# Radioprotective Action of SH Compounds

## I. Studies Based on Survival Rate Curve

Shoji KAWASAKI
*Department of Radiology, Yamaguchi University School of Medicine, Ube, Japan.*
*(Director: Prof. Dr. Ko Sakurai)*
(Received February 20, 1973)

## INTRODUCTION

Current interest has been focused on the protective effects of various chemical substances for radiation injuries.[1)2)] Previously the author has pointed out that the modifying action exists in vitamine $B_2$, $B_6$ and $B_{12}$ among vitamine B group.[3)4)5)] Such an action of vitamine $B_6$ was confirmed by Abe and Takahashi.[6)] Alexander *et al.*[7)] and Langendorff *et al.*[8)9)10)] reported a marked radioprotective effect of serotonin, in prolonging the survival time of mice and rats irradiated with lethal doses of X-ray. However, the most active compounds protecting the radiation injury were SH reagents.[11)12)13)] Concerning these reagents, there are numerous interesting reports.[14)15)16)17)18)19)20)21)] The mechanism of radioprotection of these SH compounds is known to be quite complicated and not obtained clear-cut explanation for the radiation injury. Eldjarn *et al.*[22)] pointed out the important action of SH compounds within the nucleus of cells for radiation injury.

In view this, the study of the radioprotective action of exoginous SH compounds is considered to be important for the elucidating of the biological action mechanism of radiation. In the present experiment, the radioprotective effects of glutathione (GSH), cysteamine (MEA), aminoethylisothiuronium Br•HBr (AET), 2–mercaptopropionylglycine (MPG) and dithiothreitol (DTT) were studied on the basis of mouse survival rate. These radioprotective actions of SH compounds were studied by single dose irradiation as well as repeated irradiation of sublethal doses with lapse of time after the administration of SH compounds.

## MATERIALS AND METHODS

1) *Amimals*: Male mice of dd strain at 8–10 weeks of age with body weight of 20–25 gram served for the experiments.
2) *Irradiation methods*: X-rays of 200 kVp, 17.8 mA, with filter of 1.0 mm Al, focus skin distance of 50 cm, half value layer of 1.55 mm Cu, 30 R/minute was used. The dose was measured with Victoreen R-meter type 602 chamber.

a) *Single whole body irradiation of X-ray*: Within 30 minutes before irradiation, the SH compound was injected intraperitoneally. The changes of survival rate and body weight were observed.

b) *Repeated whole body irradiation of X-ray*: Repeated irradiation with 300 R/week was performed until the animal died. SH compound was injected intraperitoneally. GSH (30 mg/animal), MEA (2 mg/animal) and AET (3 mg/animal, 6 mg/animal) were injected separately at 30 minutes before irradiation until the death of the animal. The amount of GSH injected 30 minutes before irradiation varied from 10 to 20, 30, 40 and 50 mg. The compound was injected at 6 hours, 3 hours and 30 minutes before irradiation. Intraperitoneally injection was also carried out 30 minutes, 3 hours, 6 hours and 24 hours after irradiation.

3) *SH compound*: Of SH compounds, MEA (2 mg/animal, 4 mg/animal), AET (3 mg/animal, 6 mg/animal), GSH (10, 20, 30, 40 and 50 mg/animal), MPG (5 mg/animal) and DTT (4 mg/animal) were used. Without description, these SH compounds were injected intraperitoneally 30 minutes before irradiation.

## RESULTS

### I. *Whole body single irradiation.*

1) *Changes in survival rate and body weight.* As shown in Table 1, the rate of survival at 30 days after irradiation was 75 per cent after irradiation with 400 rads, 67 per cent with 450 rads, 26 per cent with 500 rads and 8 per cent with 550 rads. After irradiation, the body weight fell suddenly to about 90 per cent of the previous body weight one day after irradiation, and followed by a gradual decrease as illustrated in Fig. 1.

After the irradiation with 400 rads, body weight decreased to 85.6 per cent of the preirradiation level at 6 days. With 450 rads, body weight was 74.5 per cent after 12 days. With 500 rads, it was 68.6 per cent after 11 days, while the body weight was 64.3 per cent 14 days after the irradiation with 550 rads. These data represent minimal weight. Therefore these findings reflect that the body weight gradually recovers after these periods. In this instance, by the increase of irradiation dose, the period of the decreased body weight is extended, thus the recovery of body weight is delayed.

Table 1. The protection afforded by SH compounds injected intraperitoneally before irradiation

| Exp. | SH Compound | Dose (rad) | Treated number | Survival number | Rate per cent |
|---|---|---|---|---|---|
| I | Control | 400 | 12 | 9 | 75 |
| | | 450 | 12 | 8 | 67 |
| | | 500 | 12 | 3 | 25 |
| | | 550 | 12 | 1 | 8 |
| | | 600 | 12 | 0 | 0 |
| II | GSH (30 mg) | 500 | 12 | 7 | 58 |
| | | 550 | 12 | 2 | 17 |
| | | 600 | 12 | 1 | 8 |
| III | MEA (4 mg) | 700 | 24 | 13 | 54 |
| | | 750 | 12 | 4 | 33 |
| | | 800 | 12 | 1 | 8 |
| IV | AET (3 mg) | 550 | 12 | 11 | 92 |
| | | 600 | 12 | 5 | 42 |
| | | 650 | 12 | 3 | 25 |
| | | 700 | 24 | 3 | 13 |
| | | 800 | 12 | 1 | 8 |
| V | AET (6 mg) | 600 | 12 | 6 | 50 |
| | | 700 | 12 | 4 | 33 |
| | | 800 | 12 | 2 | 17 |
| | | 900 | 12 | 0 | 0 |
| VI | DTT (4 mg) | 500 | 12 | 3 | 25 |
| | | 550 | 12 | 1 | 8 |
| | | 600 | 30 | 1 | 3 |
| | | 700 | 26 | 0 | 0 |
| VII | MPG (5 mg) | 500 | 12 | 9 | 75 |

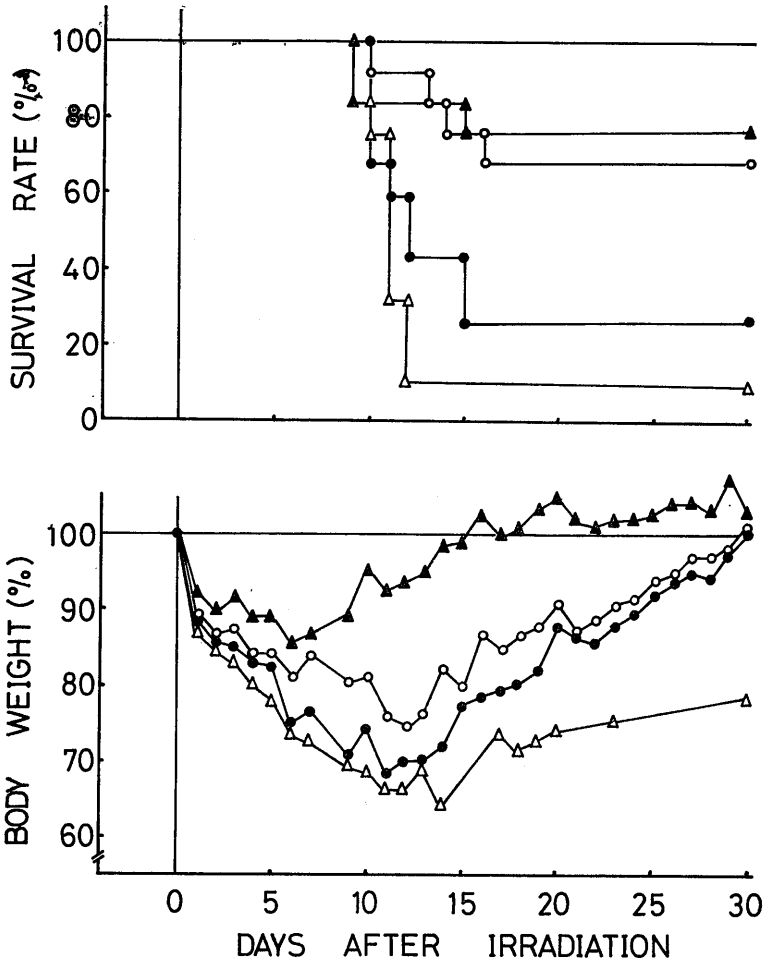


Fig. 1. Survival curves and body weight changes of mice after whole body X-irradiation.
▲——▲ 400 rads ○——○ 450 rads
●——● 500 rads △——△ 550 rads

2) *Protection by SH compounds*: The curves of the changes in the rate of survival and in the body weight after single whole body irradiation are shown. in Fig. 2 (GSH, 30 mg), Fig. 3 (MEA, 4 mg), Fig. 4 (AET, 3 mg) and Fig. 5. (AET, 6 mg). Table 1 summarizes these survival rates at 30 days after irradiation Fig. 6 shows these survival rates at 30 days after irradiation. The values of LD 50/30, LD 37/30 and LD 10/30 are obtained from the curves of Fig. 6. Dose reduction factor (DRF) of these SH compounds is calculated and indicated in Table 2.

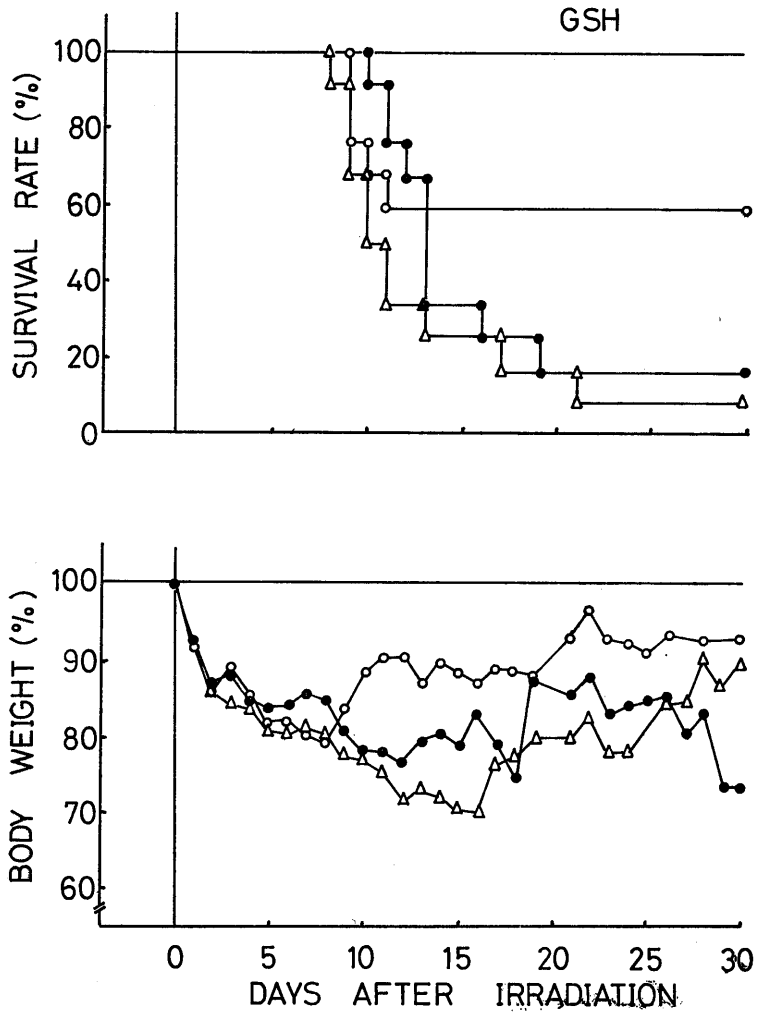


Fig. 2. Survival curves and body weight changes of mice after whole body X-irradiation with glutathione pretreatment.

○——○ 500 rads ●——● 550 rads
△——△ 600 rads

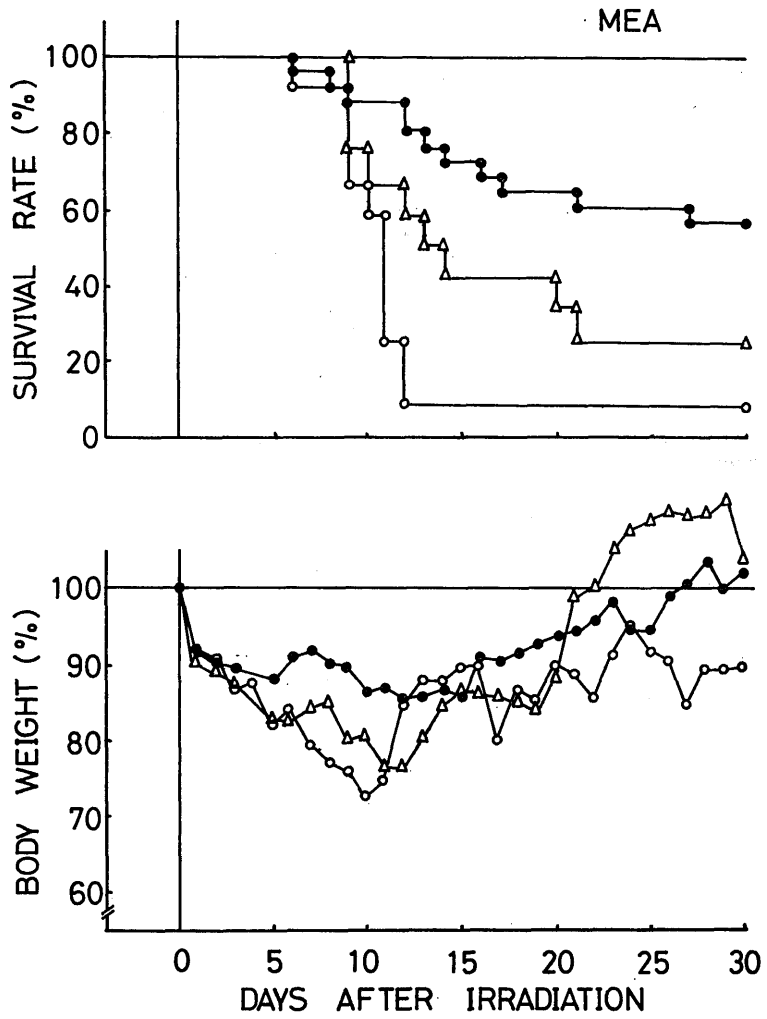


Fig. 3. Survival curves and body weight changes of mice after whole body X-irradiation with MEA pretreatment.

●——● 700 rads △——△ 750 rads
○——○ 800 rads

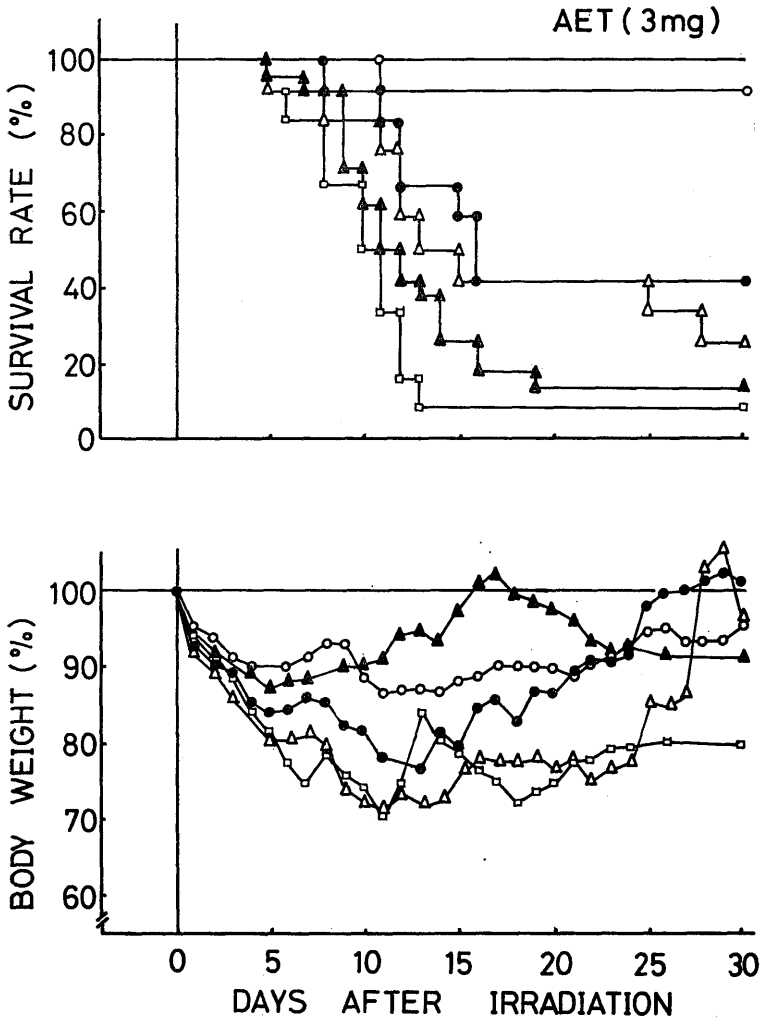


Fig. 4. Survival curves and body weight changes of mice after whole body X-irradiation with AET (3 mg/mouse) pretreatment.

○———○ 550 rads
△———△ 650 rads
□———□ 800 rads
●———● 600 rads
▲———▲ 700 rads

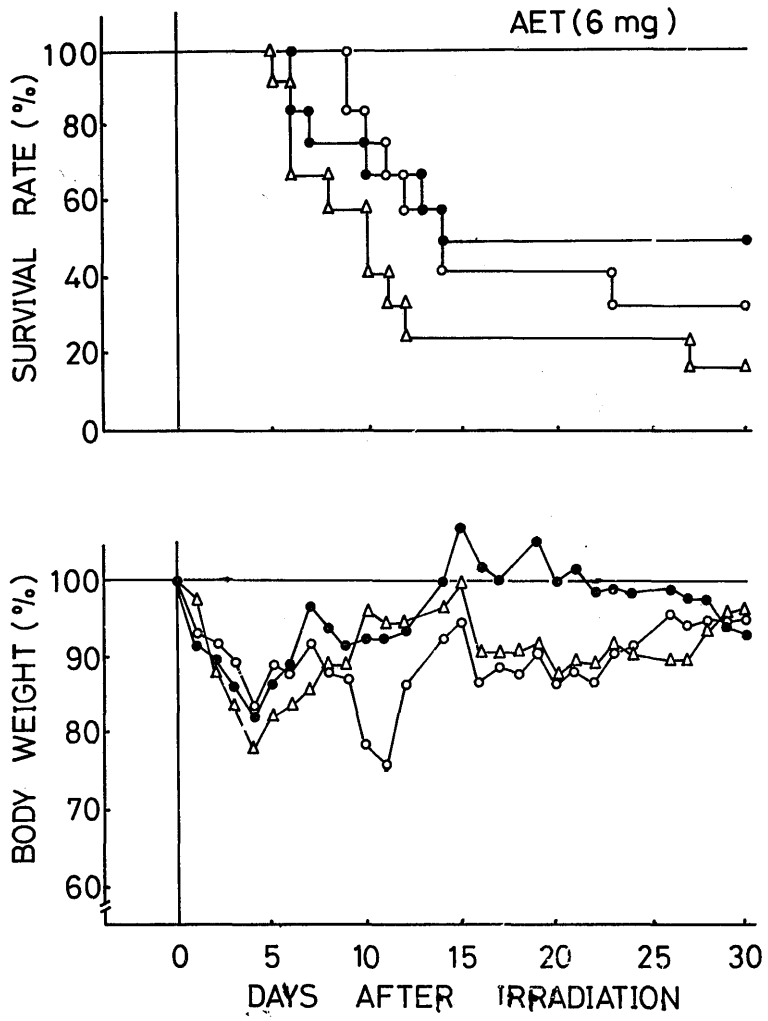


Fig. 5. Survival curves and body weight changes of mice after whole body X-irradiation with AET (6 mg/mouse) pretreatment.

●——● 600 rads ○——○ 700 rads
△——△ 800 rads

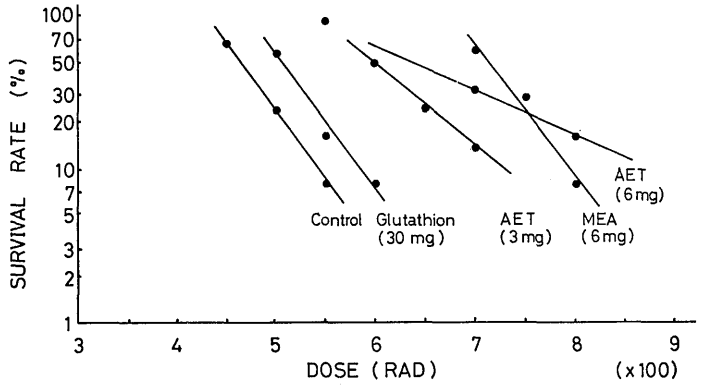


Fig. 6. Dose survival curves at 30 days in mice treated with SH compound before whole body X-irradiation.

Table 2. DRF of various SH-compounds

| SH-compound | LD50/30 | | LD37/30 | | LD10/30 | |
|---|---|---|---|---|---|---|
| | Dose (rad) | DRF | Dose (rad) | DRF | Dose (rad) | DRF |
| Control | 462 | | 477 | | 537 | |
| GSH (30 mg) | 505 | 1.09 | 520 | 1.09 | 585 | 1.09 |
| MEA (4 mg) | 712 | 1.54 | 728 | 1.53 | 792 | 1.47 |
| AET (3 mg) | 597 | 1.29 | 625 | 1.31 | 727 | 1.34 |
| AET (6 mg) | 632 | 1.37 | 677 | 1.42 | 870 | 1.61 |

In the control group (without injection of SH compounds), the value of LD 50 for GSH (30 mg), MEA (4 mg), AET (3, 6 mg) were similary calculated, yielding 505, 712, 597 and 632 rads respectively. DRF was also obtained from these data as 1.09, 1.54, 1.29 and 1.37 respectively.

The slope of the curves were quite similar between qroup treated with GSH and MEA and the control, as shown in Fig. 6. However, the groups treated with AET gave different curves from that of the control group. DRF obtained based on LD 50 scarcely changed in LD 37 and LD 10 for MEA and GSH. However, in the case of AET the value was gradually increased. A definite radioprotective effect was observed in the treatment with MPG.

Against these protective effects of the SH compounds, the rate of survival scarcely differed in the treatment with DTT from the control values.

3) *Time factor for the administration of MEA*: Radioprotective effect of 4 mg MEA was compared among the group injected 60 minutes before irradiation,

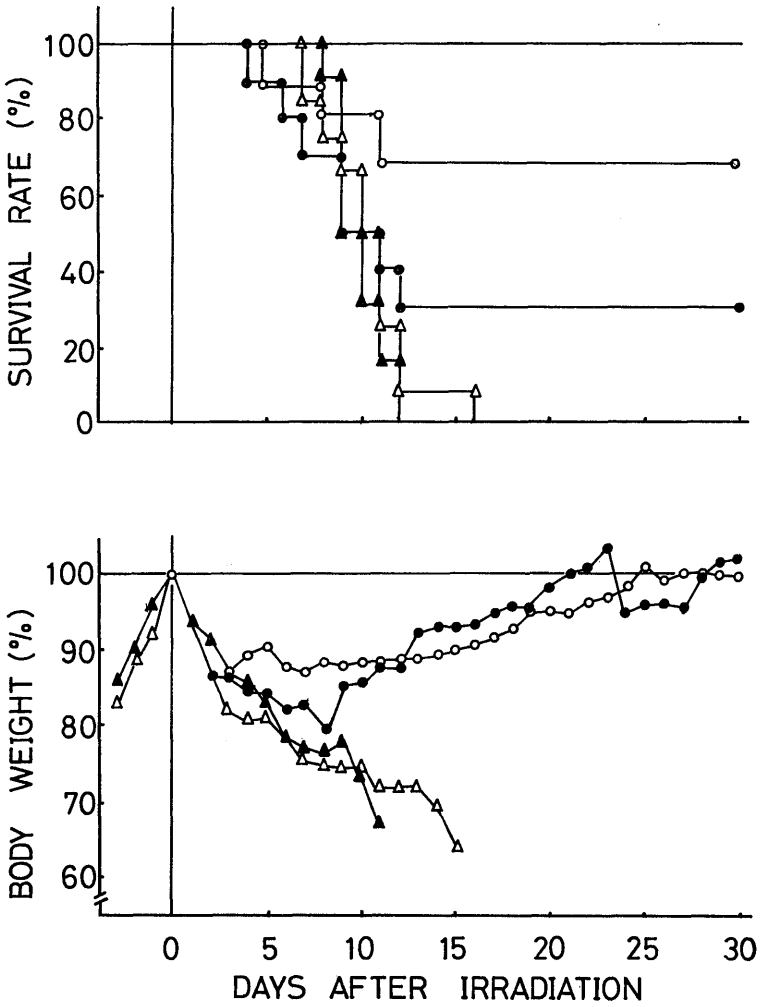


Fig. 7. Survival curves and body weight changes of mice treated with MEA at different times before and after irradiation with 600 rads.

■———■ control (600 rads only)
△———△ post-treatment at 30 minutes
○———○ pretreatment at 30 minutes
●———● pretreatment at 60 minutes

30 minutes before irradiation and 30 minutes after irradiation (Fig. 7). In the control group irradiated with 600 rads, all the animals died between 8 to 12 days after irradiation. When the drug was administered 30 minutes after irradiation, a mortality curve was similar to that of the control group and all the animal died within 16 days after irradiation. When the drug was administered 30 minutes before irradiation, some of the animals died at some time corresponding to the

time while the control group were surviving, but no animals died subsequently after 11 days. Therefore, the 30-day survival rate was 68 percent. In the group where the drug was administered 60 minutes before irradiation, a curve similar to that the control was obtained until the 12th day following irradiation but thereafter no animal died. The 30-day survival rate was 30 percent.

II. *Repeated whole body irradiation.*

1) *Effects of GSH*: Repeated irradiation with 300 R/week was performed until the animal died. Ten, 20, 30, 40 and 50 mg/animal of GSH were injected intraperitoneally 30 minutes before irradiation. Protective effect of GSH varied according to the dose of injected GSH. Average days of survival were 18.4 days in the control group, and 22.6, 23.8, 31.7, 23.0 and 20.7 days in the group treated with 10, 20, 30, 40 and 50 mg respectively. Namely, with the increase of injected dose of GSH, the protective effect improved and a considerable life-prolonging effect was observed at the dose of 30 mg. But these effects grew poorer again by further increase of the dose to 40 and 50 mg (Table 3–I).

Table 3. Chemical protection against repeated X-irradiation

| Exp. | SH compound | Treated number | Mean survival days |
|---|---|---|---|
| I | Control | 42 | 18.4 |
| II | GSH (10 mg) | 10 | 22.6 |
| | GSH (20 mg) | 10 | 23.8 |
| | GSH (30 mg) | 10 | 31.7 |
| | GSH (40 mg) | 10 | 23.0 |
| | GSH (50 mg) | 10 | 20.7 |
| III | GSH (30 mg) | | |
| | 30min. before irrad. | 10 | 28.0 |
| | 3 hrs. before 〃 | 10 | 29.7 |
| | 6 hrs. before 〃 | 9 | 23.8 |
| IV | GSH (30 mg) | | |
| | 30 min. after irrad | 10 | 22.7 |
| | 3 hrs. after 〃 | 10 | 13.9 |
| | 6 hrs. after 〃 | 10 | 22.5 |
| | 24 hrs. after 〃 | 10 | 18.9 |
| V | MEA (2 mg) | 12 | 25.4 |
| | AET (3 mg) | 12 | 32.3 |
| | AET (6 mg) | 12 | 35.7 |

* Repeated irradiation with 300 R/week was performed until the animal died.

** SH compound was injected intraperitoneally.

Intraperitoneally injection of GSH in mice at 30 minutes, 3 hours and 6 hours before the irradiation gave survival days in average of 28.0, 29.7 and 23.8 days respectively, and 18.4 days in the control group. A positive effect was observed with the administration of the drug from 30 minutes to 3 hours before irradiation. These protective effects grew less when the drug was administered earier such as at 6 hours (Table 3-II).

Injection of GSH at 30 minutes, 3 hours, 6 hours and 24 hours after irradiation gave survival days in average of 22.7, 13.9, 22.5 and 18.9 days respectively. The effect was not significant and the survival time was rather when the drug administered 3 hours after irradiation (Table 3-IV).

2) *Effects of MEA and AET*: As in the case of GSH treatment, MEA (2 mg/animal) or AET (3 or 6 mg/animal) was injected intraperitoneally 30 minutes before irradiation. Average survival days were 18.4 days in the control group, and 25.4, 32.3 and 35.7 days in the group treated with 2 mg MEA, 3 mg AET, or 6mAET respectively. Namely, a definite protective effect of AET was observed. Increase in the amount of AET administration resulted in prolongation of average survival days and in improvement of the these protective effects (Table 3-V).

## DISCUSSION

Related to the protective effect of SH compounds, there are numerous paper published.[18)23)24)25)26)27)28)] All of these papers show the increased survival rate. One of the first papers related to the GSH effect published by Chapman *et al.*[23)] reported the reduction of mortality rate and a less decrease of body weight by GSH administration before X-irradiation. The present experiment has been carried out to clarify that whether the SH compounds have similar effect for the repeated irradiation or not as observed on the single irradiation. As a result, the author reconfirmed a similar protective effect of GSH, MEA, AET and MPG in the dd strain mice but not of DTT: The value of DRF calculated from the data obtained by administration of 30 mg GSH was about 1.09. This value is quite close to the one obtained from the data of Chapman, who administered 1.6 mg/g to the mice, obtaining the value of DRF was 1.13. Furthermore similar protective effect was observed on the repeated irradiation by administration of these SH compounds before irradiation.[29)] But the effect was not observed when the SH compound was administered after irradiation. The reason for the failure action of SH compounds may be explained as follows: The accumulation of SH compound in the cells or organism prior to irradiation is necessary for radioprotective effect.[28)30)31)32)]

Another experiment of this study is focused on the point whether various SH compounds act in the similar mode or not. The results of the experiment using

the GSH, AET, MEA, MPG and DTT indicate that the mode of action of GSH and MEA was quite similar, but the one of AET differed from the above SH compounds: There can be scarcely observed any change in the slope of dose effect curve between GSH and MEA, but the one of AET is quite different from them. These data indicate that GSH and MEA protect the bone marrow and AET acts as a protective agents not only for the bone marrow but also for the intestine.[4) 33) 34) 35) 36)]

Falconi *et al.*[38)] reported the radioprotective effect of DTT with potent oxidative capacity not only before irradiation (until 72 hours) in both oxidized and reduced form. However, in the present experiment the author could not reconfirm the result of Falconi *et al.* The toxicity at the dose of 4 mg/animal of DTT might be rather dominant so that the dose response of DTT requires further studies.

## SUMMARY

The radioprotective effect of SH compounds (GSH, MEA, AET, MPG and DTT) was studied by using of 30-day survival rate in mice and obtained the following results.

1. DRF (dose reduction factor) of GSH (30 mg), MEA (4 mg), AET (3 mg) and AET (6 mg) was 1.09, 1.54, 1.29 and 1.31 at $LD_{50}$ respectively.
2. The dose-effect curve of GSH and MEA had a slope approximately similar to that of the control group. The slope was less steep for AET.
3. In administering MEA at intervals of 60 minutes, 30 minutes before irradiation and 30 minutes after irradiation, the best effect was obtained with administration 30 minutes before irradiation.
4. When the GSH administration was shifted to repeated irradiation, positive effect was obtained in the administration at 3 hours or 30 minutes before irradiation and a poorer effect in the administration at 6 hours before irradiation. Administration after irradiation gave no effect.
5. When the GSH administration was shifted to repeated irradiation, 30 mg of GSH gave the best effect.
6. Protective effect of other compounds in the dose of 3 mg, 6 mg AET or 3 mg MEA, was also observed for repeated irradiation. AET (6 mg) had the best protective effect of all the SH compounds tested.

On the basis of these experimental results, the author explained the radioprotection of SH compounds.

## ACKNOWLEDGEMENT

I am gratefful to Prof. Dr. Ko Sakurai of Yamaguchi University School of

Medicine for his helpful suggestions and criticisms throughout the work and also to Assistant Prof. Kozo Utsumi of Okayama University School of Medicine for his helpful encouragement.

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