THE ROLE OF FATTY ACIDS ON RECOVERY OF MICE EXPOSED TO TOTAL BODY IRRADIATION*

By

Yoshimasa Tanaka, Takaji Hashimoto,
Shigeaki Imai and Kiwamu Yamano
Department of Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan
(Director: Prof. M. Fukuda)

被照射マウスの回復に対する脂肪酸の意義

京都大学医学部放射線医学教室（指導：福田正教授）
田　中　敬　正，　橋　本　隆　治
今　井　重　昭，　山　野　　究

不飽和脂肪酸，とくに必須脂肪酸の放射線障害の回復に関する研究は，多数行われているが，之等のメカニズムや必要な量的関係の研究は極めて少い．著者等は，d-d 系マウスを無脂肪食群と，リノレイン酸源としてサフラワ油（1，5，10，20%），メチール・リノレイン酸（1%）を，オレイン酸源としてオリーブ油（5%）を，更に飽和脂肪酸源として大豆硬化油添加群（5%）に分ち，25日間飼育後，全身1時照射と分割照射を行い，生存率，生存日数，体重の変動及び鉄代謝を測定し以下の如き成績を得た．

1. 無脂肪群は，不飽和脂肪酸添加群に比べ，1時照射，分割照射共に，著明に生存率及び生存日数の低下を認めた．
2. しかし20%サフラワ油添加群は，10%のものに比べ延命効果が少なかつた．
3. 1%サフラワ油を加えたものは，無脂肪食群に比べ明らかな延命効果を認めたが，10%添加のものに比べ著明な差がなかつた．
4. 5%大豆硬化油を加えたものは無脂肪食群に比べむしろ有害であつた．
5. 体重変化も不飽和脂肪酸を加えたものは，無脂肪食群に比べ，減少は僅少に止まつた．
6. 強制的不飼和脂肪酸投与は（胃ゾンデによる）むしろ有害であつた．
7. 5%サフラワ油添加群のマウスの ^{59}Fe の摂取率は，無脂肪食群に比べ著明に高値を示し，必須脂肪酸は骨髄障害の回復に有効であると考えられる．

1. Introduction

Recently, a large number of substances, some chemicals[2)4)] and other complex nucleoprotein[1)3)] from tissue have been reported to accerelate animals to recover from lethal radiation. It is a very important problem to study the post-irradiation recovery in the field of radiation therapy.

The study of a correlation between the survival of animals exposed to total-body irradiation and dietary fats has been reported[5)6)7)8)]. Obese mice have been observed to survive irradiation longer than have normal mice[9)]. The polyunsaturated fatty acids especially essential fatty acids present in the

*Dedicated to Professor M. Fukuda on the Occasion of his 60th Birthday.

fat have been shown to be the effective in such recovery[10)11)12)13)]. However, little is known concerning the necessary amounts of these substance and the mechanism of such recovery. In recent years, M. Maqsood[14)] have shown that olive oil therapy has the effect of cellular regeneration of bone marrow.

The present paper has been concerned with a study of the recovery effects of dietary fat, especially essential fatty acids and the effects on the bone marrow functions of the irradiated mice. The experiment was performed with single and multiple sublethal total body ^{60}Co irradiation both. The forced feeding of polyunsaturated fatty acids was also described.

2. Experimental Methods

The experimental diets presented in Table 1 were used. Dietary component of 10% safflower oil is shown in this Table. Weanling male mice of d-d strain weighing 10–12 g were divided into several groups 30 to 40 each. The animals of control groups were fed on fat-free diets.

Safflower oil containing 69.5% linoleic acid were used as linoleate supplement diet and olive oil containing 64.6–84.4% oleic acid were used as oleate supplement diet.

Table 1. The percentage composition of the diets used

Dietary component	Linoleate suppl. diet	Fat-free diet
Casein	20	20
Sucrose	66	76
Safflower oil **	10	—
Salt mixture *	4	4

* The following composition was employed :
$Ca_3(PO_4)_2$, 410; K_2HPO_4, 206; $MgSO_4$ $7H_2O$, 120 ; NaCl, 100; Fe citrate, 26; $MnSO_4H_2O$, 4; $ZnCO_3$, 2; KI, 1 ; $CuSO_4$ $5H_2O$, 1.

** Fatty acid distribution in safflower oil, analysed by gas-liquid chromatography; C_{12} Lauric 0.1%, C_{14} Myristic 0.4%, C_{16} Palmitic 7.2%, C_{18} Stearic 3.7%, C_{18} Oleic 14.4%, C_{18} Linoleic 69.5%, C_{20} Unsaturated No. 1 2.1%, C_{20} Unsaturated No. 2 2.7%.

Fatty acid distribution in olive oil,was as follows; C_{14} Myristic 0.1–1. 2%, C_{16} Palmitic 6.9–15.6%, Stearic 1.4–3.3%, C_{18} Oleic 64.6–84.4%, Linoleic 3.9–15.0%.

As is well known, chemically refined linoleic acid is easilly oxidizable, and furthermore, large dosages may be actually toxic[15)]. On the other hand, linoleate in vegetable oil (safflower oil) hardly shows such effects. The synthetic ration was supplemented with the following components (in mg. per 100 g):

pyriodoxine hydrochloride, 1.5; nicotinic acid, 10.0; thiamine mononitrate, 1.0; riboflavin, 1.5; Ca pantothenate, 2.5; folic acid 0.15; biotine, 0.05; p-aminobenzoic acid, 30.0; inositol, 50.0; choline chloride, 100.0; vitamine E, 5 mg; vitamine A acetate, 2500 unit; vitamine B_{12}, 1.0; ascorbic acid, 37.5; vitamine K, 0.1.

The animals were fed daily, and all food not consumed 24 hours after feeding was discarded. Food and water provided ad libitum. After 25 days of feeding, all mice received an exposure of ^{60}Co-irradiation. Another groups of mice were irradiated with 250 to 400R of total-body ^{60}Co-irradiation which was repeated about once weekly until dose of 1750R (7 exposures) had been administered in experiment 2, and 1200R (4 exposures) in experiment 3.

Total body irradiation were given at the rate of 40R/min. with a ^{60}Co therapy machine. The dosage was measured on the bottom of the wooden box with a Victoreen Chamber.

In experiment 4, mice were given by stomach tube 1 ml of safflower oil and olive oil. Forced feeding was began every day after irradiation and continued for 6 days. Control animals irradiated in a similar manner were given by stomach tube 1 ml of water instead of oil.

In experiment 5, ^{59}Fe uptake were measured with the method reported before[16]. Irradiated and control mice were injected with approximately 0.1 μc of ^{59}Fe citrate at 24 hours after total body irradiation. Injection were made into the tail vein. Animals were killed at every experiment and the blood was drawn from heart puncture.

3. Experimental Results

Experiment 1. This experiments were performed to determine the comparative effects of feeding the basal fat-free diet and a similar ration supplemented with 10, 5, 1% safflower oil and 5% hydrogenated bean oil on the percentage of survival exposed to single total body ^{60}Co irradiation.

Results are summarized in Table 2 and Fig. 1. The thirty day survival rate of mice exposed to total body irradiation was significantly less on the fat free diet than on a similar ration supplemented with 10.5 and 1% safflower oil. The group supplemented with only 1% safflower oil showed significantly increased thirty day survival rate compared with fat free diet.

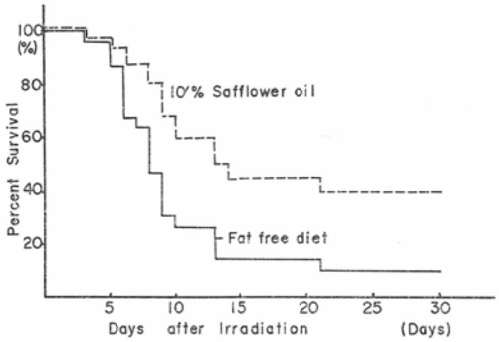

Fig. 1. Percent survival of mice exposed to 650 R total body ^{60}Co-irradiation on fat free diet and 10% safflower oil groups.

Table 2. Comparative effects of a fat free diet and a similar ration supplemented with 10, 5,1% safflower oil and 5% hardened oil on the 30 day survival rate of mice administered single total body ^{60}Co irradiation (650 R).

Dietary group	No. of animals	30 day survival rate	P (Chi-square)
Fat free diet	30	10.0	—
10% safflower oil	30	40.0	P<0.02
5% safflower oil	30	36.7	P<0.04
1% safflower oil	30	33.3	P<0.05
5% hardened oil	31	6.5	—

Experiment 2. This experiments were performed to determine the effects of supplements of 10, 20% safflower oil, 5% hardened oil on the average survival time and survival rate of mice administered fat free diet and exposed to multiple sublethal dose. After 25 days of feeding, all mice received an exposure of 250R of total body ^{60}Co irradiation which was repeated one weekly until a total dose of 1750R (7 ex-

posures). Experimental results were shown in Table 3 and Fig. 2–4.

The percentage survival of these groups were similar up to the 30th day after first irradiation, but in the succeeding days groups supplemented with safflower oil showed a highly significant increase in percent survival than fat free groups. Furthermore, mice fed with 10% safflower oil had higher survival than 20% groups.

The body weight gains in the case of mice supplemented wigh 10% safflower oil were significantly higher than those of the fat free diet as shown Fig. 4.

Table 3. Comparative effects of a fat free diet and a similar ration supplemented with 10, 20% safflower oil and 5% hardened oil on the survival rate and average length of survival of mice administered multiple sublethal doses of total-body ^{60}Co-irradiation.

Dietary group	No. of animals	Survival rate at 70 day after first irrad.	average length of survival after first irrad. (day)
Fat free diet	40	2.5%	48.5±2.4
10% safflower oil	40	70.0%	63.0±2.74
20% safflower oil	38	45.0%	57.4±3.0
5% hardened oil	40	0%	35.0±2.5

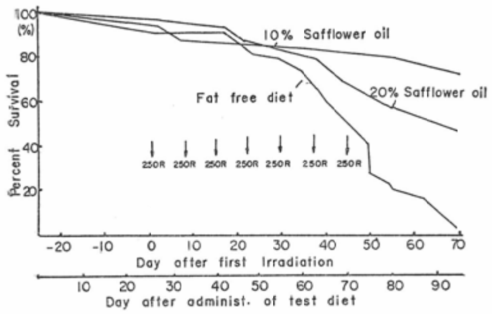

Fig. 2. Percent survival of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation on fat free diet and 10,20% safflower oil groups.

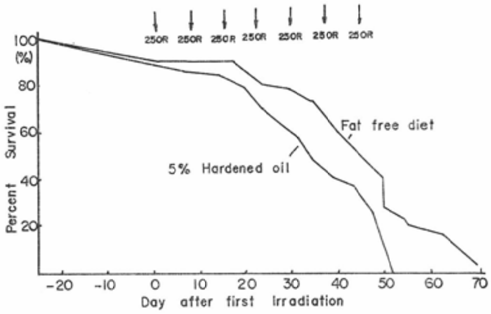

Fig. 3. Percent survival of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation on fat free diet and 5% dardened oil groups.

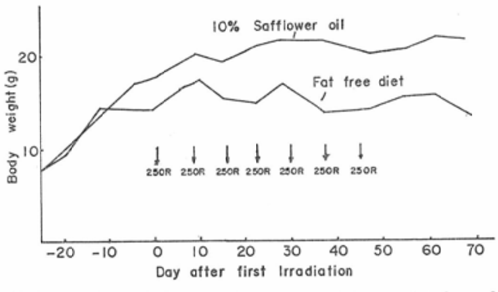

Fig. 4. Body weight (g) of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation on fat free diet and 10% safflower oil.

Table 4. Comparative effects of a fat free diet and a similar ration supplemented with 5,1% safflower oil, 5% olive oil, 1% methyl linoleate and 5% hardened oil on the survival rate and average length of survival of mice administered multiple sublethal doses of total body ^{60}Co-irradiation.

Dietary group	No. of animals	Survival rate at 45 day after first irrad. (%)	average length of survival after first irrad. (day)	P (Chi-square)
Fat free diet	38	10.5	16.2±1.87	—
5% safflower oil	39	33.4	31.2±1.89	P<0.05
1% safflower oil	39	23.0	29.8±2.02	P<0.05
5% olive oil	40	30.0	30.0±1.84	P<0.05
1% methyl linoleate	38	23.6	28.2±1.96	P>0.05
5% hardened oil	38	0	14.9±0.8	—

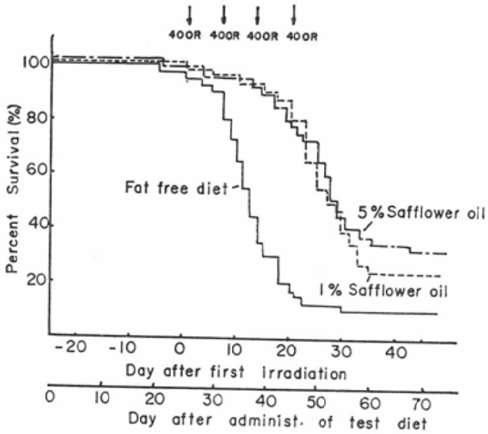

Fig. 5. Percent survival of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation (4 exposure) on fat free diet and 1,5% safflower oil groups.

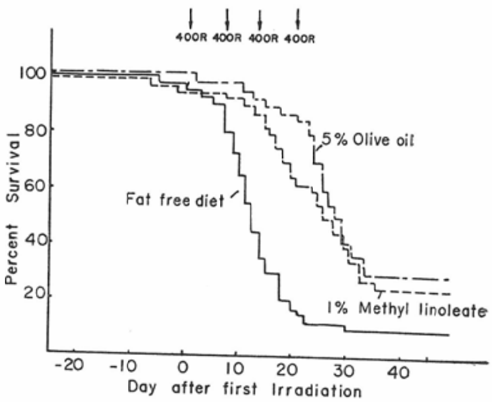

Fig. 6. Percent survival of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation (4 exposures) on fat tree diet, 5% olive oil and 1% methyl linoleate groups.

Experiment 3. This experiment was performed about the same method as exp. 2. The effects of supplements of 1, 5% safflower oil, 5% olive oil, 1% methyl linoleate and laboratory chow (Oriental) on mice exposed to multiple sublethal irradiation were examined. After 25 days of feeding, all mice received an exposure of 400R of total body^{60}Co irradiation which was repeated one weekly until a total dose of 1600R (4 exposures). Experimental results are shown in Table 4 and Fig. 5–8.

The mice fed with these polyunsaturated fatty acid had significantly higher survival than fat free diet groups. It is very interesting to find that the groups supplemented with only 1% safflower oil had a remarkable effect and not different from 5% safflower oil.

Experiment. 4. The forced feeding groups (water, olive oil and safflower oil) given 850R total body irradiation are presented in Fig. 9. Each groups consisted of 30 mice with an average weight close to 20 g. (7 weeks). The percent survival of mice with forced fed olive oil and safflower oil were much lower than water as shown Fig. 9. The mice fed oil had more server diarrhea and lower food consumption than irradiated control forced fed water.

Experiment. 5. Radioiron was injected at one day after total body irradiation with 300R and

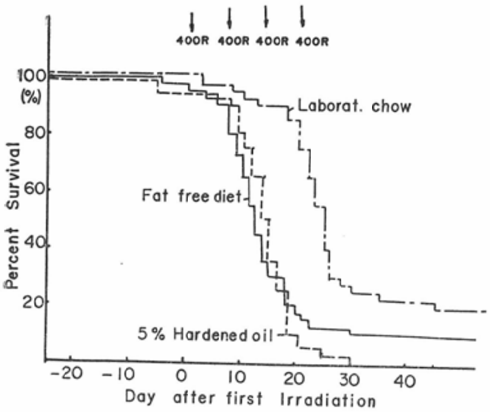

Fig. 7. Percent survival of mice exposed to multiple sublethal doses of total dody ^{60}Co irradiation (4 exposures) on fat free diet, laborat. chow and 5% harden oil groups.

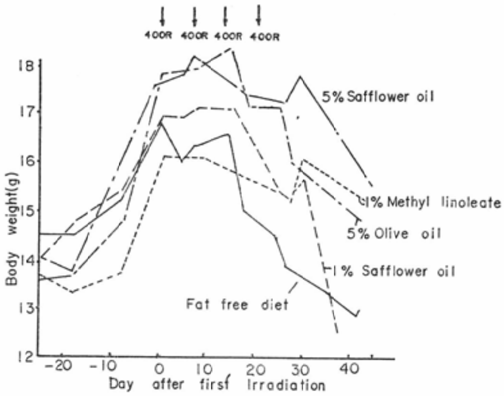

Fig. 8. Body weight of mice exposed to multiple sublethal doses of total body ^{60}Co irradiation on fat free diet, 1,5% safflower oil, 5% olive oil, and 1% methyl linoleate groups.

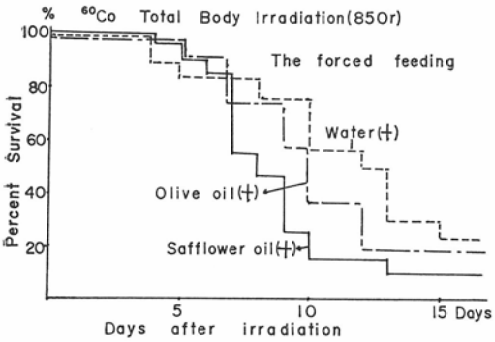

Fig. 9. Percent survival of mice exposed to total body ^{60}Co irradiation and forced-fed olive oil, safflower oil or water.

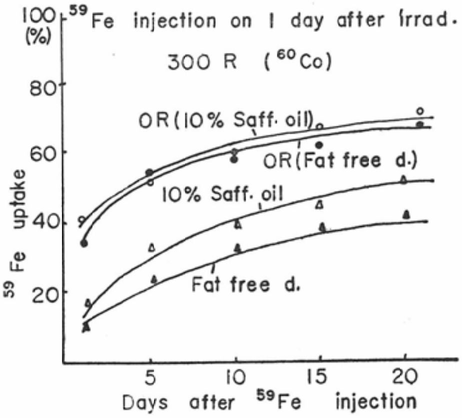

Fig. 10. The comparative effect of a fat free diet and a similar ration supplemented with 1% safflower oil on ^{59}Fe uptake of mice exposed to ^{60}Co total body irradiation with 300 R.

hereafter ^{59}Fe uptake was measured as shown Fig. 10.

After about 4 to 6 days in non-irradiated mice, the uptake of ^{59}Fe in red cells reached its maximum value of around 60% of the injected amount. There were no significant difference between non-irradiated 10% safflower oil group and fat free diet. In the irradiated groups, however, the uptake was depressed very markedly initially and then rose to a constant maximum value at a level lower than that of a group of control animals. The ^{59}Fe uptake on irradiated mice supplemented with fat free diet were more markedly depressed than 10% safflower oil groups.

4. Discussion

It is clear from these data that the mice supplemented with olive oil, safflower oil and methyl linoleate were more radioresistant than the mice fed with fat free diet. The mice supplemented with hardened oil (hydrogenated bean oil) were without effect or rather detrimental. i.e. the percentage survival were decreased than fat free diet groups. The recovery effect of olive oil, safflower oil and methyl linoleate appears to be due to its essential fatty acid content.

Duel[13]), Cheng[16]), et al have demonstrated that rats on fat free diets supplemented with as little as 2% cottonseed oil or 10 mg methyl linoleate are much more radioresistant than animals on a fat free diet when these animals are subjected weekly to repeated sublethal dose of radiation. B.H.Ershoff[7]) also reported that the average survival time of mice exposed to multiple sublethal doses of total body X-irradiation was significantly less on a diet free of fat than on a smilar ration supplemented with 10% cottonseed oil. The essential fatty acids are believed to help animals because of their bactericidal or reticuloendothelial system stimulating actions[14])[17]). Recently, Stuart, Biozzy, Stiffel, Halpern and Mouton (1960)[18]) described that olive oil and glyceryl triolate caused an intense stimulation of phagocytic functions in adult male mice. The stimulating activity is very marked and in some instances the index of phagocytic function was increased ten fold. Further, Maqswood and Ashikawa[14]) reported that olive oil therapy accelerated the rate of cellular regeneration and repopulation in the bone marrow, spleen and thymus of X-irradiated male mice. It may be interest to mention here that Nagai et. al.[19]) demonstrated polyunsaturated fatty acid especially linoleic acid have a role to enhance the resistance to bacterial infections in mice and rats. It appear that stimulation of phagocytic functions and resistance to bacterial infection promote the recovery from radiation injury.

In our experiment, there was no significant difference between content of 1 and 10% safflower oil in percent survival but decreased in 20% safflower oil on the contrary. Cheng[10]) reported that the protective effect of fat was approximately as satisfactory when cottonseed oil was incorporated in the diet at a 2% level as when it comprised 15 or 30% of the diet. The effect of dietary protein and fat on mortality after administration of ^{32}P to mice has been studied by Cornatzer et al.[20]) According to their data, a high protein, high fat diet has a detrimental effect, optimal survival was obtained with a diet containing 10% protein and 5% fat.

Recently, Akin et al[21]) reported that oral forced feeding of fat to rats exposed to various types of irradiation was detrimental to survival. There is evidence that any post-irradiation forced feeding, not only of fats, is detrimental.[22])[23]).

The percent survival of mice fed hardened oil supplement was less than that of animals on the fat free diet as shown experiment 1, 2 and 3. This results are in good agreement with the report of Deuel[24]) and B.E.Ershoff[7]) that the addition of hydrogenated cocconut oil to a fat free diet hastened the appearance of symptoms of essential fatty acid deficiency in the rat.

It is clear from our data that the linoleic acid help the bone marrow function to recover from radiation damage. In brief, the mechanism of post-irradiation recovery of these oil consists in the stimulating effect on reticuloendothelial system or hematopoetic organ and the increase of phagocytic function.

From these mechanisms, it facilitates the increase of resistance to bacterial infections in animal and the rate of regeneration in the irradiated mice by restoring the impaired cell functions and the reticuloendothelial system.

5. Conclusion

The role of polyunsaturated fatty acids especially linoleic acid and oleic acid in the radiation protection and recovery effects in mice were studied. Diets supplemented with safflower oil (1, 5, 10 and 20%), olive oil (5%), methyl-linoleate (1%) hydrogenated bean oil (5%) and fat free diets were employed.

Percent survival, average survival time and body weight were obtained after ^{60}Co irradiation. The following conclusions were obtained;

1) Percent survival of mice exposed to single and multiple sublethal doses of total body irradiation was significantly less on a fat free diet than on a smilar diet supplemented with 1, 5 and 10% safflower oil, methyl-linoleate and olive oil.

2) Safflower oil at 20% level of feeding was less protective effect than 10% safflower oil.

3) The groups supplemented with only 1% safflower oil were markedly effective compared with fat free diet and not significantly different from 10% safflower oil groups.

4) Hardened oil (hydrogenated bean oil) at a 5% level of feeding was rather detrimental.

5) The body weight gains in the case of polyunsaturated fatty acid treated animals were significantly higher than those of fat free diet groups.

6) The effect of forced feeding of safflower oil and olive oil was detrimental.

7) The ^{59}Fe uptake on irradiated mice supplemented with fat free diet were more markedly depressed than 10% safflower oil groups.

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