Acute Death of Mice during Massive Gamma Irradiation-III

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大線量照射によるマウスの致死効果（第3報）

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^{60}Co γ線をＩＣＲマウス（雌）に短時間大線量照射を行なつて，その急性致死効果を心電図法により観察した．照射線量率と照射中致死に要する全線量との関係を10 R/min から40 kR/min の範囲にわたつて調べ，中間線量率域では線量率が低下すれば照射中致死線量もより少くてすむ結果を得た．また照射中致死に要した時間とその全線量の間には，log-log グラフ上でほぼ直線的関係を得たが，上記と関連して線量率依存性が若干あることを認めた．

フェノバルビタールが致死を遅らしめるのに有効であることはすでに報告したが，2.5 mgを照射約10分前に腹腔内に注射した結果，40kR/minの線量率照射において，照射中致死に要する線量の比は2.6倍であつた．

心電図は照射開始前から死の確認に至るまで連続観察を行ない，先に藤井[3]が報告した“初期動揺”（照射開始後短時間内に始まる心電図基線の著しい動揺で，ある時間後平静に復し心電図形も見かけ上元に戻つた状態になる現象）について，静まるまでの時間からこの間に受けた照射線量を求めた．線量率にかかわりなく1.8 kR を得たが，これは平均生存時間-線量曲線における3.5日効果を与える線量で，初期動揺が終つた時点でもはや再生しえないことを再確認した．バルビタールの麻酔効果は，この初期動揺の遷延をももたらすが，その程度は全く照射中致死における延引の割合に等しい結果であつた．

心電図上における心搏と全身痙攣時の筋電パルス数を測定した．心搏パルス数は一定間隔毎に，照射開始から致死までを見ると全体に増加の傾向があり，バルビタール注射においてもほぼ同様であつた．筋電パルスは照射中異なる時期での痙攣についても，強度の差はあれ，パルス数には一定傾向はなかつた．

一定線量照射後の平均生存時間については既報同様指数関係を認めたが，線量率によつては余り差がないという結果であつた．

Introduction

Considering the types of radiation death following massive gamma irradiation, acute death is of special interest. Some of the reports on this subject have been made by W. Langham et al.[7] (1956), J.B. Storer et al.[9] (1957), H.L. Andrews[2] (1958) and R.G. Allen et al.[1] (1960), but there have been no studies so far on the intrinsic nature of acute death.

Takeshita and Fujii[10] (1963) observed electrocardiograms of mice until death during irradiation at an exposure rate of 8 kR/min, and documented a logarithmic correlation between mean survival time after irradiation and the exposures ranging from 16 kR to 100 kR. Detailed histologic study[4] (1963) of several organs of these mice revealed marked hyperemia.

Fujii[3] (1965) reported observations at exposure rates of 15 kR/min and 30 kR/min. A marked fluctuation of the baseline noted on electrocardiograms immediately after initiating irradiation was called "initial pitching", and the average dose delivered by the time of disappearance of this fluctuation was considered to relate to the 3.5 day effect of B. Rajewsky[8] (1955).

In these studies acute radiation death was accompanied by tonic convulsions, but the mechanism thereof is still entirely unknown. Fujii stated that the logarithmic shortening of mean survival time after irradiation by dose was similar to an amplified oscillation phenomenon with feedback and due to increased stress to an irreversible state, which ultimately led to death. Now if this concept were applied to the death which occurred during massive irradiation and such an accelerating factor were responsible, a dose rate dependency can be anticipated.

The present study was performed to determine the dose rate effects for death during irradiation, including lower exposure rate irradiation, and to observe the electrocardiographic changes at exposure rates higher than those previously reported[10][3].

Methods

ICR-JCL female mice over 7 weeks of age, obtained from Nippon CLEA K. K., were used. The animals were irradiated at high exposure rates in the ^{60}Co Gamma-ray Irradiation Facility of the Japan Atomic Energy Research Institute (JAERI), Tokai -Establishment. Irradiation of the animals at lower exposure rates was performed using the telecobalt unit at Hiroshima University Research Institute for Nuclear Medicine and Biology. Exposure rates used in the experiments are shown in Table 1.

The sources referred to in this table are basket-type radiation sources for high exposure rates, with radiocobalt containers arranged cylindrically. Exposure rates inside and outside the source were provided

Table 1. Cobalt-60 gamma-ray exposure rates

Date	Location	Source (kCi)	Exposure rate	
			Inside the source (kR/min)	Outside the source (kR/min)
Nov. 1966— Oct. 1967	JAERI	16	22	4.4 at 15 cm— 0.16 at 80 cm
Dec. 1967	Hiroshima Univ.	2*	—	0.1 at 41 cm— 0.01 at 1.3 m
Jun. & Nov. 1968	JAERI	15**	40	5.7 at 15 cm— 0.52 at 60 cm

*In the telecobalt unit **Newly installed in 1968

by the JAERI Facility but at short distances from the source some corrections were necessary due to geometrical considerations.

To determine the time until death during irradiation inside the source, mice were affixed to electrocardiographic plates of our special design previously described[10]. Continuous electrocardiograms in lead I were obtained from the beginning of irradiation. The time of appearance of tonic convulsions electromyographically and on electrocardiograms indicated the time of death. These tracings were used in the analyses of initial pitching, heart rate and electromyographic frequency. For irradiation outside the source, mice were placed in individual lucite cases at distances from 15 cm to 80 cm. Each animal's death was ascertained by periscope. For irradiation at lower exposure rates, mice were placed in a special cage in an air-conditioned room and given sufficient solid feed and water. Radiation was administered continuously until death.

Mice were irradiated in the range of 10 R/min to 40 kR/min. Time until death during irradiation and the total exposure were recorded for each group.

For irradiation inside the source, groups of 5 mice, each fixed to an electrocardiographic plate, were protected by a lead shield in the cave prior to irradiation. Their maximum pre-exposure did not exceed 80 R. For irradiation outside the source, the most proximal animal was estimated to have received a pre-exposure of about 200 R. In each case, these pre-exposures were considered negligible compared with the massive doses they eventually received.

To control convulsions, 5 mg phenobarbital, the LD_{50} for acute toxicity, and a half dose of it were injected intraperitoneally about 10 to 20 minutes before irradiation. Chemical agents such as 5-hydroxytryptamine (5-HT) and 2-β-aminoethylisothiourea (AET) were given to observe their protection against radiation in doses double those normally used.

Mean survival time following irradiation was calculated from the midpoint of radiation exposure, for each mouse received the dose at rates of 0.44, 2.8 and 22 kR/min. These techniques were essentially the same as reported previously[10].

Results

1. Total exposure and time until death during irradiation

Results are shown as untreated groups at exposure rates of 3.1, 22 and 40 kR/min in Table 2. The total exposures of 110 kR at 3.1 and 22 kR/min were in good agreement with our previously reported data[10], but that at 40 kR/min was lower than expected from these data.

Results for the groups irradiated outside the source are summarized in Figures 1 and 2. Figure 1 shows the correlation between the exposure rate and time until death during irradiation; while Figure 2 shows the relation between the exposure rate and the total exposure until death during irradiation. As shown in Figure 1, there is a linear relation on a log-log graph except for two groups at the highest and the lowest exposure rates in the region used. Grossly this linear relation has a gradient of -0.85, and there may be a dose rate dependency, considering the fact that it was not -1.

In Figure 2, a considerable difference is noted in the total exposure until death during irradiation for rates of 0.1 kR/min to 6 kR/min, and that is a decreasing trend of the total exposures at lower rates. Low values were obtained in the total expsoure at the rates higher than 10 kR/min. These were irradiated in-

Table 2. Total exposure and time until death during irradiation

Exposure rate and drugs (mg)	Number of mice	Time until death during irradiation (min)	Total exposure (kR)
3.1 kR/min			
Untreated group	20	36 ± 4	110 ± 10
Phenobarbital (5)	10	59 ± 5	180 ± 10
5-HT (2)	5	38 ± 4	120 ± 10
AET (8)	5	33 ± 2	100 ± 10
22 kR/min			
Untreated group	14	5.1 ± 0.8	110 ± 20
Phenobarbital (5)	10	9.8 ± 0.3	220 ± 10
Phenobarbital (2.5)	10	8.0 ± 1.3	180 ± 30
5-HT (2)	5	5.0 ± 0.5	110 ± 10
AET (8)	5	5.1 ± 0.3	110 ± 10
40 kR/min			
Untreated group	34	1.9 ± 0.5	76 ± 20
Phenobarbital (2.5)	10	5.0 ± 0.4	200 ± 20

Fig. 1. Relation between the exposure rate and time until death during irradiation.

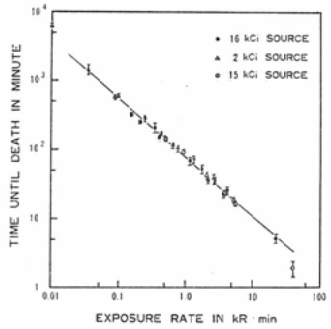
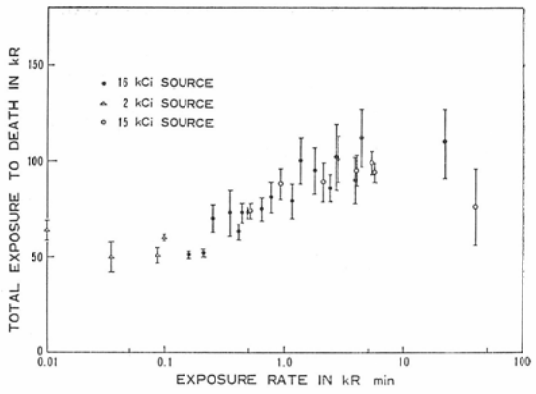


Fig. 2. Relation between the exposure rate and the total exposure until death during irradiation.



side the source and may not be comparable to those groups irradiated outside the source for their exposure conditions.

The effects of anticonvulsives and some chemical radiation protection agents are shown in Table 2, and evaluated by the ratio of time until death during irradiation, compared with untreated groups. Preliminarily injecting 5 mg phenobarbital per mouse, ratios of 1.6 at an exposure rate of 3.1 kR/min and 2.0 at 22 kR/min were obtained; while ratios were 1.6 at 22 kR/min and 2.6 at 40 kR/min respectively with a dose of 2.5 mg which was enough to anesthetize and in safety. Phenobarbital was generally ineffective at total exposures of 220 kR or more which indicated that extension of time until death during irradiation by the effect of anesthesis could not be expected beyond these exposures. No effects were demonstrated with 5-HT or AET.

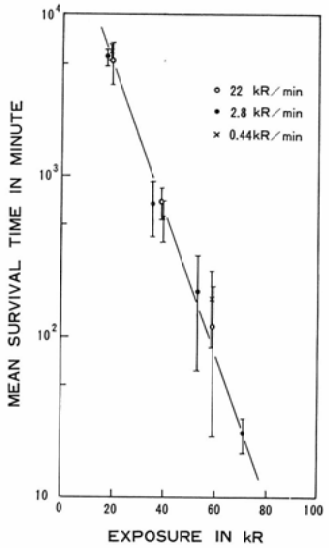
2. Mean survival time following irradiation

Mean survival times of mice in groups of 10, irradiated from 17 kR to 71 kR at rates of 0.44, 2.8 and 22 kR/min are shown in Figure 3. Since no significant differences were noted among these groups their data were combined and plotted. Mean survival time satisfies the equation

$$\text{M.S.T. (min)} = Ce^{-KD} \qquad (1)$$

where C is 40,000 min and D is in kR units, $K = 0.102 \pm 0.008$.

Fig. 3. Mean survival time following irradiation at 17 kR to 71 kR at rates of 0.44, 2.8 and 22 kR/min.



3. Electrocardiographic observations

3.1. Initial pitching

Initial pitching on electrocardiograms immediately after starting irradiation was confirmed, and is shown in Table 3. The patterns of individual differences of this phenomenon are shown in Figure 4.

The total exposure delivered until disappearance of initial pitching was 18 kR, in agreement with results of Fujii[8]. Initial pitching was noted in all animals irradiated at 40 kR/min following the injection of 2.5 mg phenobarbital, but was late, indicating an extension of time until death as the effect of the drug.

Initial pitching began after starting the irradiation at from 6 kR to 8 kR (Table 4), but the onset time and dose were diverse as shown in Figure 4. The pitching was vigorous at onset, and gradually became

Table 3. Total exposure and time until disappearance of initial pitching on electrocardiograms

Exposure rate (kR/min)	Number of cases	Time until disappearance of initial pitching (sec)	Total exposure (kR)
22	14	50 ± 10	18 ± 4
40	34	26 ± 4	18 ± 3
22*	10	68 ± 16	25 ± 6
40*	7	41 ± 5	27 ± 3

*Following the administration of 2.5 mg phenobarbital

Fig. 4. Initial pitching on electrocardiograms during irradiation at a rate of 40 kR/min; the arrow indicates faint convulsions (No. 24).

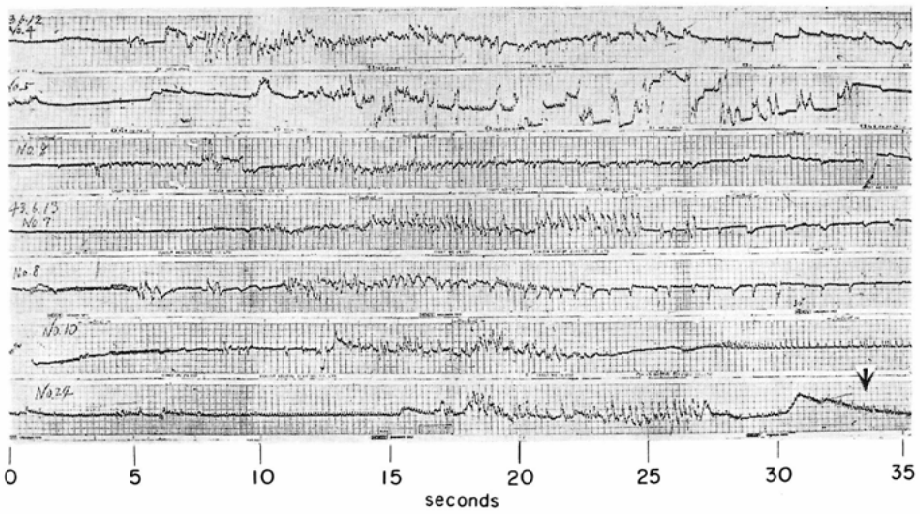


Table 4. Comparison of onset of initial pitching on electrocardiograms

Exposure rate (kR/min)	Onset time of initial pitching (sec)	Exposure until onset (kR)
22	17 ± 10	6.2 ± 3.7
40	13 ± 4	8.7 ± 2.5

cyclic, repeated and intermittent, and eventually returned to a stable pattern or nearly so. This was clearly compared with electromyographic patterns that appeared at the time of convulsions as shown with the arrow in the case of No. 24.

3.2. Heart rate

Figure 5 shows the heart rate from the beginning of exposure to death during irradiation at rates of 22 kR/min and 40 kR/min. Cases with temporary decrease in heart rate at the beginning of irradiation are shown by dotted lines, and they were about 40 per cent of the total. They were observed to have less chance for subsequent recovery to their initial heart rates. The majority of the cases had increased heart rates when irradiation was begun.

All cases showed a marked decrease in heart rate following convulsions and, depending on their severity, they led directly to death.

In Figures 6a and 6b are shown heart rates for the groups irradiated at 22 kR/min and 40 kR/min following the administration of 2.5 mg phenobarbital, including cases of 5 mg injected group. Some showed a temporary decrease in heart rate with irradiation, but the general tendency for it to increase with irradiation occurred as in the untreated cases.

3.3. Electromyographic frequency

The electromyographic frequency was obtained by microscopic observations of tonic convulsions immediately before death and several repeated faint convulsions during irradiation, which were recorded on

Fig. 5. Heart rate until death during irradiation at rates of 22 kR/min and 40 kR/min.

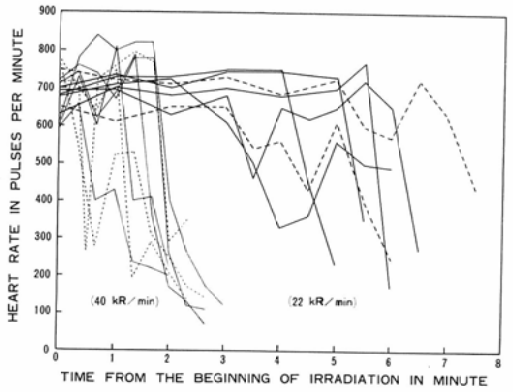


Fig. 6a. Heart rate until death during irradiation at 22 kR/min with corresponding values after phenobarbital.

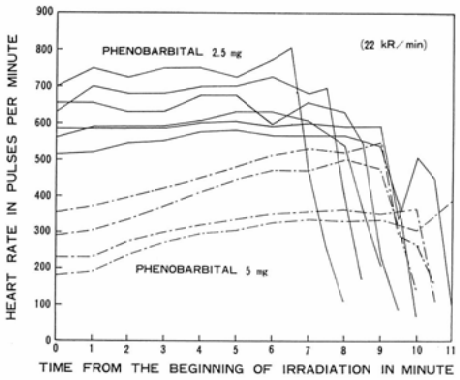


Fig. 6b. Heart rate until death during irradiation at 40 kR/min following phenobarbital.

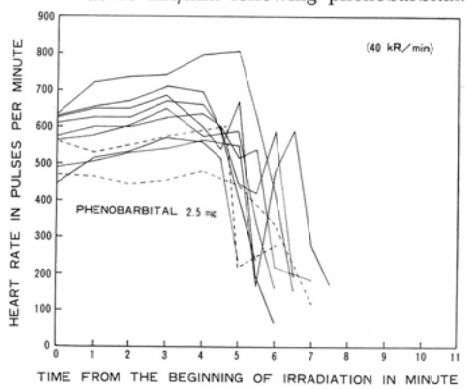
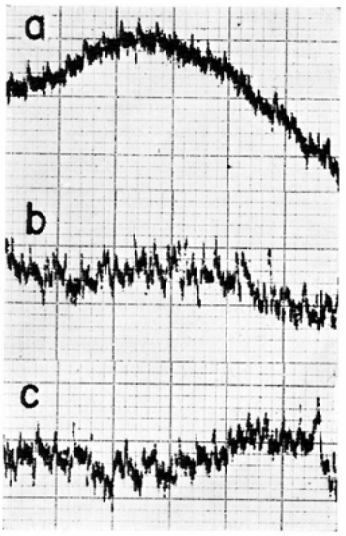


Fig. 7. Electromyographic frequency due to several repeated faint convulsions and tonic convulsions immediately before death during irradiation: (a) at 0.6 min after, 24 kR, 175 ± 12 cps; (b) at 1.2 min after, 48 kR, 155 ±16 cps; and (c) at 1.8 min after, 72 kR, 142 ± 8 cps.



electrocardiograms.

With irradiation at 40 kR/min, convulsions usually lasted 1 to 3 seconds except for that immediately before death. The frequency during a single episode of convulsions was counted in 0.24 second segments.

They were shown as a typical case in Figure 7, but did not demonstrate any definite tedency however. The average of these frequencies was 170 ± 13 cycles per second in the groups irradiated at 40 kR/min.

Discussion

Langham et al.[7] discussed the acute symptoms of radiation damage in a comprehensive study using total exposure of 1 kR to 200 kR at rates of 1.3 kR/min to 7.5 kR/min. Storer et al.[9] reported that the log of mean survival time (Y) in the range of 10 kR to 150 kR (X) can be expressed as $Y = a + bX$. Experiments supporting this have been performed on monkeys by Allen et al.[1], and Andrews[2].

Takeshita and Fujii[10] also determined the pattern of acute death which occurred during irradiation by observing electrocardiograms. They demonstrated that 111 ± 4 kR was the total exposure in mice either to the head or the whole body at the rate of 8 kR/min, and that mean survival time after irradiation was related to the exposure as mentioned above. Detailed histologic examinations of Fujii and Takeshita[4] (1963) revealed hyperemia and small hemorrhagic foci in numerous organs, but did not determine the direct cause of death. Later, Kurosawa et al.[6] (1966) using guinea pigs and Kido[5] (1967) using dogs reported nearly the same results by histologic examinations following irradiation of up to 30 kR.

Fujii[3] extended his studies to the irradiation of mice at high exposure rates of 15 kR/min and 30 kR/min. Total exposure until death during irradiation and mean survival time after irradiation were studied. One hundred and fifty kR appeared to be the limit of the exposure leading to death when the animals were treated with chlorpromazine. His findings of initial pitching on electrocardiograms were instrumental in linking the areas of intestinal death and cerebral death, considering the relation between mean survival time after irradiation and dose in association with the 3.5 day effect.

In the present study, with the exposure rate of 40 kR/min, death seemed to have been produced earlier than expected from results at 22 kR/min. If any exogenous factor were responsible, it may have been the relatively high temperature in the cave, especially during the warm season in which the study was conducted. In the group injected with phenobarbital, however, death during irradiation was marekdly delayed and the total exposure delivered as high as 200 kR, the highest yet observed injecting 2.5 mg of it. The aforementioned effect of temperature may therefore have been negligible.

Drugs of 5-HT and AET were administered in amounts double those usually used to protect against sublethal exposures, but they were found to be ineffective against the acute effects produced in the present study.

Acute death within 3 or 4 days after irradiation has not been found to differ markedly among certain strains of mice. In the equation (1) to calculate mean survival time following irradiation, C is the gross extrapolation of respective averages to zero, and K is the linear portion of the curve through this point on a semilog graph. These factors were in good agreement with previously reported values[10].

The cause of initial pitching on electrocardiograms is unknown. Occasionally, mild convulsions were superimposed on this fluctuation of the baseline, but initial pitching was essentially different from the convulsions as shown in Figure 4. It may be due to marked movements of the body, which were not detectable on electrocardiograms before irradiation. Considering the exposure delivered and the time until onset of the pitching, a cause for the pitching phenomenon could not be demonstrated. The total exposure at the disappearance of initial pitching was 18 ± 3 kR at rates of both 22 kR/min and 40 kR/min.

The pattern of electrocardiographic pulses following recovery of the pitching appeared the same as prior to irradiation or showed only slight changes in which the spikes appeared mildly increased upward (R) or downward (Q or S). Similar fluctuations were also seen in anesthetized mice, extended in time and disappeared later in proportion to the delay of their death. This suggests that changes in electrical potential are amplified and repeated until the development of some essential physiological change or extreme damage, and are completely irreversible in ordinary situations.

Heart rate usually increased with irradiation and changed with time after faint convulsions. In some mice irradiated at 40 kR/min, heart rate rapidly decreased and this continued without recovery to their normal rates, and tonic convulsions occurred and they had shorter survival times during irradiation.

Suppression of initial pitching and tonic convulsions before death seems to be more directly associated with decreased heart rate produced by anesthesia, which may be related with a state of hypoxia, and to be effective in delaying death during irradiation.

In the groups irradiated at a rate of 22 kR/min following various doses of phenobarbital, some differences were shown between the degree of anesthesia and the delay of death.

Changes in heart rate are not felt to be essential in the study of acute death; the shape of electrocardiographic pulses may be more important. These tracings were obtained only in lead I: therefore, the analysis of cause of death was inconclusive.

The electromyographic frequency did not show any trend with laps of time during irradiation except tonic convulsions at the terminal stage.

This is a general summary of the findings obtained from the series of investigations to date. A number of phenomena were brought to light, but it was not possible to determine the mechanism of acute death. There were many restrictions posed by the isolated structure for ^{60}Co gamma irradiation for high exposure rates, i.e. the size of the animals irradiated, the number of mice, and pre- and post-treatment procedures. Since a more powerful gamma-ray source is not yet available, we will pursue these studies relative to the effects of irradiation localized to body parts, using other high energy radiation apparatus.

Summary

The effects of massive doses of rapidly delivered ^{60}Co gamma-rays on mice were studied. The relation between the exposure rate and the total exposure leading to death during irradiation was established in the range of 10 R/min to 40 kR/min. Delay of acute death was demonstrated using phenobarbital injections. Analysis of electrocardiograms of mice from the beginning of irradiation until their death, revealed a pitching phenomenon at the electrocardiogram baseline during the early period of irradiation. The change in heart rate and electromyographic frequency in convulsions during irradiation were measured. Mean survival time of mice irradiated was also determined and expressed as an exponential function of the given dose.

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References

1) Allen, R.G., Brown F.A., Logie, L.C., Rovner, D.R., Wilson, S.G. Jr. and Zellmer, R.W.: Radiat. Res. 12: 532–559, 1960.
2) Andrews, H.L.: Radiat. Res. 9: 469–477, 1958.
3) Fujii, K.: Nipp. Act. Radiol. 25: 30–46, 1965.
4) Fujii, K. and Takeshita, K.: Nipp. Act. Radiol. 23: 300–307, 1963.
5) Kido, C.: Nipp. Act. Radiol. 27: 68–86, 1967.
6) Kurosawa, H., Moriya, H., Takanashi, S. and Agari, N.: Nipp. Act Radiol. 26: 454–457 and 1173–1179, 1966.
7) Langham, W., Woodward, K.T., Rothermel S.M., Harris, P.S., Lushbaugh C.C. and Storer, J.B.: Radiat. Res. 5: 404–432, 1956.
8) Rajewsky, B.: Radiation death in mammals, Radiobiology Symposium 1954, edited by Z.M. Bacq and P. Alexander (New York: Academic Press), 1955.
9) Storer, J.B., Harris P.S., Furchner, J.E. and Langham, W.H.: Radiat. Res. 6: 188–288, 1957.
10) Takeshita, K. and Fujii, K.: Nipp. Act. Radiol. 23: 40–47, 1963.