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MICRONUCLEUS TEST IN MICE FED ON AN IRRADIATED DIET

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A mutagenicity study was carried out in mice fed on a γ-irradiated diet. As an indicator of mutagenic activity, we observed an incidence of micronuclei in erythrocytes. The average body weight of the mice fed on the diet irradiated to dose range of 400–1,000 kGy decreased, and the mice fed on the 800–1,000 kGy-irradiated diet died during the period from 8 to 14 days after the start of feeding. On the other hand, when the mutagenic activity of the irradiated diet was tested by observing occurrence of micronucleus in erythrocytes, no significant increase was recognized. These results indicated that the irradiated diet had no mutagenic activity, even though it possessed a toxic effect on the growth of mice.

Key words: micronucleus test, erythrocytes, food irradiation, mouse.

INTRODUCTION

Food irradiation is one of important techniques for preservation of foods because of its sterilizing activity. The irradiation induces radiation chemical reactions in the foods, and the radiation chemical reactions may lead to the production of toxic substances. Extensive studies have been carried out on the toxic activity of irradiated foods. In 1976 the Joint FAO / IAEA / WHO Expert Committee stated that "The Committee was presented with evidence on the great similarity in radiolytic products in related foods treated with radiation doses of the order of 10 kGy. It is considered, therefore, that it is possible to generalize to a considerable extent about the irradiation chemistry of foods" (Joint FAO / IAEA / WHO Expert Committee, 1977). Furthermore, in 1981 the Committee stated that "For doses up to 10 kGy the radiolytic products were unlikely to have *a priori* considerable carcinogenic or mutagenic potential. Consequently, testing could be appropriately reduced" (Joint FAO / IAEA / WHO Expert Committee, 1981). On the other hand, for doses over 10 kGy the data for carcinogenic or mutagenic potential of irradiated foods were insufficient to state any

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conclusion (Joint FAO / IAEA / WHO Expert Committee, 1981).

To assess the mutagenic potential of irradiated foods which were irradiated over 10 kGy, a number of studies could be referred. Many sorts of mutagenic products were detected in the irradiated foods by radiation chemical techniques (Schubert, 1969, Aiyar & Subba Rao, 1977, Basson et al., 1977, Thomas & Beyers, 1979). Some reports gave positive evidence for the mutagenic activity of irradiated foods in micro-organisms (Muenzner & Renner, 1975, Ishidate et al., 1981, Niemand et al., 1983), mammalian cells (Berry et al., 1965, Shaw & Hayes, 1966, Hills & Berry, 1967), mice (Moutschen-Dahmen et al., 1970, Leonard et al., 1977), and rats (Vijayalaxmi, 1975, Vijayalaxmi & Sadasivan, 1975). On the contrary, some reports showed no evidence for the mutagenic effects in mice (Reddi et al., 1977, Chauhan et al., 1975, Levinsky & Wilson, 1975), and rats (Eriksen & Emborg, 1972, Chauhan et al., 1976). This discrepancy may be attributed to the difference of detection methods depending on both the biological species and concentrations of produced mutagens (Conning, 1983). The mutagenic potential of the radiolytic products is also considered to be different between species. Thus, experiments using mammalian are recommended (Elias, 1983). As tests using mammalian are usually less sensitive than the method using micro-organisms, we have used as high irradiation dose as possible to increase the concentration of radiolytic products, and also employed a micronucleus test as used a sensitive method for the detection of mutagenicity.

MATERIALS AND MHTHODS

Animals

Female C3H / He mice (4 weeks old) were obtained from Shizuoka Laboratory Animal Center, Shizuoka, Japan and maintained under CV condition. The mice were allowed for free access to tap water. The room temperature was kept at 20±1℃ throughout the experiments. A diet (MB-1) was obtained from Funabashi Farms, Chiba, Japan, and irradiated. The mice were fed on a irradiated diet from 28th day after birth and continued throughout the experiments.

Irradiation and diet preparation

The diet consisted of proteins (24.5%), lipids (4.4%), fibers (3.6%), ash content (5.5%), water (7.0%), water-soluble non-nitrogen content (55%) and traces of vitamins. This was exposed to 100–1,000 kGy of γ-rays using a 2,000 Ci ^{60}Co gamma source at a dose rate of 17 kGy / hr. The dose rate was determined by Fricke dosimeter (Fricke & Hart, 1966).

Micronucleus test

The micronucleus test was carried out 7th day after the start of feeding mice with irradiated diet essentially according to the method by Schmid (1976). Immediately after sacrifice of the mice, both femora were removed and freed from muscle by the use of gauze and fingers. The proximal end of the femur was carefully shortened

with scissors until a small opening to the marrow canal became visible. One fifth ml of calf serum was injected into the canal using a syringe with 26 gauge needle. Then the femur was submerged in the serum which filled a centrifuge tube. By gentle aspirations and flashings, the marrow was forced out through the opening around the needle. The tube was centrifuged at 1,000 rpm for 5 min. The supernatant was drawn off with a pipette. The cells in the sediment were carefully mixed by repeated aspiration into the capillary part of a pipette. A small drop of the viscous suspension was put on the end of a slide and spread. The preparations were air dried at room temperature and then stained sequentially with undiluted May-Gruenwald's solution for 3 min and subsequently with 1 / 2 diluted May-Gruenwald's solution for 2 min. The stained preparations were shortly rinsed twice with distilled water, counter-stained with 1 / 6 diluted Giemsa's solution for 10 min and rinsed with distilled water. Remained water was rejected by blotting to fillter papers and by dipping in methanol. The preparations were mounted after xylene for 5 min. Five animals were taken as an experimental group and about 2,000 erythrocytes were scored from each animal.

Results

The average body weight of the mice fed on irradiated diet was plotted against the days after the beginning of feeding (Fig. 1). When the mice were fed on the diet

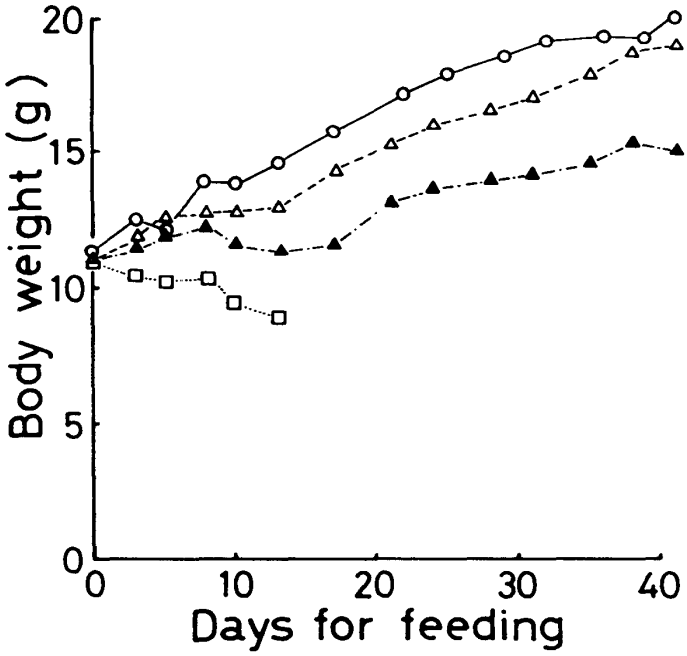

Figure 1 Average body weight of mice fed on irradiated diets.
○; Control, △; 100 kGy, ▲; 400 kGy, □; 1000 kGy.

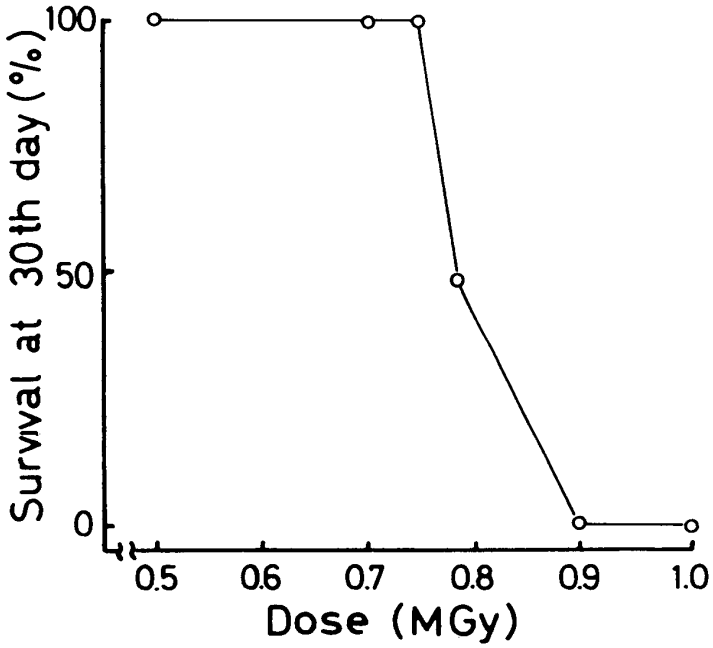

FIGURE 2 Percent survival of mice which fed on irradiated diets for 30 days (n =7−14).

irradiated with 400 to 1,000 kGy, the average body weight tended to decrease. The mice fed on 1,000 kGy-irradiated diet died during the period from 8 to 14 days after the start of feeding. On the other hand, there is no influence on the average body weight of mice when the diet was irradiated with γ-rays below 100 kGy. Figure 2 shows the survival of mice fed on irradiated diets for 30 days. When the mice were fed on the diet exposed to irradiation doses over 800 kGy, they died during the period from 8 to 14 days.

Table 1 shows the data on the frequency of polychromatic erythrocytes. The

TABLE 1 Frequency of polychromatic erythrocyles and erythrocytes with micronuclei in mice fed on the irradiated diet

γ-Ray dose to diet (kGy)	0	700	800	900	1000
% Polychromatic-erythrocytes ± S.D.	52.30 ±4.07	50,22 ±4.23	51.18 ±10.11	44.06 ±5.88	46.74 ±6.76
% Erythrocytes with micronulei ± S.D.	0.34 ±0.14	0.39 ±0.13	0.43 ± 0.14	0.41 ±0.14	0.18 ±0.18

data suggest that there is no alteration in the maturation of erythrocytes. This table also shows the data on the incidence of micronuclei in the bone-marrow of the fed mice. It is evident that the irradiated diet induced no significant increase in the incidence of micronuclei in the erythrocytes.

Discussion

When the mice were fed on the diet treated with γ-ray of 700 −1,000 kGy, their average body weights were drastically decreased. Nutritional change in the irradiated foods was reported in these high dose ranges (Murray, 1983). It was suggested that the nutritional change in the foods affected the body weights of animals. On the other hand, it was indicated that there is no effect on the maturation of erythrocytes (Table 1). As micronuclei are formed at the time of maturation of erythrocytes in a bone marrow, detection of mutagen was thought to be possible even if the side effect is observed (Schmid, 1976). No increase in the incidence of micronuclei strongly suggested that the irradiated diet had no mutagenic activity because radiolytic products would exist in high concentration in the irradiated diet. These results are comparable to those of Reddy et al. (1981) who reported lack of micronucleus formation in bone-marrow cells of mice fed on irradiated diet for 7 days. In contrast to these studies, an increase in the chromosomal anomalies in bone-marrow cells was observed in rats which were fed on irradiated wheat (Vijayalaxmi & Sadasivan, 1975). These data with the bone -marrow cells of rats which were fed on irradiated wheat were, however, not confirmed by George et al. (1976). The lack of cytogenetic effects in the present investigation provides an additional evidence for the lack of mutations in test animals.

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