000 R and 1,000 R (Fig. 1). The scattered dose of the shielded area was measured about 2% of the dose of the irradiated area. Moreover, the reliability of this experimental method was confirmed by means of roentgenograms to make sure that the above-mentioned three regions were correctly irradiated.

3. Procedure for injecting parotin:

Three milligrams of parotin manufactured by Teikoku Zoki Seiyaku K.K. (Teikoku Internal Organs Pharmaceutical Co., Ltd.) was resolved in a mixture amounting to 20 ml of 6.7 ml sterile distilled water and 13.3 ml sterile physiological Saline solution, and 0.1 ml of the resultant solution was subcutaneously injected into the back of rats. This dosage, equivalent to 0.1 mg/kg parotin, is within the normal therapeutic range applicable for treating the periodontal disease cases of human beings. The injection was made on the experimental rats twice per week for a period of 24 weeks.

4. Measurement:

Measurements were made by means of X-ray films of the longitudinal length of the tibia and the transversal width of the diaphysis immediately above the peroneal connection of the tibia, each belonging to the irradiated hind leg, eight times, namely, before irradiation, 2 and 4 weeks, 2, 3, 4, 5 and 6 months after irradiation respectively. The control rats, which had not been subjected to irradiation, were measured by the same method at the same periods.

5. Method of histopathological examination:

At three different times of 1, 3 and 6 months after irradiation, the irradiated rats were sacrificed by decapitation, immediately followed by extraction of the left hind leg bone, which was fixed in 10% formalin solution. The fixed specimens were decalcified in mixture of potash alum and nitric acid, and stained with four types of staining fluid, i.e., hematoxylin and eosin, hematoxylin and Van Gieson, PAS, and Azan-Mallory, to be subjected to microscopic observation. The control rats, free from irradiation, were treated in the same fashion for microscopic observation at the same periods.

III. Results of Experiment

1. Effect of X-rays on the hind leg bone of juvenile rats:

1) Changes of the longitudinal length and transversal width of the tibia:

As shown in Fig. 2, 3 and 4, the changes of the longitudinal length of the tibia was characterized by the retardation of growth in proportion of the increase of the irradiation dosage. Classified by the irradiated regions, Group WB was affected to the highest degree, followed by Group E and Group D, the latter of which revealed the lightest damage caused by irradiation. The percentages of the growth of the irradiated groups after 6 months compared with the control group are given in Table I. To be specific, by the irradiation of 1,000 R, Groups WB, E and D accounted for 86.4%, 89.7% and 95.5% respectively. with 2,000 R, the same groups showed 82.9%, 89.0% and 93.1%; with 5,000 R, 72.2%, 83.0% and 92.3% respectively.

The transversal width of the diaphysis diminished in all the groups of WB, E and D, with the increase of X-ray dosage. However, with reference of different regions irradiated, the effect was found to be the

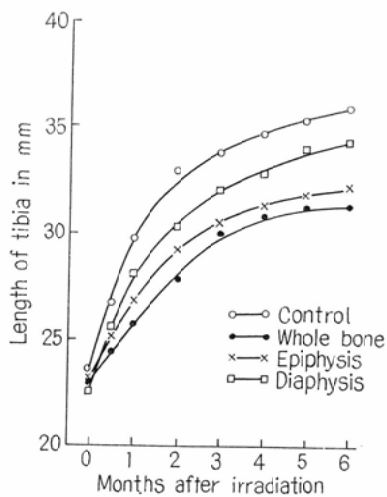


Fig. 2. Changes of the longitudinal lengths of tibia selectively irradiated with 1,000 R (One-month-old Rats).

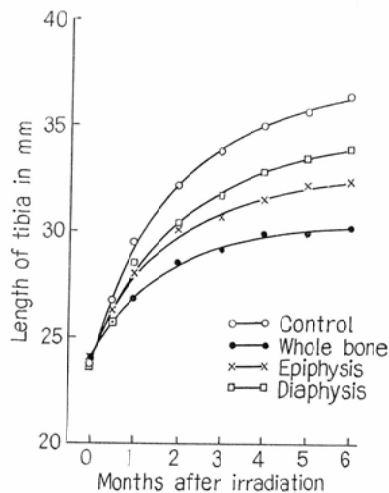


Fig. 3. Changes of the longitudinal lengths of tibia selectively irradiated with 2,000 R (One-month-old Rats).

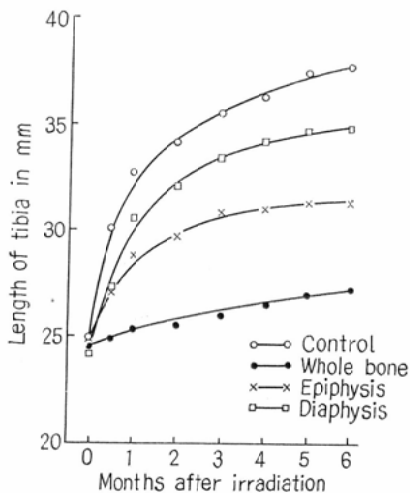


Fig. 4. Changes of the longitudinal lengths of tibia selectively irradiated with 5,000 R (One-month-old Rats).

Table 1. Growth rate (%) of the longitudinal length of tibia six months after selective irradiation (One-month-old Rats)

Irrad. Regions	Dosis		
	1,000 R	2,000 R	5,000 R
Whole bone	86.4	82.9	72.2
Epiphysis	89.7	89.0	83.0
Diaphysis	95.5	93.1	92.3

Table 2. Growth rate (%) of the transversal widths of tibia six months after selective irradiation (One-month-old Rats).

Irrad. Regions	Dosis		
	1,000 R	2,000 R	5,000 R
Whole bone	80.9	71.4	66.6
Epiphysis	95.2	89.1	84.4
Diaphysis	90.5	83.5	80.0

most intense in Group WB, followed by Group D and Group E. With the growth of the irradiated groups at 6 months compared with the control group, Group WB, E and D accounted for 80.9%, 95.2% and 90.5% respectively, by the irradiation of 1,000 R. With 2,000 R, the corresponding percentages were 71.4%, 89.1% and 83.5%, and with 5,000 R, the same were 66.6%, 84.4% and 80.0% (See Table 2).

2) Radiographic appearances:

In addition to the diminishing of the longitudinal length of the tibia, it was found that the tibia curved, the transversal width of the tibia was shortened, and the fibula was reduced in dimensions. Group WB showed a tendency to thinning in the fibula as a whole; Group E revealed in particular the thinning around the tibia attachment in the proximity of the fibula; Group D exhibited the thinned diaphysis of the fibula. These changes came to be perceived 2 months after the irradiation with 1,000 R, while the manifestation was as early as one month after irradiation with 2,000 R or 5,000 R. Especially in the groups irradiated with 5,000 R, the shape was alike to the core of a lead pencil in the diaphysis in Group WB and D, and in the vicinity of the proximate tibia attachment in Group E. In some cases, free ending appearances were observed. Typical radiographs are shown in Fig. 5

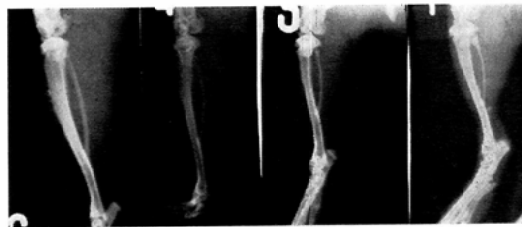


Fig. 5. Radiographic appearances six months after 5,000 R irradiation (control, diaphysis, epiphysis and whole bone, from left to right).

3) Histopathological findings:

In the epiphyseal cartilage, such manifestations were seen, as thinned cellular layers, swelling of cells, picnosis or necrosis, reduction and disappearance of cells, which was accompanied by the increased and hyaline degeneration of cartilaginous stromata. These changes were visible in Group WB and E and became more conspicuous with the higher dosage of irradiation to the extent that trabecular dysplasia and a decrease of osteoblasts came to be observed.

In the diaphysis, such phenomena were observed in Group WB and Group D as the capillary hyperemia in the bone cortex, periosteal hypertrophy, and the expansion of the Haversian canal. However, very little change was observed in the epiphyseal cartilage of Group D, in which the epiphysis had not been

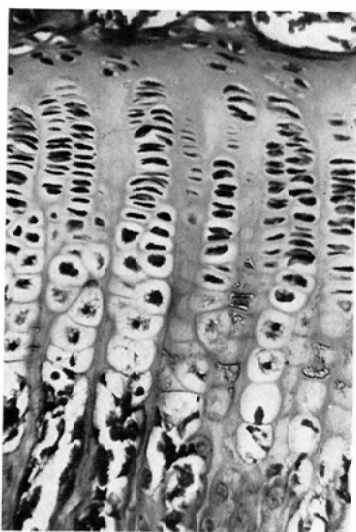


Fig. 6. Epiphyseal cartilage of control rats: Orderly arranged cellular columns of the epiphyseal cartilage and invasion of the vascular connective tissue.

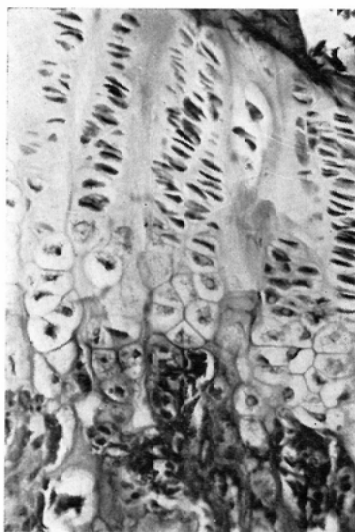


Fig. 7. Three months after 1,000 R irradiation (epiphysis only): Decrease of cartilage cells, slight disorder of column-formed arrangement, flattening of proliferation layer cells, increase of cartilage stromata, and irregularity of the preliminary calcification layer.

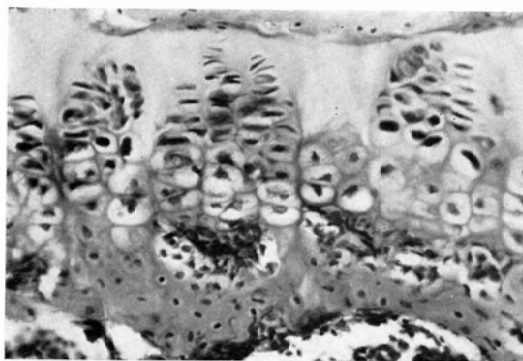


Fig. 8. Six months after 2,000 R irradiation(epiphysis only): Disorder and shortening of column-formed arrangement, bleeding, and poor formation of preliminary calcification layer.

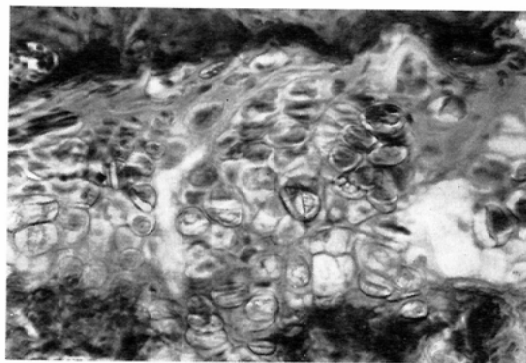


Fig. 9. Six months after 5,000 R irradiation (epiphysis only): Layers of cartilage cells are indistinguishable. Degeneration and necrosis of all cartilage cells. Remarkable necrotic changes of cartilage stromata.

irradiated. A few cases are shown in Fig. 6 to 10.

2. Effect of X-rays on the hind leg bone of mature rats:

1) Changes of the longitudinal length and transversal width of the tibia:

As for the changes of the longitudinal length of the tibia, no remarkable difference was noted among the control group and Group WB, E, and D by the irradiation of 1,000 R and 2,000 R, as shown in Fig. 11 and 12. In the irradiation with 5,000 R, however, a difference, slight as it was, was detected between

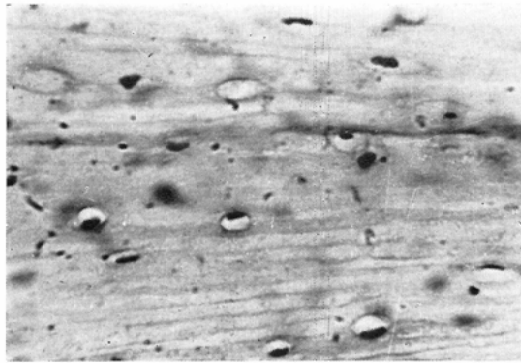


Fig. 10. Three months after 5,000 R irradiation (diaphysis only): Pycnosis of osteocytes, vacuolar degeneration and necrosis are visible.

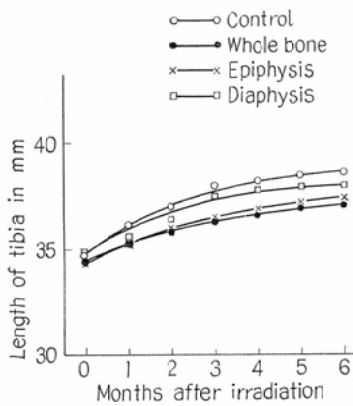


Fig. 11. Changes of the longitudinal length of tibia selectively irradiated with 1,000 R (Three-month-old Rats).

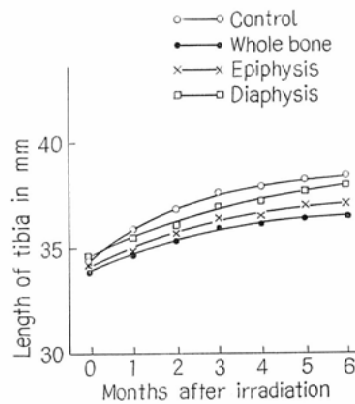


Fig. 12. Changes of the longitudinal length of tibia selectively irradiated with 2,000 R (Three-month-old Rats).

Group WB and the control group, while Group E and WB presented some retardation of growth, with the exception of Group D which was hardly differentiated from the control group.

The comparison with immature rats was made in respect of growth retardation by the irradiation of 5,000 R, as shown in Table 3, revealed the fact that mature rats suffered very little retardation of growth. Statistically speaking, the growth rate of immature rats in Group WB was 72.2%, while that of mature rats was 91.6%. In Group E, 83.0% in immature rats was contrasted with 94.2% in mature rats. In Group D, the corresponding percentages were 92.3% versus 98.1%.

Such was the case also with the transversal width of the diaphysis. Statistically speaking, immature rats accounted for 66.6% in Group WB, 84.4% in Group E, and 80.0% in Group D, while mature rats gave 90.7% in Group WB, 96.3% in Group E and 92.6% in Group D.

Table 3. Comparison of the growth rate (%) of tibia, between one- and three-month-old Rats six months after selective irradiation with 5,000 R.

	Dosis and Age Irrad. Regions	5,000 R	
		One-month-old Rats	Three-month-old Rats
Longitudinal Lengths	Whole bone	72.2	91.6
	Epiphysis	83.0	94.2
	Diaphysis	92.3	98.1
Transversal Widths	Whole bone	66.6	90.7
	Epiphysis	84.4	96.3
	Diaphysis	80.0	92.6

2) Radiographic appearances:

Diminishing of the longitudinal length and transversal width of the tibia was not remarkable in mature rats. Beside, mature fibula did not reveal any abnormality observed in the juvenile fibula, such as thinning, pencil like figure, and curvature of the tibia.

3) Histopathological findings:

Major pathologic changes were a slightly irregular arrangement of epiphyseal cartilage cells, a slight degeneration of cells of the proliferation layer, and the lowered stainability of cartilaginous stromata. These effects were obvious in Group WB and E, but less with lower dosage of X-rays. Very little change was observed in group D. Six months after the irradiation, there were some cases where epiphyseal cartilage cells were partially lost, with only stromata left. Generally the difference of mature rats from the control group was not so evident as that of immature rats.

3. Effect of the salivary gland hormone on the bony radiation injuries:

While irradiating X-rays on the left hind leg bone, a subcutaneous injection of parotin was made on the dorsum, and the effects on the longitudinal length of the tibia were studied. Including the group to which parotin was not injected, the following combinations were considered:

Non-injected rats	Right hind leg bone	Control	Group C.
Non-injected rats	Left hind leg bone	Only irradiation	Group X.
Injected rats	Right hind leg bone	Only parotin	Group P.
Injected rats	Left hind leg bone	Irradiation plus parotin	Group X + P.

The denominations of the above groups will be used in the following description.

1) Changes of the longitudinal length of tibia:

(a) Group irradiated with 1,000 R:

As shown in Fig. 14, no changes was noted among the groups one month after the irradiation, but Group X began to show the retarded growth of the longitudinal length of the tibia in three months. After six months, only Group X showed a slight retardation of growth, but Group P and X + P re-vea-

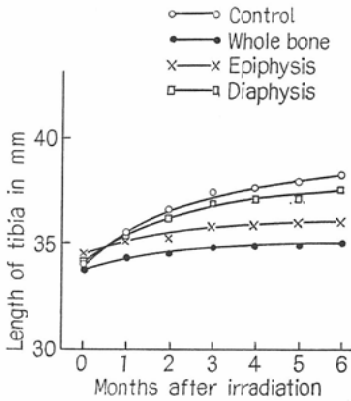


Fig. 13. Changes of the longitudinal length of tibia selectively irradiated with 5,000 R (Three-month-old Rats).

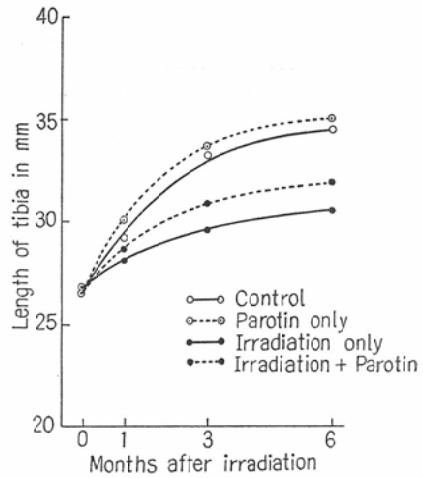


Fig. 15. Effect of Parotin on growth of the longitudinal length of tibia irradiated with 2,000 R.

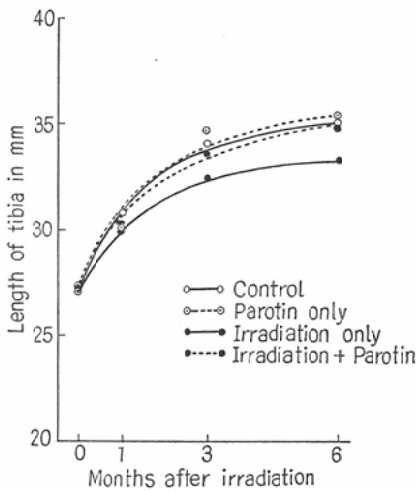


Fig. 14. Effect of Parotin on growth of the longitudinal length of tibia irradiated with 1,000 R.

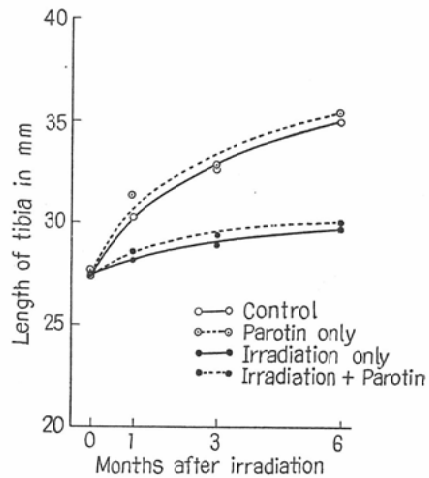


Fig. 16. Effect of Parotin on growth of the longitudinal length of tibia irradiated with 5,000 R.

led hardly and difference from Group C. The growth rates of different groups in six months are expressed by the following formula:

$$P \geq C \geq X + P > X$$

(b) Group irradiated with 2,000 R:

As shown in Fig. 15, Group C and P did not show any significant difference. Group X presented very little growth after the irradiation, while Group X + P gave a somewhat higher rate of growth than Group X. The rates of growth in six months are as follows:

$$P \geq C > X + P > X$$

(c) Group irradiated with 5,000 R:

As illustrated in Fig. 16, the development of the longitudinal length of the tibia formed a similar curve for Group C and P, while very little growth was noted after irradiation for Group X and X + P. The growth rates in six months are shown in the following:

$$P \geq C \gg X + P \doteq X$$

2) Radiographic appearances:

The tibia of the irradiated rats was found to be shorter, the bone cortex increased in radiolucency, and some cases showed even a slight curvature. Besides, the fibula was seen to be extremely thin, losing its proper curve and straightening itself like a wire.

In the cases medicated with parotin, the longitudinal length was elongated and the opacity of diaphyseal cortex was apparent, compared with the unmedicated groups. These observations were obtained not only in the tibia but also in the fibula. Moreover, such findings were observed to Group X + P irradiated with 1,000 R, but the pictures were blurred in the group irradiated with 2,000 R and absent in the group irradiated with 5,000 R. (Fig. 17).

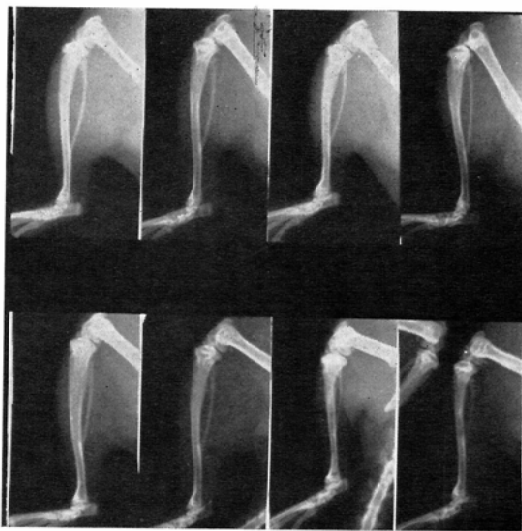


Fig. 17. Effect of Parotin on the recovery from radiation damage of bones.
Upper: three months after 1,000 R irradiation.
Lower: six months after 2,000 R irradiation.
(control, Parotin only, irradiation plus Parotin, from left to right)

3) Histopathological findings:

(a) Group P:

The formation of bone trabeculae in Group P was accelerated up to three months far more than Group C. Osteoblasts did not vary greatly in number, but were found enlarged in size. No large difference was noted from Group C in six months (Fig. 18.).

(b) Group irradiated with 1,000 R:

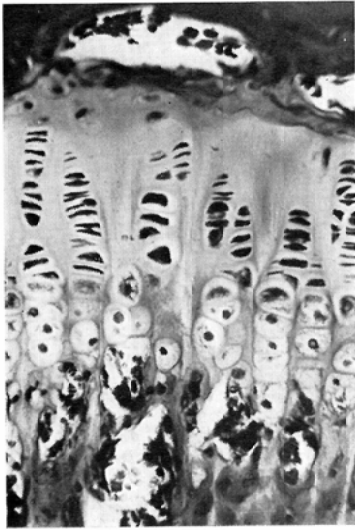


Fig. 18. Three months after administration of Parotin: Conspicuous univasion of vascular connective tissue. Hypertrophic [cells of proliferation layers.

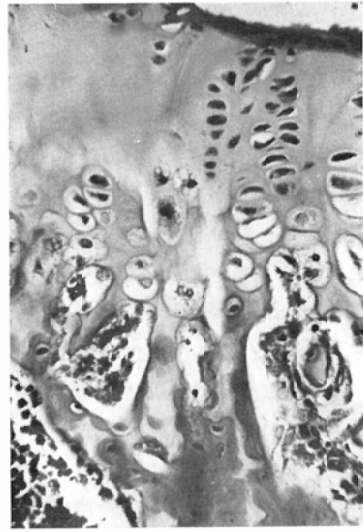


Fig. 19. Three months after 1,000 R irradiation (whole bone): Decrease in number of the cartilage cells, increase of stromata, remarkable disorder of column-formed arrangement.

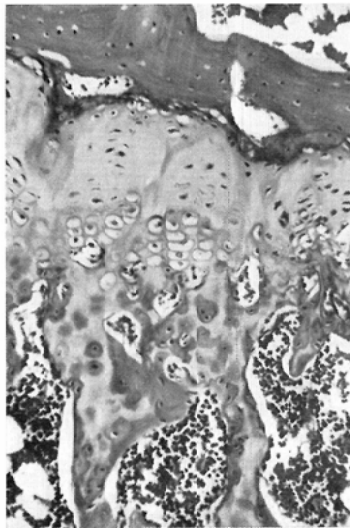


Fig. 20. Six months after 1,000 R irradiation (whole bone): Disorder of column-formed arrangement and bone trabeculae, and necrosis of cartilage cells.

Group X: One month after the irradiation, the cartilage cellular layer of the epiphysis is thinner than that of Group C, and the column-formed layer cells turned flat. Preliminary calcification layers were scanty and osteoblasts were decreased in number. After three months, the cartilage cellular layer was somewhat thicker than at one month and the cell itself appeared larger in size, but the number of cells dropped

to a small extent. The column-formed arrangement was a little disturbed and the number of osteoblasts was very small. After six months, the cartilage cellular layer was found to be considerably thick, but the column-formed arrangement was very irregular. Cellular necrosis was observed and the stromata were partially reduced in stainability (Fig. 19 and 20).

Group X + P: After one and three months, bone trabeculae were poor in this group compared with Group C, but better than Group X. The cartilage cellular layer was thick, and the column-formed arrangement was less irregular.

The disappearance and picnosis of the nuclei of proliferation layer cells were slight. Preliminary calcification layers developed, and the number of osteoblasts did not make a great difference from Group C. After six months, the irregularity of cartilage cell arrangement was much lower than Group X. The number and size of osteoblasts were large (Fig. 21 and 22).

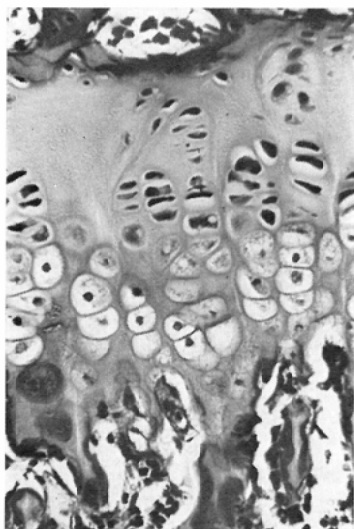


Fig. 21. Three months after administration of Parotin (whole bone irradiated with 1,000 R): Column-formed arrangement of the cases administrated with Parotin is more regular than that of the cases without Parotin, and the disappearance and necrosis of nuclei are reduced in number. A large numbers of osteoblasts are seen.

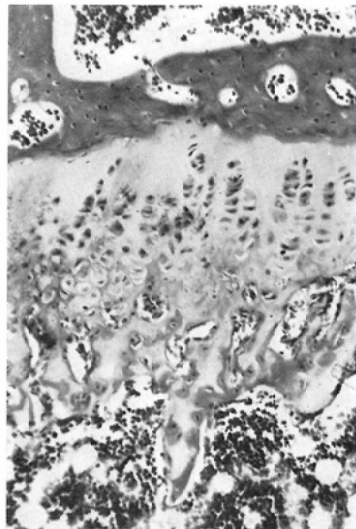


Fig. 22. Six months after administration of Parotin (whole bone irradiated with 1,000 R): An increased numbers of cartilage cells and osteoblasts, compared with the cases of irradiation only, are seen.

(c) Group irradiatee with 2,000 R:

Group X: One month after the irradiation, no great difference was made from the group irradiated with 1,000 R, and some animals exhibited thicker cartilage cell layers than the group irradiated with 1,000 R. The column-formed layer, in general, was shorter and preliminary calcification layers were thinner than Group C.

In spite of bone trabecular formation observed, the number of osteoblasts was small. After three months, foam cell layers were particularly thin, and proliferation layers grew thinner and irregular in arra-

ngement. Preliminary calcification layers were irregular, with the epiphyseal line disturbed. Osteoblasts degenerated. After six months, the stainability of cartilage cells reduced, and the arrangement of column-formed layers turned irregular, showing degenerated pictures. On account of the very poor formation of preliminary calcification layers, it looked like immediately transit to the bone trabeculae. The epiphyseal line was obscure (Fig. 23).

Group X + P: After one month, the cells of column-formed layer were picnotic and irregular in their arrangement, and the formation of preliminary calcification layers and bone trabeculae were hampered. After three months, cartilage cells were poor in stainability and irregular in column-formed arrangement. Formation of preliminary calcification layers was somewhat affected. The osteocytes of bone trabeculae were picnotic, and osteoblasts, partly detected, but were degenerated.

After six months, the epiphyseal line was clear; the irregularity of the column-formed arrangement of cartilage cell layers and the picnosis of cells were observed, though to a slighter degree than at three months. Osteoblasts were found to be large (Fig. 24).

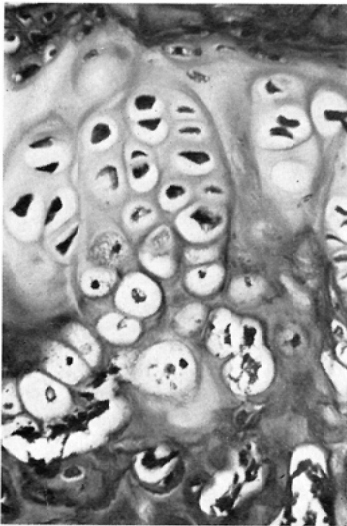


Fig. 23. Three months after 2,000 R irradiation (whole bone): Numerical decrease of cartilage cells, irregular preliminary calcification layers, and deranged epiphyseal line are seen.

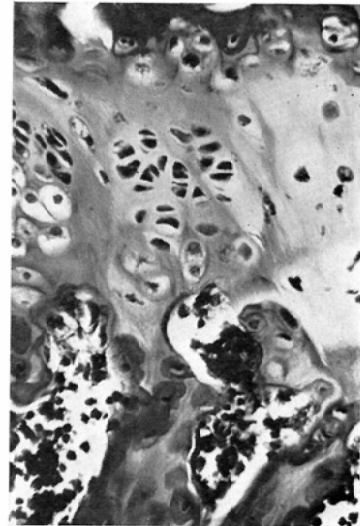


Fig. 24. Three months after administration of Parotin (whole bone irradiated with 2,000 R): Disorder of column-formed arrangement and malformation of preliminary calcification layers are seen.

(d) Group irradiated with 5,000 R:

Group X: One month after the irradiation, cartilage cell layers were thin and the column-formed arrangement was extremely disordered. All cartilage cells were swollen, and these nuclei were picnotic or necrotic. The osteocytes were intensely atrophied, and osteoblasts were thinned and degenerated. After three months, cartilage cell layers were thin and the column-formed arrangement was so disturbed that it partially contained mosaic structures. The stainability of cartilage cells reduced, with preliminary calcification layers narrowed in width, and it looked like transit to the osteoid tissues. After six months,

layers of cartilage cells were not distinguishable from one another. The stainability of cartilage cells sharply declined, and the nuclei were necrotic. The circumference of cartilage changed into osteoid tissue. Preliminary calcification layers were almost indistinct, cartilage cell layers shifting immediately to bone trabeculae. The stainability of osteocytes was enormously reduced (Fig. 25).

Group X + P: After one month, the degeneration of cartilage cells was intense, with the cells of column-formed layer destroyed except a small amount of remainder. Formation of preliminary calcification layer was poor. The cell picnosis of bone trabeculae was somewhat slighter than Group X. Osteo-

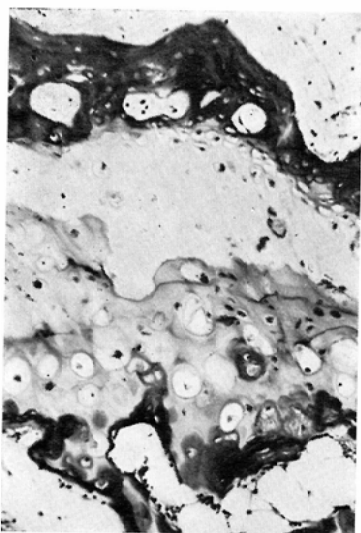


Fig. 25. Six months after 5,000 R irradiation (whole bone): Remarkable drop in stainability and necrotic cartilage cells are seen. Each layer is indistinguishable. Preliminary calcification layers are mostly lost.



Fig. 26. Six months after administration of Parotin (whole bone irradiated with 5,000 R): Degree of the damages are mostly similar to those in Fig. 25.

blasts were thinned and degenerated. After three months, cartilage cell layers grew fairly thick, but calcification was observed in the presence of column-formed layers. The number of bone trabeculae was very small, but osteoblasts a little increased in number and size. After six months, the stainability of cartilage cells dropped lower than at three months, and the stromata which had been calcified in the place of column-formed layers was found to be necrotic. Bone trabeculae were hardly formed (Fig. 26).

IV. Discussion

1. Irradiation dosage and growth of bones:

Recently Phillips and Kimeldorf¹⁴⁾ has clarified, in pursuit of the relation between age and dosage of irradiation, by mathematical formulations which give a quantitative analysis, that growth retardation was intensified in the case of the younger bones and the larger dosage.

In the present experiment, the larger retardation of bone growth under the higher levels of irradiation has been verified for juvenile bones. In particular, the growth of the longitudinal length of the tibia seems

to have been affected to a considerable extent. Also the transversal width of the diaphysis suffered noticeable damage from irradiation, resulting in a general growth retardation of bones affected.

Iwagiri¹⁵⁾ has stated that the changes caused by irradiation are a retarded growth in the longitudinal length of the tibia and a dwindling in size of the diaphysis. Caneghem et al.¹⁶⁾ have reported that bone shortenings are not observed in chickens if the dosage is under 800 R, but that a slight damage is noticed with 1200 R and the damage increases in proportion to the increase of dosage, and that the lowest limit of irradiation damage is estimated at 800 R. Kasai⁹⁾ has set the same limit in 600 R as the result of his experiment on new-born rats. These reports all concern observations carried out for 1–2 months after their radiation. In contrast to the preceding tests, the present experiment was based on a prolonged observation as long as six-months, and it has been known that bone shortening is effected even with 1,000 R. It is likely therefore, that the lowest limit may be lower.

2. Regions of irradiation and bone growth:

As in the foregoing description, both longitudinal length and transversal width were reduced remarkably by WB-irradiation. UnByder E- or D-irradiation, the dwindling of tibia as well as partial diminishing in dimensions of fibula appeared corresponding to the regions of irradiation. These findings are similar to those reported by Rubin et al.¹⁷⁾

Brooks and Hillstrom,¹⁸⁾ Bisgard and Hunt¹⁹⁾ maintain that bone growth is stopped by irradiation of 1,500 R on the epiphysis. Likewise, Caneghem et al.¹⁶⁾ have reported that irradiation with 2,000 R on the epiphysis produces a similar result.

In recent years, an increasing variety of the studies has been made in the medical application of RI, resulting in copious achievements in the investigation of the so-called "bone seeker" or "cartilage seeker". It has been confirmed by autoradiography that the cartilage seeker localize in the epiphysis and that the bone seeker localize in the metaphysis. The present research has also clarified that irradiation damage on the bones is more conspicuous in epiphysis than in diaphysis. This view suggests that endochondral bone formation taking place in the epiphysis is more sensitive to irradiation than osteoblastic osteogenesis.

3. Irradiation damage versus age:

In the present research, a comparative study was made between juvenile rats one month old and somewhat mature rats three months old, with the result that the three-month-old rats were affected less irrespective of the method of irradiation.

In practice, Montag⁷⁾ has reported an instance of a child whose right forearm bone was deformed, shortened and atrophied by X-irradiation with 440 R.

Mature bones are not necessarily unaffected as reported by Akimoto²⁰⁾ who observed a slight shortening of the longitudinal length of femur and tibia in the group of rabbits whose hind leg bones were frequently subjected to irradiation. On the other hand, Takagishi²¹⁾ has reported a case of uterine cancer, in whom a metastasis in bones was suspected on completion of radiation treatment on the uterus, followed by a large-dosed irradiation on the pelvis, which resulted two and half years later in a fracture of the femoral neck. Similar cases have been noted in the form of fractures of the rib and clavicle caused by the radiation therapy for breast cancer²²⁾⁻²⁶⁾.

The mechanism of genesis of radiation damage to the bone is a very controversial problem. Dahl²⁷⁾

proposes that decalcification of epiphysis and metaphysis may be the ultimate cause of subsequent bone deformity, bone thinning, necrosis and even spontaneous fractures. Furthermore, some researcher lays a stress on the disturbance of blood vessels, supposing that blood vessel disturbance may fortify the mechanism²⁸⁾.

4. Histological findings:

Without distinction of the dosage and region of irradiation, age, and the number of days after irradiation, the histological pictures obtained are essentially the same, accompanied by such pathologic changes, to varying degrees, as the degeneration and necrosis of cartilage cells, the hyalinic degeneration and necrosis of the cartilaginous stromata, the decrease or vanishment of osteoblasts, the degeneration of diaphyseal cortex and osteocytes, and congestion in the capillaries. Generally speaking, the pathologic changes are most intensified by WB-irradiation, while epiphysis and diaphysis are strongly affected by E-and D-irradiation respectively. With 1,000 R irradiation, recovery of roughly normal pictures is apparent in six months after irradiation, but no recovery is observed if the dosage is raised to 2,000 R or higher. Juvenile bones are affected more remarkable than mature bones.

5. Damage- preventive and therapeutic effects of parotin:

Retardation caused by irradiation in the growth of bones has been confirmed by the present study and its predecessors. Meanwhile, some experiments have been attempted to develop the preventive means for radiation damage done to the bones. Yamaguchi²⁹⁾, one of the researchers in this field, carried out X-ray irradiation ranging from 50 R to 1,000 R on the hind leg bones of rats. Prior to the irradiation, he injected subcutaneously dl- cis-2-amino-cyclohexanthiol (ACT cis) or dl-trans-2-amino-cyclohexanthiol (ACT trans), both credited to prevent radiation damage, with the result that the medicated group suffered less radiation damage than the unmedicated group. A similar method was adopted by Ebisuda³⁰⁾, who conducted a study of the effects of combined uses of ACT and VK, ACT and VB₁₂ etc. In addition, Irie et al³¹⁾ have reported that radiation damage to the bone is alleviated by the administration (through injection) of mercaptoethylguanidine 1/2 H₂SO₄ (MEG 1/2 H₂SO₄) previous to irradiation.

The above ACT, MEG, VK and VB₁₂ are the agents considered to be capable of reducing the mortality of irradiated animals if they are administered prior to irradiation. In addition, a few other hormones like ACTH and thyroxin are regarded effective to prevent radiation damage.

Parotin used in the present experiment is one of the hormones, but its potency as preventive of radiation damage has not been established. This agent essentially exerts an influence directly or via the pituitary system on the cartilage cells of the epiphysis, accelerating the growth and development of bones. For this reason, this agent was tested to know whether it had a therapeutic effect on the radiation damage of bone.

As far as the results of this experiment are concerned, it is confirmed that the administration of parotin somewhat accelerated the growth of legs on the non-irradiated side as indexed by the longitudinal length of the tibia, and that it greatly alleviated the damage to the legs on the irradiated side. This favorable action of the agent is endorsed histologically, as the parotin-injected group showed, even on the irradiated legs, only a slight necrosis of bone trabeculae and a regular column-formed arrangement of cartilage cells of the epiphysis.

It is generally noted that the tissue characterized by active cell-division is highly susceptible to radia-

tion damage but it recovers rapidly from the damage. This recovery is generally considered to be caused by the replacement of damaged cells or tissues with undamaged cells or tissues.

The experimental object of this research is immature bones, which are vigorously great metabolic tissue cells, however, affected only a little by irradiation of a comparatively low dose. It is inferred that parotin have a favorable effect on the tissues and cells which have got a relative slight damage of radiation and they serve to alleviate the general damage. This seems to indicate an advisable use of parotin at X-ray treatment of infants for the purpose of lessening a disturbance of bone growth that will occur at a late stage.

V. Summary

In the hind limbs of the young and adult rats, 3 methods of irradiation were applied, to the whole hind limbs, the epiphysis alone, and the diaphysis alone, using single irradiation of 1,000 R, 2,000 R and 5,000 R respectively. The course of the appearance of radiation disturbance in the bone was studied over a 6 month period after irradiation with measurement and interpretation of X-ray picture and histopathological picture. Following results were obtained.

1. The radiation disturbance in the bone was intensified in the case of the younger bone and the larger dosage. The disturbance was most intense when the hind limb was irradiated as the whole, followed by irradiation on the epiphysis alone, and further by irradiation on the diaphysis alone.

2. The lower limit of the dose to cause the disturbance appeared to be less than 1,000 R.

3. Subcutaneous injection of salivary gland hormone (parotin) twice a week after the irradiation alleviated the developmental defect of the bone due to the irradiation of 1,000 R, as demonstrated by measurement of X-ray picture and histological finding. When the dose was over 2,000 R, the disturbance was so intense that no effect of parotin was evident.

Acknowledgement

Grateful acknowledgement is made to Prof. S. Tarusawa for his constant interest and guidance in this investigation. The author also wishes to thank the Director of 2nd Department of Pathology, Prof. S. Katsura, and the Director of Orthopedic Surgery, Prof. T. Igari for the valuable suggestions and advice.

(This work was presented at 19th, 20th and 25th General Meeting, Japan Radiological Society.)

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