Radioprotective Effect of Chitosan in Sub-lethally X-ray Irradiated Mice.

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The radioprotective effect of chitosan was studied in mice following whole-body X-ray irradiation. C3H/He mice were exposed to 7 Gy, and their survival rates were examined. The survival rates of chitosan-diet mice were about 20% higher than those of mice on a standard diet, and the rates dropped sharply to a plateau at day 10 after X-ray irradiation. The chitosan-diet mice had an increased weight ratio of spleen to body within the experimental period. The leukocyte, thrombocyte, and erythrocyte counts as well as the hematocrit and hemoglobin levels were recovered significantly and more rapidly in the chitosan-diet mice than the standard-diet mice at day 14 after irradiation. The scavenging abilities of chitosan were evaluated by the ESR spin-trapping method. These observations suggested that chitosan led to hematopoietic activation and leukocytogenesis in mice after sub-lethal dose irradiation, and that the biological response might be caused by radical trapping or scavenging.

INTRODUCTION

It has been reported that many substances, such as ginseng extract1; alcohols like glycerol2; protein-associated polysaccharides, such as AM-53; and AM218; bacterial extracts, such as Broncho-Vaxon5; heat-killed lactobacillus6; synthetic trehalose dicymonomyleolate7; 16,16 dimethyl prostaglandine E28; and OK-4329; offer protection against irradiation effects. Sato et al.10 reported that sodium tungstate decreased the hematocrit value and the survival rate in Wistar-strain male rats which had been exposed to 60Co γ-rays. They supposed that sodium tungstate activated macrophages to stimulate hematopoietics. Cherupally et al.11 summarized a list of various categories of radioprotectors and their mechanisms of action. Radioprotective agents generally have a role as free radical scavengers or hematopoietic stimulants. Metallothionein, the synthesis of which can be induced by metalloelement administration, is a known radical scavenger, and metallothionein is considered to act as a radioprotector by scavenging radicals11,12.

Chitin is a structural polysaccharide composed of a β-1,4-linked N-acetylglucosamine residue and a cellulose–like biopolymer, which is distributed widely in nature, especially in crustaceans, insects, fungi and yeast. Chitosan is the main derivative of chitin. There are several other derivatives of chitin that are currently in use or have potential applications in the commercial sector13–15. For example, in agriculture its derivatives are used as nematocides and feed ingredients for the treatment of seeds and fruits and for soil improvement. Although the degree of deacetylation of chitosan has been found to influence biological activities, its basic biokinetic behavior in animals is not yet clear. Chitosan is thought to be an unabsorbable food fiber and, in general, it is not absorbed through the gastrointestinal tract. Nishimura et al.16 investigated the intestinal absorption of 14C-chitosan in rats. They reported that chitosan underwent digestion into low-molecular-weight substances within the gastrointestinal tract, and that it was distributed extensively in rat tissues. However, an intensive biokinetic study of chitosan is necessary in order to understand how it is metabolized. Chitosan and its derivatives are not toxic, and are also known to be natural chelating agents. Nishimura et al.17 reported that chitosan could be used as a drug to reduce the bioavailability from gastrointestinal absorption of ingested radiostrontium.

However, the correlation between the incidence of radioprotection effects and the survival rate after being fed chitosan is poor. In the present study, mortality and physiological observations were investigated in chitosan-fed mice in order to evaluate how chitosan affected the survival rates of mice exposed to X-
rays; also, the scavenging ability of chitosan was evaluated by ESR spectrometry.

**MATERIALS AND METHODS**

**Animals and X-ray irradiation**

C3H/He male mice of 6 weeks of age were purchased from SLC Inc. (Japan). Two hundred mice were used to observe the survival curve, and another 100 mice, including 20 non-irradiated mice, which were fed a standard diet, were prepared for blood checks. The mean body weight 40 days before X-ray irradiation was 20.5 ± 0.6 g and about 29 to 32 g at the time of X-ray irradiation. The mice were separated into two groups. One group (140 mice) was fed a cubed diet, which contained 5% w/w of insoluble chitosan (Chitosan-F, for feed ingredients, Kimitsu Chemical Industries, Japan). The other group (160 mice) was fed a standard cubed diet (MF, Oriental Yeast Co., Japan) and water ad libitum. The mice were kept for 40 days on each diet, and then were exposed to 7 Gy of whole-body X-ray irradiation, except for the 20 mice specified above. The survival rates of these mice were observed within 30 days after irradiation.

**Hematology and spleen weight**

Blood was collected from a heart puncture under ether anesthesia, and the spleen weight was measured 0, 8, 14 and 21 days after irradiation. The erythrocyte count, leukocyte count, hematocrit value and hemoglobin concentration were evaluated on an automated hematology analyzer (Sysmex Co., F-820, Japan). The hematology analysis was carried out using a one-way factorial ANOVA test with multiple comparisons (SAS Institute Inc., USA). Animals were treated under the Guide for the Care and Use of Laboratory Animals in National Institute of Radiological Sciences.

**Electron spin resonance (ESR)**

Sample: Chitosan was diluted with 1 N acetic acid to provide a 1% w/w solution. Chitin and chitosan consisting of β-1,4-linked N-acetylglucosamine residue and 20 mM of N-acetylglucosamine (MW: 221.21, Wako Pure Chemical Industries, Ltd., Japan) was used for the reference material. The scavenging abilities of chitosan were evaluated by the ESR spin trapping method18–22, in which 5,5-dimethyl-1-pyrroline-N-oxide (DMPO, Labotec, Tokyo) was used as a spin trap.

Superoxide anion radical generation system: Superoxide anion radicals (O₂⁻) were generated with a hypoxanthine-xanthine oxidase system.

Hydroxyl radicals generation system: Hydroxyl radicals (·OH) were generated from the reaction of Cu(en)₂ and H₂O₂ using an ultraviolet-ray (UV) system.

ESR measurements: For measuring super oxide and hydroxyl radical scavenging, a free radical monitor (JES-FR30S, JEOL, Japan) was used. This compact, sensitive ESR spectrometer has a function to normalize all spectra for accurate calculations using manganese oxide as an internal standard; manganese oxide provided a constant signal to which all peak heights were compared. A sample peak height was divided by the MnO peak height to give the relative peak height. ESR measurements were made under the following conditions: magnetic field, 335.8 mT; power, 4 mW; modulation frequency, 9.4 GHz; modulation amplitude, 0.079 mT; response time, 0.03 s; sweep time, 2 min. ESR spectra were measured at 23°C. A data analysis was performed using a computerized program (version 5.2 for FES-FR 30) connected to the free radical monitor.

**Statistical analysis**

The statistically significant difference in ratio of the spleen to body weight and in the recovery of haemophysiological markers in murine peripheral blood were determined by comparing the individual values at each time after irradiation with the respective control values (each time) by using Dunnett’s test23.

**RESULTS**

**Effect of chitosan on the survival of irradiated mice**

Figure 1 shows survival curves of mice after exposure to 7 Gy whole-body X-ray irradiation. Deaths of the irradiated mice were observed from about 10 days after irradiation. Chitosan-diet mice showed a significantly lower mortality rate than that of standard-diet mice (P < 0.001). The survival ratios 30 days after irradiation were 50% and 30% for the chitosan-diet mice and the standard-diet mice, respectively.

**Effect of chitosan on the recovery of body and spleen weight**

The body weight of irradiated mice decreased to about 77% of the normal weight on day 14 in the standard-diet mice (Fig. 2). The decreased body weight for irradiated mice receiving chitosan was about 95% of the normal weight. The body weight of the irradiated mice was not significantly different after day 21. The spleen-to-body weight of irradiated mice of about 0.26% on day 0 decreased to 0.1% on day 14, and increased to 0.69% on day 30.

![Fig. 1. Effect of chitosan on the survival of irradiated mice. Solid triangle, chitosan diet; solid circle, standard diet.](image_url)
21 for the standard-diet mice. The ratio of the spleen-to-body weight of irradiated mice decreased to 0.23% on day 14 and increased to 0.97% on day 21 for the chitosan-diet mice (Fig. 3). The spleen weight of both irradiated mice groups tended to increase from day 8 until day 21, and went back to a normal level on day 30.

**Effect of chitosan on hemogram after X-ray irradiation**

Figure 4 shows the leukocyte patterns after irradiation with a 7 Gy dose for both diet groups. The number of leukocytes in the chitosan-diet mice decreased rapidly to $2.8 \times 10^6/\mu l$ after irradiation on day 8, then increased until they reached $45 \times 10^6/\mu l$ on day 14. The number of leukocytes of the chitosan-diet group increased from $45 \times 10^6/\mu l$ on day 14 to $958 \times 10^6/\mu l$ on day 21 after X-ray irradiation, but with wide individual differences. However, the leukocyte counts in standard-diet mice decreased to $3.7 \times 10^6/\mu l$ and $4.8 \times 10^6/\mu l$ on days 8 and 14 after X-ray irradiation. The number of leukocytes of the standard-diet group increased progressively from $4.8 \times 10^6/\mu l$ on day 14 to $813 \times 10^6/\mu l$ on day 21 after X-ray irradiation. In non-irradiated mice, no marked change in the number of leukocytes was observed during the experimental period.

Figure 5 shows that in the chitosan-diet mice the erythrocytes decreased to $630 \times 10^6/\mu l$ on day 8, and gradually recovered to a value of $730 \times 10^6/\mu l$ on day 14. For the standard-diet group, the counts decreased to a minimal value of about 40% ($340 \times 10^6/\mu l$) on day 14 and slowly recovered thereafter. But the counts were not significantly affected from day 14 in either diet group.

Figure 6 shows the thrombocyte counts. In irradiated mice the thrombocyte counts decreased to the minimum value, which was $5 \times 10^4/\mu l$ for the standard-diet mice on day 8. The counts decreased to $0.8 \times 10^4/\mu l$ on day 14, and then slowly increased thereafter to $55 \times 10^4/\mu l$ on day 21. On the other hand, the counts of the chitosan-diet mice reached an almost normal level on day 14. Figures 7 and 8 show that the recoveries of the hematocrit and hemoglobin levels were enhanced by the chitosan during the experimental period, but the counts of the standard-diet mice remained lower; they were especially lower on day 14.

These data showed that chitosan induced a significant increase in the ratio of the spleen-to-body weight and the level of hematocrit and hemoglobin in irradiated mice.
Table 1 shows scavenging abilities of chitosan and reference material (N-acetylglucosamine). The values are expressed as % formation of O$_2^-$ or ·OH. The scavenging ability of O$_2^-$ in chitosan and the reference material was very low and the respective % formations of O$_2^-$ were 83 and 100%. However, the scavenging ability of ·OH in chitosan was high, and the respective % formations in chitosan and the reference material produced by the reaction of Cu(en)$_2$ were 20 and 84%. Also, the respective % formation of ·OH in chitosan and reference material produced by the reaction of H$_2$O$_2$ using an ultraviolet-ray system were 40 and 64%.

**DISCUSSION**

Many substances which exert radiation-protection effects in mice have been reported. Yonezawa’s group reported that a single intraperitoneal injection of partially purified ginseng extract after X-ray irradiation of 6.5–6.75 Gy significantly increased the 30-day survival ratio in mice$^{24,25}$. They also found that a Shigoka extract prepared from *Acanthopanax senticosus* increased the survival ratio in mice$^{26}$. They supposed that the increase in the survival ratio in mice by the two extracts was closely related to the recovery of the thrombocyte count and prevention of the hemorrhagic tendency. In this experiment, the survival rates in chitosan-fed C3H/He mice were at least 20% higher than those of the standard-diet group after 7Gy of X-ray irradiation; it was also clear that the initial recovery rate varied inversely with the received diets. In the experiment reported here, somewhat different patterns of illness, death, and recovery emerged between the chitosan-diet group and standard-diet group after X-ray irradiation. Initially it had been expected that the two curves would coincide for bone-marrow death, where it was presumed that the conditions of the bone marrow would contribute markedly to the radiation effect. Therefore, the survival rate depended strongly on the diet factors, particularly chitosan, in this study.

In general, mice subjected to a single whole-body exposure of a sub-lethal dose develop anorexia and diarrhea and lost weight prior to a death$^{27}$. The radiation-induced decrease in body weight, which appeared on day 14 for both diet groups may reflect the presence of a severe physiological burden. The decrease in the body weight was not very severe on day 14 for chitosan-fed mice, even though a radiation-induced decrease in the body weight appeared in both diets groups during the experimental period (Fig. 2). The onset and progressive severity of this decreasing trend in body weight following irradiation was likely to be related to the physiological burden, and reflected the ability of chitosan in the diet to prevent body weight loss. In studies with mice given ginseng extract, the weight changed only slightly post irradiation$^{28}$. Therefore, the results in this study suggested that chitosan in the diet protected mice from radiation.
effects, including body weight loss.

Quantitative relationships between the radiation dose and time dependent changes in the weight of various tissues have been reported\(^2\). Any tissue showing a loss of cellularity as a result of radiation exposure is a potential candidate for use as a quantitative indicator. Commonly used tissues include spleen, thymus and testis. There is an initial intense breakdown of cells after radiation exposure, followed by atrophy of the organs due to cell depletion, and then a gradual recovery. Previously, Bond et al.\(^3\) reported that histological evidence of injury was most easily produced in hemopoietic cells after irradiation. They reported that hemapoiesis gradually reappeared by days 10 to 14, and the bone marrow might actually appear hyperplastic; a similar sequence occurred in lymphoid tissues, such as the spleen. In the present experiments, chitosan stimulated rapid recovery of the spleen weight of irradiated mice from days 8 to 21, while the events in standard-diet mice followed a somewhat slower time course. Similar results were observed by Takeda et al.\(^4\) after the injection of ginseng extract as a radioprotective material. Takeda et al.\(^4\) also reported that the extracts stimulated the recovery of the splenic weight from day 6 in irradiated mice. The present findings were in agreement with the earlier results, and suggested that chitosan may decrease the extent of loss as well as stimulate the recovery of splenic weight in irradiated mice.

Other measured data, such as the numbers of blood cells, hematocrit counts and incidence of mitotic figures, can be quantified with the radiation dose. Chitosan induced a rapid increase in the leukocyte counts as one of the parameters tested from days 8 to 21 in irradiated mice (Fig. 4). In the standard-diet group, however, the leukocytes counts increased slowly compared with the chitosan-fed mice after irradiation. These results suggested that the stimulated recovery of leukocytes was one of the important factors for the restoration of radiation effects. Moreover, the leukocyte counts after exposure seemed likely to have resulted from stimulation of the leukocytogenetic system by the chitosan.

A significant depression in the number of thrombocytes was seen following X-ray irradiation. In this study, thrombocytes, like leukocytes, also showed an initial increase, followed by a gradual decline to very low levels in the chitosan diet group, and were the same level as in the standard diet group. The minimum values occurred at day 8, followed by a fairly rapid recovery to near normal levels at 14 to 21 days in the chitosan diet mice. It was previously reported that the mortality within this period generally results from hemopoietic injury, leading to hemorrhage, and a radiation-induced decrease in thrombocytes appeared by day 14 post irradiation\(^2,3\), and showed that the level of thrombocytes was enough to prevent and/or protect the mice from progressive mortality. Therefore, the rapid increase in thrombocytes counts following irradiation was likely to be related to the onset of a hemopoietic effect, including hemorrhage for mice on the chitosan diet. The trends of the hemoglobin and hematocrit levels, which are regarded as being one of the important causes for hemopoietic damage after irradiation, were similar to other parameters in the chitosan-diet mice, and a similar finding has been discussed previously\(^4\). These results suggested that chitosan acted as a specific stimulus to hematopoietic and leukogenic regeneration.

On the other hand, radioprotective agents generally have a role as free radical scavengers. Metallothionein, the synthesis of which can be induced by metalloelement administration, is a known radical scavenger, and metallothionein is considered to act as a radioprotector by scavenging radicals\(^5\). In these experiments, the scavenging abilities of chitosan were evaluated by the ESR spin trapping method. The scavenging ability of \(\text{O}_2^-\) in chitosan was very low. This means that chitosan is not an inhibitor of \(\text{O}_2^-\). However, the scavenging ability of \(\text{OH}^-\) in chitosan was high. This suggested that chitosan is a radical scavenger of \(\text{OH}^-\). The % formation of \(\text{OH}^-\) produced by the reaction of Cu(en)\(^2+\) was lower than that of \(\text{H}_2\text{O}_2/\text{UV}\) using an ultraviolet-ray system. This result indirectly suggested that chitosan probably acted by chelating the Fe(II) or Cu(I) ion in the Fenton reaction. Hannyou et al.\(^6\) investigated the radical scavenging activity of pectic oligosaccharides, chitooligosaccharides, gulcosamine and chitosan by ESR. They showed that low-molecular-weight chitosan had a high radical scavenging ability for \(\text{OH}^-\) and a low radical scavenging ability for \(\text{O}_2^-\). These results were in complete agreement with the present results.

In conclusion, the present observations suggested that chitosan led to hematopoietic activation and leukocytenogenesis in mice after sub-lethal dose irradiation, and that the biological response might be caused by radical trapping or scavenging.

### REFERENCES

1. Yonezawa, M. (1976) Restoration of radiation injury by intraperitoneal injection of ginseng extract in mice. J. Radiat. Res. 17: 111–113.
2. Worm, K. H., Klimeczuk, U. and Schulte-Frohlinde, D. (1993) Radiosensitization and radioprotection of \(E. coli\) by alcohols. Int. J. Radiat. Biol. 64: 485–495.
3. Rea, A., Guenechea, G., Bueren, J. A. and Magando, G. (1992) Radioprotection mediated by the haemopoietic stimulation conferred by AM-5: a protein-associated polysaccharide. Int. J.

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**Table 1.** Scavenging abilities of chitosan and reference material against \(\text{O}_2^-\) and \(\text{OH}^-\). The values are expressed as % formation of \(\text{O}_2^-\) or \(\text{OH}^-\)

| Material             | % formation of \(\text{O}_2^-\) | % formation of \(\text{OH}[\text{Cu(en)}_2]\) | % formation of \(\text{OH}[\text{H}_2\text{O}_2/\text{UV}]\) |
|----------------------|-------------------------------|---------------------------------|---------------------------------|
| Chitosan             | 83                            | 20                              | 40                              |
| N-Acetylglucosamine  | 100                           | 84                              | 64                              |

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Rad. Biol. 62: 65–72.

4. Guenechea, G., Albella, B., Bueren, J. A. Magando, G., Tuduri, P., Guerrero, A., Pivel, J. P. and Real, A. (1997) AM218, a new polysaccharide induces radioprotection in mice when administered shortly before irradiation. Int. J. Radiat. Biol. 71: 101–108.

5. Fedorocko, P., Brezani, P. and Mackova, N. O. (1992) Radioprotection of mice by the bacterial extract Broncho-Vaxom®: haemopoietic stem cells and survival enhancement. Int. J. Radiat. Biol. 61: 511–518.

6. Furuse, M., Tsuneoka, K., Uchida, K. and Nomoto, K. (1997) Acceleration of granulocytic cell recovery in irradiated mice by a single subcutaneous injection of a heat-killed Lactobacillus casei preparation. J. Radiat. Res. 38: 111–120.

7. Landauer, M. R., McChesney, D. G. and Ledney, G. D. (1997) Synthetic trehalose dicorynomycolate (S-TDCM): Behavioral effects and radioprotection. J. Radiat. Res. 38: 45–54.

8. Walden, Jr., T. L. and Farzaneh, N. K. (1995) Radioprotection by 16,16 dimethyl prostaglandin E2 is equally effective in male and female mice. J. Radiat. Res. 36: 1–7.

9. Joshima, R., Ohara, H. and Aoki, Y. (1992) The effect of OK-432 upon erythropoietic recovery in sub- lethally irradiated mice: A preliminary report. J. Radiat. Res. 33: 290–300.

10. Sato, K., Ichimasa, M., Miyahara, K., Sugiomi, M., Nishimura, Y. and Ichimasa, Y. (1999) Radioprotective effects of sodium tungstate on hematopoietic injury by exposure to 10Co γ-rays in Wistar rats. J. Rad. Res. 40: 101–113.

11. Cherupally, K. K. N, Dillip, K. P.and Nomura, T. (2001) Radioprotectors in radiotherapy. J. Radiat. Res. 42: 21–37.

12. Murata, R., Nishimura, Y., Hiraoka, M., Abe, M. and Satoh, M. (1995) Manganese chloride treatment does not protect against acute radiation injury of skin or crypt cells. Radiat. Res. 143: 316–319.

13. Muzzarelli, R. A. A., Jeniaux, C. and Goody, G. W. (Eds) (1986) Chitin in Nature and Technology, Plenum Press, New York

14. Gebelein, C. G. (Ed) (1986) Advances in Biomedical Polymers, Plenum Press, New York

15. Machinami, R., Kifune, K., Kawaide, A. and Tsurutani, R. (1991) A histological study of the fate of chitin suture material after intramuscular suturing. Med. Sci. Res. 191–392.

16. Nishimura, Y., Watanabe, Y., Hong, J. M., Takeda, H., Wada, M. and Yukawa, M. (1997) Intestinal absorption of 14C-chitosan in rats. Chitin Chitosan Res. 3: 55–61.

17. Nishimura, Y., Takada, H., Inaba, J., Ishii, K., Watarai, K. and Mustusaka, N. (1994) Effect of natural chelating agents on the intestinal absorption of radiostrointium in rats. Radiat. Protect. Dosim. 53: 331–334.

18. Rossen, G. M. and Rauckman, E. J. (1984) Spin trapping of superoxide and hydroxyl radicals. Methods Enzymol. 105: 198–209.

19. Janzen, E. G. (1971) Spin trapping. Accounts Chem. Res. 4: 31–40.

20. Janzen, E. G. (1984) Spin trapping. Methods Enzymol. 105: 188–198.

21. Fridovich, I. J. (1970) Quantitative aspect of the production of superoxide anion radical by milk xanthin oxidase. Biol. Chem. 245: 4053–4057.

22. Ueda, J., Ikota, N., Hanaki, A. and Ozawa, T. (1994) Synthesis of new oligopeptides against active oxygen species. Biochem. Mol. Biol. Int. 33: 1041–1048.

23. Cheung, S.H. and Holland, B. (1992) Extension of Dunnett’s multiple comparison procedure with different simple sizes to the case of several groups. Comput. Stat. Data Anal. 14: 165–182.

24. Yonezawa, M. (1976) Restoration of radiation injury by intraperitoneal injection of Ginseng extract in mice. J. Radiat. Res. 17: 111–113.

25. Yonezawa, M., Katoh, N. and Takeda, A. (1981) Restoration of radiation injury Ginseng extract. II. Some properties of the radioprotective substances. J. Radiat. Res. 22: 336–343.

26. Yonezawa, M., Katoh, N. and Takeda, A. (1989) Radiation protection by Shigoka extract on split-dose irradiation in mice. J. Radiat. Res. 30: 247–254.

27. Abrams, H. L. (1951) Influence of age, body weight and sex on susceptibility of mice to the lethal effects of X-radiation. Proc. Soc. Exp. Biol. Med. 76: 729–732.

28. Takeda, A., Yonezawa, M. and Katoh, N. (1981) Restoration of radiation injury by Ginseng. I. Responses of X-irradiated mice to ginseng extract. J. Radiat. Res. 22: 323–335.

29. Woodward, K. T. and Rothermel, S. M. (1956) Observation on gastrointestinal function after X-ray and thermal colon exposure. 1. Effect on the progress of barium, metal, body weight, and survival. Radiat. Res. 5: 441–449.

30. Kohn, H. I., Kallman, R. F. and Berdijis, C. C. (1957) Late effects of whole-body X-irradiation in the mouse some gross and histologic aspects of the development of morbidity prior to the terminal state, with special reference to the gonad, uterus, heart, kiver, kidney, and submaxillary gland. Radiat. Res. 7: 407–435.

31. Bond, V. P., Silverman, M. S. and Cronkite, E. P. (1954) Pathogenesis and pathology of post-irradiation infection. Radiat. Res. 1: 389–400.

32. Nakamura, W., Kankura, T. and Eto, H. (1971) Occult blood appearance in feces and tissue hemorrhage in mice after whole body X-irradiation. Radiat. Res. 48: 169–178.

33. Nakamura, W., Furuse, T. and Kasuga, T. (1976) Comparative studies on radiation-induced thrombocytopenic death in SPF and CV mice of same strain. The 16th Int. Cong. of Hematol., Abst., p. 352.

34. Storer, J. (1966) Acute response to ionizing radiation. In: Biology of the Laboratory Mouse. 2nd ed., pp. 427–446, Ed. Staff of the Jackson Laboratory/E. L. Green, McGraw-Hill, New York

35. Masutahara, J., Shida, T., Ishioka, K., Egawa, S., Inada, T. and Machida, K. (1986) Protective effect of zinc against lethality in irradiated mice. Environ. Res. 31: 558–567.

36. Hanmyou, K., Namikawa, H., Saito, T., Ohkami, H. and Tazawa, K. (1999) The radical scavenging activity of pectic oligosaccharides from apple pectin evaluated by electron spin resonance (ESR). J. Nursing Toyama Med. Pharm. Univ. 2: 48–57.