PAKISTAN JOURNAL
OF
SCIENTIFIC AND INDUSTRIAL RESEARCH

Vol. 5, No. 1 January 1962

# POSTIRRADIATION PROTECTION AND RECOVERY

## Part VI.—Effects of Esters of Unsaturated Fatty Acids on Gonads of X-irradiated Male Mice

M. MAQSOOD*

*Donner Laboratory of Nuclear Biophysics and Medical Physics, Lawrence Radiation Laboratory, University of California, Berkeley, U.S.A.*

(Received August 28, 1961)

Administration of esters of certain unsaturated fatty acids, *i.e.* methyl oleate, and methyl stearate, within an hour following whole -body x-irradiation of male mice at 625 r [$LD_{50(30)}$], accelerated the rate of cellular regeneration in the testes of these animals when compared with the untreated x-irradiated ones, on the 30th day postirradiation. On the other hand esters of certain other unsaturated fatty acids adversely affected the rate of cellular regeneration in the testes of x-irradiated mice. These differences may, in part, be due to differences in the composition of the esters of unsaturated fatty acids used in these experiments. There are indications that lipid therapy, by restoring the cell functions, accelerates the rate of cellular regeneration in the x-irradiated animals.

Recently, Maqsood[17] has described the beneficial effects of olive oil therapy on the rate of cellular regeneration in the sex organs of x-irradiated male mice. Ashikawa[3] reported that the administration of esters of certain unsaturated fatty acids increased the survival rate while others adversely effected the survival rate in the x-irradiated male mice. No information on the effects of these agents on the gonads of x-irradiated animals is available. An understanding of the mechanism of action of various therapeutic agents in the organisms will materially contribute in developing new agents which will have practical applications in the field of radiation protection. The effects of intraperitoneal administration of esters of unsaturated fatty acids on the sex organs of x-irradiated male mice are described in the present paper. The earlier papers of the present series deal with the effects of olive oil therapy on the haematopoietic organs, liver functions, gonads, and male fertility of x-irradiated male mice.[15–18]

*Visiting Research Scientist from Pakistan. Appointment supported by the Internatinal Cooperation Administration under the world wide research scientists programme administered by the U. S. National Academy of Sciences, Washington, D.C.

Now at CENTO Institute of Nuclear Science, Tehran, Iran, on deputation from Pakistan Atomic Energy Commission.

## Materials and Methods

*Mice.*—Webster strain of male Swiss white mice, 6-7 weeks old and weighing 26 ± 4 g. were used for x-irradiation experiments. The animals were caged individually in glass jars. The mice were fed *ad libitum.*

*X-irradiation and Dosimetry.*—The x-ray source was from a Phillips 250 Kv, 25 ma deep therapy unit operated at 200 Kvp, with a 0.5 mm. Cu and 1.0 mm. Al filters. Each mouse received whole-body radiation in a single exposure of 625 r [$LD_{50(30)}$] and a total of 40 mice were x-irradiated simultaneously. The dose rate was adjusted to 20 r/minute. To minimize back scattering, the canisters were separated from the irradiation positioning wheel by means of a 2-inch long aluminum spindle and the wheel was electrically rotated at 3 r.p.m. (Fig. 1).

The experimental groups of mice received a single intraperitoneal injection of 1 ml. of methyl oleate, methyl stearate, methyl linoleate and methyl palmitate, within an hour following irradiation. The mice were killed on the 30th day postirradiation. The testes, seminal vesicles, thymus and spleen from each animal were re-

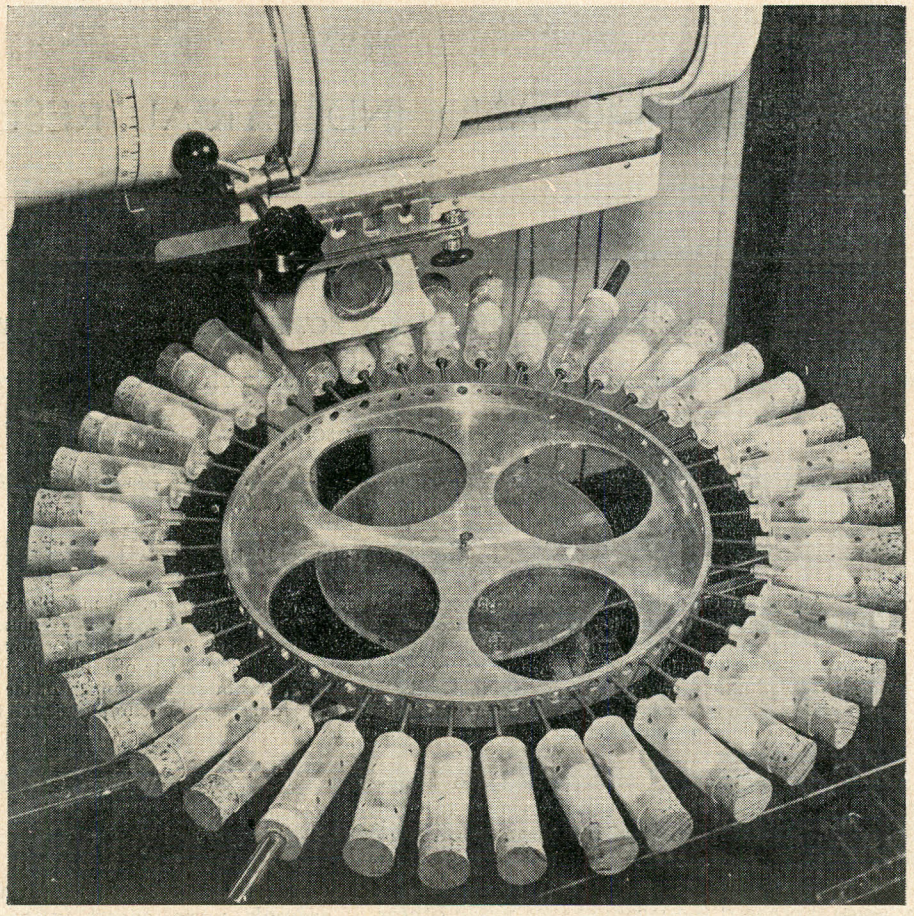

Fig. 1.—Philllip's deep-therapy unit with experimental setup for simultaneously exposing male mice to uniform whole-body x-irradiation. The irradiation positioning wheel, lucite mouse canister and rotator are shown.

moved and weighed separately. The organs were then fixed in Bouin's fluid. The tissues were sectioned at 6-7 microns and stained with H and E stain. The standard error of the mean for each group was estimated $SE = \left[ \sqrt{\frac{Ed^2}{n\,(n-1)}} \right]$.

## Results

### TESTES

*Organ Weight.*—The data in Table 1 show that the administration of methyl oleate and methyl stearate to the x-irradiated male mice resulted in a significant increase in the weight of the testes when compared with the testes weight of the methyl linoleate and methyl palmitate treated x-irradiated male mice. Furthermore, the testes weight of the methyl oleate and methyl stearate treated x-irradiated mice was significantly higher than that of the x-irradiated mice. The testes weights of the methyl linoleate and methyl palmitate treated groups and untreated x-irradiated group of mice did not differ significantly on the 30th day following whole body x-irradiation [$LD_{50/30}$)]. The testes weight of the experimental mice was significantly lower than that of the control group on the 30th day following x-irradition (Table 1).

*Histology.*— Histological examination of the testes of the experimental mice indicated that methyl oleate and methyl stearate treatment accelerated the rate of cellular regeneration and repopulation in the seminiferous tubules when compared with the x-irradiated group (Figs. 2 and 3). A number of the seminiferous tubules showed active cellular regeneration. On the other hand methyl linoleate and methyl palmitate treatments adversely affected the rate of cellular regeneration in the seminiferous tubules (Figs. 4 and 5). The seminiferous tubules showed varying degrees of degenerative changes and giant cells (Fig. 4).

### SEMINAL VESICLES

*Organ Weight.*—The weight of the seminal vesicles of the methyl oleate and methyl stearate treated x-irradiated mice was significantly higher

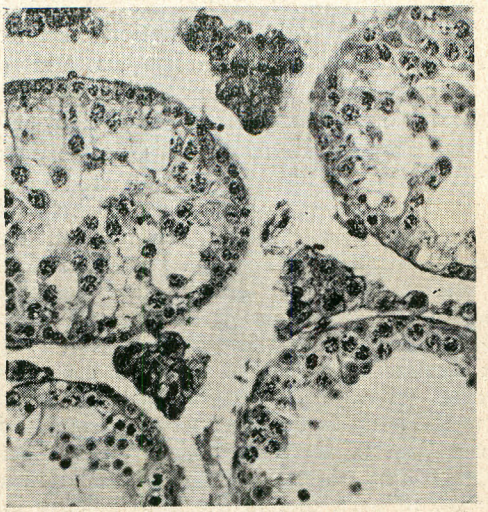

Fig. 2.—Testis section of x-irradiated mouse on the 30th day following whole-body x-irradiation [$ID_{50}(30)$], showing the degree of spermatogenic acitivity. The seminiferous tubules are shrunken in size and some show degenerative changes while in others the regeneration processes had started. × 300

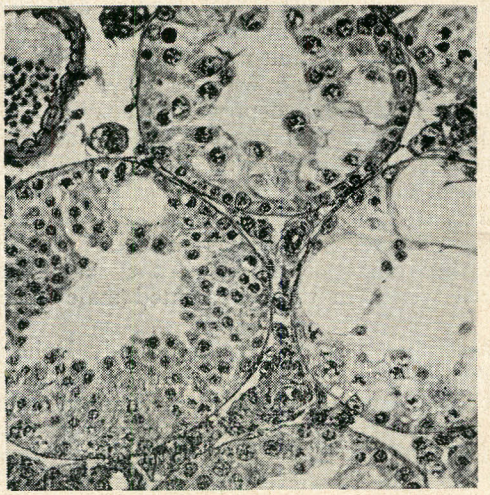

Fig. 3.—Testis section of methyl oleate treated x-irradiated mouse showing some cellular regeneration and repopulation in the seminiferous tubules. Compare with Figs. 1 and 3. × 300

when compared with the methyl linoleate/methyl palmitate treated x-irradiated or untreated x-irradiated male mice on the 30th day following x-irradiation (Table 1). The seminal vesicles weight of the experimental mice was significantly lower than that of the control group.

*Histology.*—The seminal vesicles of the methyl oleate/methyl stearate treated x-irradiated mice showed active secretory activity when compared with the methyl linoleate/methyl palmitate treated x-irradiated or untreated x-irradiated ones. His-

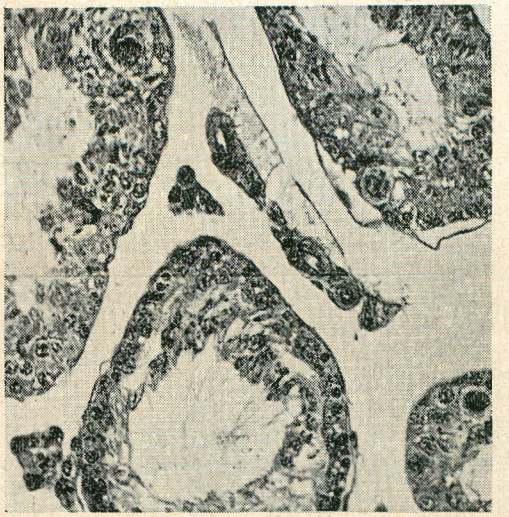

Fig. 4.—Testis section of methyl linoleate treated x-irradiated mouse showing marked degenerative changes on the 30th day postirradiation. × 300

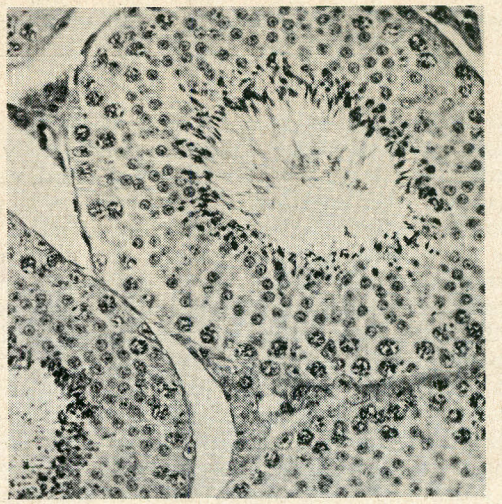

Fig. 5.—Testis section of a young mouse showing active spermatogenesis. Compare with Fig. 1. × 300

tologically the seminal vesicles of the methyl oleate/methyl stearate treated x-irradiated mice did not differ markedly from that of the control mice. The seminal vesicles of methyl linoleate and methyl palmitate treated x-irradiated mice showed arrested secretory activity and some cells showed degenerative changes.

## Discussion

The results obtained in the present series of experiments indicated that the administration of esters of certain unsaturated fatty acids, *i.e.* methyl oleate, methyl stearate accelerated the rate

TABLE 1.—EFFECTS OF ADMINISTRATION OF ESTERS OF CERTAIN UNSATURATD FATTY ACIDS ON WEIGHTS OF GONADS, THYMUS AND SPLEEN OF X-IRRADIATED MALE MICE [LD50(30)] ON THE 30TH DAY POSTIRRADIATION.

| Treatment | No. of animals | Body wt. (g.) ± SE | Testes (mg.) ± SE | Seminal vesicles (mg.) ± SE | Thymus (mg.) ± SE | Spleeen (mg.) ± SE |
|---|---|---|---|---|---|---|
| Control | 10 | 36 3 ± 4.2 | 281.4 ± 14.3 | 296.5 ± 17.6 | 41.2 ± 3.4 | 104.3 ± 10.4 |
| X-irradiated | 8 | 33.5 ± 5.1 | 96.3 ± 12.7 | 125.3 ± 16.8 | 36.3 ± 2.9 | 87.5 ± 13.6 |
| Methyl oleate | 8 | 34.1 ± 4.7 | 128.5 ± 14.8 | 242.5 ± 14.9 | 38.4 ± 3.7 | 98.4 ±12.1 |
| Methyl stearate | 9 | 34.6 ± 3.8 | 134.3 ± 13.9 | 238.3 ± 16.4 | 40.1 ± 3.6 | 102.6 ± 11.8 |
| Methyl linoleate | 7 | 30.4 ± 4.3 | 87.5 ± 11.8 | 114.5 ± 16.3 | 34.5 ± 3.4 | 89.1 ± 12.6 |
| Methyl palmitate | 8 | 31.2 ± 5.4 | 89.6 ± 12.7 | 118.3 ± 17.8 | 33.6 ± 2.8 | 82.5 ± 11.7 |

SE = Standard error.

of cellular regeneration and repopulation in the gonads of male mice following whole-body x-irradiation [LD $_{50(30)}$]. On the other hand, esters of certain other unsaturated fatty acids, *i.e.* methyl stearate, methyl linoleate adversely affected the rate of cellular regeneration in the gonads of x-irradiated male mice. These differences may, in part, be due to the differences in the composition of the esters of unsaturated fatty acids, used in these experiments. It may be of interest to mention here that radiation oxidizes fatty acids according to their degree of unsaturation and the order of oxidation is: linoleic (18:2) oleic (18:1) stearic (18:0).[7] Furthermore, Ashikawa[3] reported a decrease in the concentration of unsaturated fatty acid components with a concomitant increase in the saturated fatty acids. Maqsood [19] reported that the administration of mineral oil and powdered glass adversely affected the rate of cellular regeneration in the gonads of x-irradiated male mice.

Denel et al.[8] and Cheng et al.[6] have demonstrated an increased susceptibility to radiation injury in rats which were kept on a fat-free diet. This applies to both single and repeated whole-body x-irradiation. Methyl linoleate, when incorporated at a 1 percent level in the fat-free ration also increased survival of x-irradiated mice over that on the fat-free diet.[10] Alekseeva[1] reported that the yeast cells rich in fats were much more radio-resistant than those poor in fats. Altman et al.[2] reported a ten-fold increase in protein breakdown following whole body x-irradiation in animals. Increased excretion of nitrogenous and other catabolic products in animals following whole body x-irradiation have been reported by Caster and Armstrong.[5] Hence, if this post-irradiation tissue breakdown is partly due to increased caloric demand, fat treatment should help irradiated animals by alleviating their transient energy need and mitigate catabolism. Furthermore, there are indications that animals are temporarily incapable of utilizing carbohydrates following irradiation.[11] Under these conditions it is likely that therapeutic doses of lipids at certain time intervals following irradiation, will be of added use to the body tissues in restoring their functions. In the light of these observations the beneficial effect of lipid therapy in the x-irradiated mice may be, in part, due to the lipid helping in the restoration of various altered metabolic processes which are essential for normal cell growth and cell functions.

It is well recognized that RES phagocytic function may be altered by various colloids.[4] Stuart et al.[21] reported that olive oil and glyceryl trioleate caused an intense stimulation of phagocytic functions in adult male mice; glyceryl monooleate had no effect, whereas ethyl oleate and stearate markedly depress this function. These findings suggest that the functional changes described here are due to the intra-cellular accumulation of fat, and the chemical nature of the fat determines the effect produced. The therapeutic effects of lipids may be, in part, due to the stimulating effect of lipids on the reticulo-endothelial system in the x-irradiated male mice.

Recently, Patt,[20] Maisian and Doherty,[14] Kalkwarf,[13] Eldjarn and Pihl,[9] Hollaender[12] and others have discussed the mechanism of action of various protective agents against ionizing radiation. It is likely that no single mechanism will account for the *in vivo* action of all of the known protective chemicals. The surprising thing, however, is that some sort of biological saturation appears to be a requirement for protection in all instances and that the upper limit of protection seems independent of chemical agent and of postulated mechanism. The problem with regard to the therapeutic effect of agents like esters of certain unsaturated fatty acids, present a rather complicated picture and, as such, its mechanism of action in post-irradiation protection and recovery appears to be a complex biochemical phenomenon intricately linked with a number of physiological processes. In brief, it appears that lipid therapy facilitates the rate of regeneration in the x-irradiated mice [LD$_{50(30)}$] by restoring the cell functions and the reticulo-endothelial system.

**Acknowledgements.**—Thanks are due to Dr. J. H. Lawrence, M. D., D.Sc., Director, Donner Lab. and Professor C. A. Tobias, Ph. D. for their continued guidance. Technical assistance of Dr. J. K. Ashikawa, Miss M. Barbour and Miss B. Kullgren is acknowledged.

## References

1. V. M. Alekseeva, Biofizika, **3**, 101 (1958).
2. K. I. Altman, G. L. Haberland, K. Schreier and L. H. Hempelmann, Radiation Research, **5**, 466 (1956).
3. J. K. Ashikawa, *The Lipid Syndrome in Mammalian Radiobiology*. UCRL - 9592, Berkeley (1961).
4. G. Biozzi, B. N. Halpern, B. Benacerraf and C. Stiffel, *Physiopathology of Reticulo-Endothelial System* (Blackwell, Oxford, 1957), p. 204.
5. W. O. Caster and W. D. Armstrong, Proc. Soc. Exp. Biol. Med., **91**, 126 (1956).
6. A. L. S. Cheng, R. Mark, R. Slater and H. J. Denel, J. Nutrition, **52**, 637 (1954).
7. A. Chevallier and C. Burg, Action des radiations ionisantes sur les lipides, in *Radiobiology Symposium* (Liege, 1954), ed. by Z. M. Bacq and P. Alexander (Butterworths Scientific Publications, London, 1954). p.1.
8. H. J. Denel, A. L. S. Cheng, G. D. Kryder and M. E. Bingemann, Science, **117**, 254 (1953).
9. L. Eldjarn and A. Pihl, Mechanisms of protective and sensitizing action, in *Mechanisms in Radiobiology*, Vol. II. ed. M. Errera and A. Forssberg (Academic Press, New York, 1960) p. 231-268.
10. B. H. Ershoff, Radiation Research, **13**, 704-711 (1960).
11. G. C. Hevesy and A. Forssberg, Nature, **168**, 692 (1951).
12. A. Hollaender, *Radiation Protection and Recovery* (Pergamon Press, New York, 1960), pp. 1-392.
13. D. R. Kalkwarf, Nucleonics, **18**, 76-81 (1960).
14. J. R. Maisin and D. G. Doherty, Federation Proc., **19**, 564-572 (1960).
15. M. Maqsood and J. K. Ashikawa, J. Fertil. and Sterlity, **12**, 452-458 (1961).
16. M. Maqsood and J. K. Ashikawa, Postirradiation protection and recovery. I. Effects of lipids on haematopoietic organs. Internat. J. Rad. Biol., (in press).
17. M. Maqsood, Postirradiation protection and recovery. II. Effects of lipids on liver of x-irradiated male mice. Medicus, **23**, 1-4 (1961).
18. M. Maqsood, Postirradiation protection and recovery. III Effects of lipids on male sex organs. Medicus, **23**, 49-72 (1961).
19. M. Maqsood, Postirradiation protection and recovery. V. Effects of glass and mineral oil on gonads of x-irradiated male mice. Medicus (in press).
20. H. M. Patt, Federation, **19**, 549. (1960).
21. A. E. Stuart, G. Biozzi, C. Stiffel, B. N. Halpern and D. Mouton, Brit. J. Exp. Pathol., **39**, 599-604 (1960).