OUKA
Osaka University Knowledge Archive

Title	The effect of divided X-ray irradiations upon the percent survival and organ weights of mice
Author(s)	上野, 陽里
Citation	日本医学放射線学会雑誌. 1966, 26(4), p. 356-361
Version Type	VoR
URL	https://hdl.handle.net/11094/17160
rights	
Note	

Osaka University Knowledge Archive : OUKA

https://ir.library.osaka-u.ac.jp/

Osaka University

The effect of divided X-ray irradiations upon the percent survival and organ weights of mice*

Yori UENO

Department of Experimental Radiology (Director: Professor T. Sugahara), Faculty of Medicine, Kyoto University, Kyoto

マウス生存率および臓器重量におよぼす二分割X線照射の影響

京都大学医学部放射能基礎医学教室（主任：菅原努教授）

上野陽里

放射線照射をうけた生体の放射線感受性は，照射直後にいちぢるしく増強された後，時間とともに振動しながら正常の放射線感受性に戻る．このことは生存率を放射線感受性の示標とすることによつて認められている．本実験は，このような放射線感受性の照射後変化が，臓器重量の変化によつて認められるかどうかを追求した．dd/YF マウスを用いて，条件照射と第2照射の合計線量が700Rになるよう二分割照射をおこなつた．照射間隔は4時間から10日までである．第2照射30日目の生存率と，生存したマウスについての体重，胸線，脾，睾丸，肝の重量をもとめた．結果は，生存率を放射線感受性の指標としたばあいに，放射線感受性の振動的変化の出現は線量の分割の割合，間隔に依存した．あるばあいには，放射線感受性は正常より低下した．すなわち，抵抗性を獲得した．臓器重量は30日後に生存したマウスからだけ測定したにもかかわらず，ある条件下で二分割照射すると，生存率の振動的変化に対応した振動的変化をしめした．これらの臓器重量の変化は，これらを構成する細胞が条件照射で同期された後におこる，細胞周期にもとづく放射線感受性の差によつて生じたものであるかもしれない．

INTRODUCTION

Many experimental evidences /1, 2, 4, 9, 11, 12, 15, 16/ have shown that the radiosensitivity of mammals irradiated with a sublethal dose does not return monotonously to the preirradiated level, and, furthermore, under some conditions the radiosensitivity becomes rather less than that of unirradiated mammals. In other words, this means that the residual injury does not fade out monotonously. As to this point, Kallman /6/ and Kallman and Silini /7/ have reported extensive data from the kinetic aspect: mortality, LD 50/30 and the residual injury were used as indices to express radiosensitivity. They speculated that the bone marrow injury and its recovery play a predominant role in the change of radiosensitivity. There are, however, some possibilities that cell injury and its recovery in other organs play a role as well. The present paper describes experiments designed to determine the effect of previous irradiation on radiosensitivity of mice, using changes in organ weights as the index of radiosensitivity, and to compare the changes in organ weights with the changes in survival rate.

MATERIALS AND METHODS

Male mice of the strain dd/YF were used for experiments at the average age of 60 days. Body weights of the mice were within the range of 17 to 23 g. From one day prior to the conditioning irradiation, mice

※Contribution No. 22 from Department of Experimental Radiology Faculty of Medicine, Kyoto University, Kyoto.

were kept separately in individual plastic cages. Eleven to twenty-four mice were assigned for the experimental groups, groups with single dose irradiation contained 13 to 20 mice, and the unirradiated control group contained 24 mice. The organ weights of control mice were measured at 60 days of age. A total of 318 mice was used for the entire study. Laboratory chow and water were given *ad libitum*. Mice were observed for 30 days after the second irradiation. Deaths of mice were scored daily. At the 30th day, all survivors were killed by cervical dislocation and the weight of body, thymus, spleen, testicle, liver and brain with cerebellum and pons were measured. In all cases, no deaths occurred during the interval between two irradiations. A total dose of 700 R was used for all the series, and fractionations were designed as follows;

1st series; conditioning dose of 150 R and second dose of 550 R.

2nd series; conditioning dose of 300 R and second dose of 400 R.

3rd series; conditioning dose of 350 R and second dose of 350 R.

All irradiations were carried out at 11:00 a.m. to avoid the influence of the circadian changes in response to radiation /11/.

The physical factors for irradiation were 24 mA, 190 kvp, 1.0 mm Cu and 0.5 mm Al filtration, target-to-mouse distance 50 cm; the exposure dose rate at the position of mouse was approximately 62.3 R/min. Radiation doses were monitored during irradiation with the Victoreen Radocon dosimeter with the probe No. 601.

RESULTS

1. The changes in percent survival by fractionation

Three series of experiments were carried out, using the second doses of 350 R, 400 R and 550 R. The 30-day survivors of mice irradiated with single doses of 350 R, 400 R and 550 R were 100.0%, 84.6% and 28.6%, respectively for the mice of dd/YF use in the present experiment. The 30-day survivals to be expected from conditioning irradiations with 350 R, 300 R and 150 R would therefore be virtually nil. The percent survivals of three series are illustrated in Figure 1. As shown there, in the 2nd and 3rd series the curves of percent survival fell at 48 hours after the conditioning irradiations and thereafter increased, in spite of no fall in the 1st series. And particularly in the 1st series, the percent survival in the group irradiated with the second dose only was rather less than that of the groups irradiated with fractionated doses 2—10 days apart. This phenomenon will be discussed later.

2. The changes in thymic weight by fractionation

As illustrated in Figure 2, the thymic weight was significantly lower in the group irradiated with the interval of 2 days than in others. Interestingly, this curve appears qualitatively similar to the curve of percent survival. The vertical lines shown in Figure 2 and the others represent the standard deviations for organ weights. And, in the present study, the five percent probability level was used to discuss the significant difference.

3. The changes in the splenic weight by fractionation

In the 2nd series, the splenic weight in the group irradiated with intervals between doses of 2 and 10 days were remarkably small. The results are illustrated in Figure 3. Like the results on thymic weight, the correlation between the low splenic weight and the low percent survival in the group with the interval of 2 days is interesting.

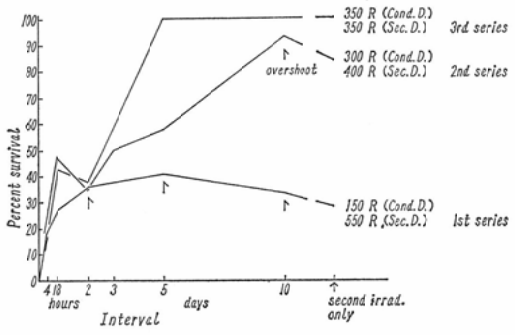

Fig. 1 Fluctuation in the percent survival at 30th day after second irradiation

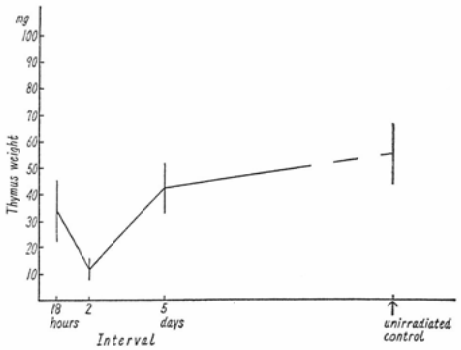

Fig. 2 Fluctuation in the thymic weight of survivors at 30th day after second irradiation (2nd series)

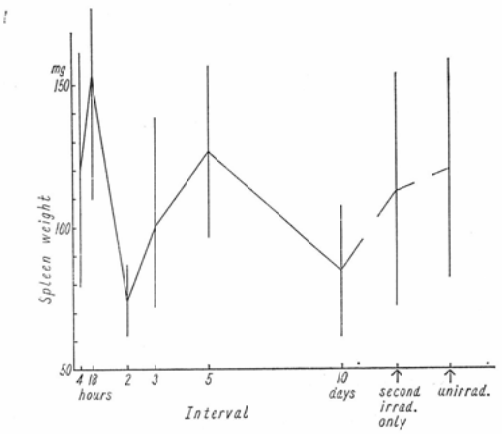

Fig. 3 Fluctuation in the splenic weight of survivors at 30th day after second irradiation (2nd series)

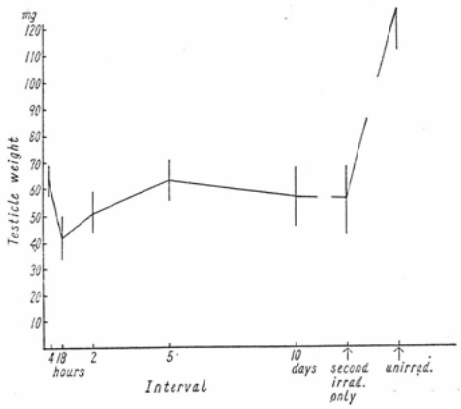

Fig. 4 Fluctuation in the testicular weight of survivors at 30th day after second irradiation (1st series)

4. The changes in testicular weight by fractionation

The testicular weight described here was measured at the 30th day after second irradiation. Therefore, this weight would be expected to be the minimum one in the course of changes in testicular weight produced by second irradiation, as shown by Kohn and Kallman /8/. In the 1st series (Figure 4), the testicular weights in all irradiated mice were significantly smaller than in unirradiated controls, but the fractionated group with the interval of 4 hours had significantly heavist testes than the group with the interval of 18 hours. In the other series, the average testicular weight increased with increasing interval between conditioning and second doses.

5. The changes in liver weight by fractionation

The average liver weight was remarkably modified by fractionation especially in the 2nd series. The average liver weights in the groups irradiated with the interval of 18 hours were significantly larger than that in the group irradiated with the interval of 4 hours and 2 days. The 3- and 5-day groups had significantly heavier livers than animals receiving only the second dose and also the 10-day group, in the 2nd series, as illustrated in Figure 5.

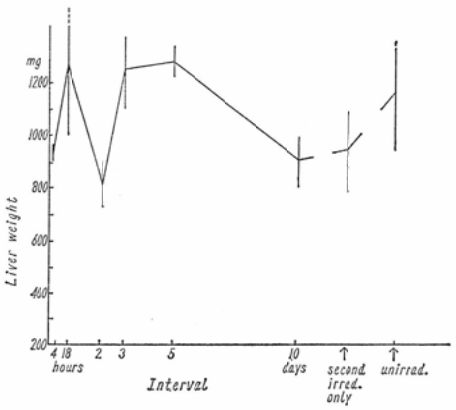

Fig. 5 Fluctuation in the liver weight of survivors at 30th day after second irradiation (2nd series)

6. The changes in the body weight and brain weight by fractionation

There were no remarkable change in body weight and brain weight compared with those in unirradiated mice.

DISCUSSION

The results illustrated in Figure 1 show that the fluctuation of radiosensitivity after conditioning irradiation pointed out by Kallman /6, 7/, occurred in the present experiments. In order to produce these fluctuations during the interval of less than 5 days, it appears necessary to irradiate with conditioning doses of at least 300 R. It was of interest for us to compare the fluctuation of organ weights in the 2nd series, in which the fluctuation of percent survival was similar to that of fluctuation of thymic, splenic and liver weights in the groups with intervals of less than two days. Because the organ weights were measured only in the surviving mice, we are unable to exclude the factor of selection by killing sensitive animals as responsible for these effects. The conclusions that have been shown are attractive, however, and merit careful consideration. The further experiments, in which the factor of selection is able to be excluded, are under consideration.

It is necessary to examine the question of why the fluctuation of percent survival are not the same in the three series of experiments, in spite of the using the same total dose of 700 R. Though it is impossible to answer this question exactly on the bases of the present results, it can be concluded that the proportion in the size of the divided doses as well as the interval between the two exposures are both important factors modifying the results.

The phenomenon that the conditioning irradiation induces greater radioresistance at some days after irradiation is noticeable in the data of the 1st and 2nd series (Figure 1). The mechanism responsible for this remains to be determined; it has been proposed by Lajtha /10/ that the increase of radioresistance is related to the overshoot of cell proliferation in the blood forming system. In the present data, there was no overshoot of radioresistance using the organ weight as the index of radiosensitivity. That is, the organ weights of mice irradiated with fractionated doses were not larger than those of mice received the second doses only, except the case of liver in the 2nd series. This problem should be investigated further.

The thymic weight is affected by various stress. As thymic weight was measured 30 days after the

second irradiation in the present experiment, it is quite likely that the weight was affected by many other factors /3, 14/ in addition to irradiation. A similar problem arises in interpreting the fluctuation of splenic weight. As reported by Hayakawa et al. /5/, the splenic weight changes unmonotonously after irradiation. Therefore, the weights of spleen and thymus may be used only with great caution as indices of radiosensitivity. In spite of this fact, it appears likely that these two organs show a radiosensitivity similar to that shown by precent survival under the same conditions (in the group with the interval less than 2 days in the 2nd series). This is based on the observation of similar changes in the weight of the liver, an organ which is comparatively independent of influences by physiological factors.

The testicular weight is a comparatively stable index, similar to the liver weight, and the testicular weight loss has a well-defined relationship to the exposure dose as described by Kohn and Kallman /8/. In the present report, the testes were comparatively small in the group with the interval of 18 hours between fractions. This suggests that the stem cell of germ cell system is relatively radiosensitive 18 hours after the conditioning irradiation in the 1st series. As one possible mechanisms responsible for this result, it may be that the conditioning irradiation initiates cell repopulation in the testes; this, however, requires confirmation. It must be remarked that the present result of reduced radiation effectiveness is in contrast to the findings of Kohn and Kallman /8/ who reported no change in effectiveness when lower doses were fractionated over comparable intervals. This discrepancy demands careful reinvestigation before the present data can be accepted, though the differences in the conditions of both experiments might induce these discrepancies.

The pattern of changes of liver in the 2nd series is similar to that of the spleen in the 2nd series.

In conclusion, fluctuations were found in organ weights of surviving mice irradiated with divided doses, and some of these were similar to the fluctuation of percent survival. Though the mechanism as to the fluctuation of percent survival is not yet explained, it may be considered that the cells of the bone marrow were synchronized after the conditioning dose as suggested by Kallman /6/. Starting from this hypothesis, it can be assumed that the fluctuation of the weights of thymus, spleen, testes and liver may be caused by the synchronization of cells constituting these organs, especially since the fluctuation is of a similar pattern to that of percent survival, despite the fact that the weights were measured only in the surviving mice selected by X-ray irradiation.

SUMMARY

1. The radiosensitivity of mice did not recover to the normal level gradually with the time after irradiation, but returned to normal with fluctuations. In some cases, it was less than the normal level.

2. By measuring the weight of spleen, thymus, liver and testes of survivors 30 days after second irradiation, the radiosensitivity of mice at each time after the conditioning dose was measured. In some conditions of these experiments, the fluctuation of radiosensitivity shown by the percent survival corresponded to that shown by organ weight.

3. The data were discussed.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Porfessor T. Sugahara for his valuable advice and comments concerning this manuscript, and to thank Mr. T. Tanaka and Miss. K. Fujimoto for their technical

assistance. The author is grateful to Dr. R.F. Kallman for valuable advice and comments concerning this manuscript.

REFERENCES

1. Betz, H.: The importance of the resistant phase of the organism at the time of application of a lethal dose of X-radiation, Comp. rend. soc. biol., (1950), 1439—1442.
2. Cronkite, E.P., Sipe, C.R., Eltholtz, D.C., Chapamn, W.H. and Chambers, F.W. Jr.: Increase tolerance of mice to lethal X-radiation as a result of previous sublethal exposures, P.S.E.B.M., **73**, (1950), 184—186.
3. Cross, A.M., Davies, A.J.S., Doe, B. and Leuchars, E.: Time of action of the thymus, Nature, **203**, (1964), 1239—1241.
4. Dacquisto, M.P.: Acquired radioresistance, a review of the literature and report of a comfirmatory experiments, Radiation Res., **10**, (1959), 118—129.
5. Hayakawa, J., Muramatsu, S., Yamada, J. and Tsuchiya, T.: The splenic and thymic weight response to whole body X-irradiation in three strains of mice, Nipp. Acta Radiol., **24**, (1964). 370—376.
6. Kallman, R.F.: Recovery from radiation injury, a proposed mechanism, Nature, **197**, (1963), 557—560.
7. Kallman, R.F. and Silini, G.: Recuperation from lethal injury by whole-body irradiation, L. Kinetics aspects and the relationship with conditioning dose in C57Bl micen Radiation Res. **22**, (1964), 622—642.
8. Kohn, H. and Kallman, R.F.: The effect of fractionated X-ray dosage upon the mouse testis I. Maximum weight loss following 30 to 240 r given in 2 to 5 fractions during 1 to 4 days, J. Nat. Cancer Inst., **15**, (1955), 891—899.
9. Kohn, H.I. and Kallman, R.F.: Acute lethality studies with the rat: The LD_{50} death rate and recovery rate, Radiation Res., **7**, (1957), 86—97.
10. Lajtha, L.G.: Response of bone marrow stem cells to ionizing radiation, Current topics in radiation research, Vol. 1. North-Holland Publ. Com., Amstherdam, Ed. by Ebert, M. and Howard, A., (1965), 139—164.
11. Mole, R.H.: Quantitative observations on recovery from whole body irradiation in mice I. Recovery after single large doses of radiation, Brit. J. Radiol. **29**, (1956), 563—569.
12. Paterson, E., Gilbert, C.W. and Mathews, J.: Time intensity factors and whole body irradiation, Brit. J. Radiol., **25**, (1952), 427—433.
13. Pizzarello, D.J., Isaak, D. and Chua, K.E.: Circadian rhythmicity in the sensitivity of two strains of mice to wholebody radiation, Science, **145**, (1964), 286—291.
14. Pospisil, M.: Effect of adrenal hormones on radiation thymolysis, Folia biologica (Praha), **7**,(1961), 217—219.
15. Raper, J.R.: Effects of total surface beta irradiation, Radiology, **49**, (1947), 314—324.
16. Rugh, R. and Wolf, J.: Fetal conditioning against X-irradiation lethality, Radiation Res., **7**, (1957), 462—463.