The Protective Effect against X-Irradiation of Methyl Lineolate in the Rat[1]

Harry J. Deuel, Jr., Amber L. S. Cheng, George D. Kryder, and M. E. Bingemann[2]

Department of Biochemistry and Nutrition, University of Southern California, Los Angeles

In previous work from this laboratory (*1*), it was shown that rats receiving a diet containing as small a proportion of cottonseed (Wesson) oil as 2% were much more resistant to injury from x-irradiation than were animals on a fat-free regimen. Although no differences in survival could be noted when the rats receiving 0 or 30% of cottonseed oil in the diet were subjected to a single lethal dose of 650 r and 850 r, a marked variability in survival time obtained when animals on these dietary regimens were subjected to repeated sublethal doses of x-irradiation in amounts of 300 r at weekly intervals. The groups in which fat was present in the diets lived for a significantly longer period after being subjected to x-irradiation than did the control group receiving a fat-free ration.

In addition to the level of fat, which is an important factor in protection from x-irradiation, sex has likewise been shown to play a significant role. In one series of tests (II) carried out on mature rats, the cottonseed-oil regimen protected the male and female rats equally well. On the other hand, in another series (III), in which vigorous young animals received diets containing 0, 2, or 30% cottonseed oil, the protective effect of the cottonseed oil was much less marked in the female than in the male animals.

The length of survival was found to be the most satisfactory index in evaluating the effectiveness of the several dietary factors. Thus, in Series II, the male rats receiving 15% or 30% of cottonseed oil in their diet survived 70 ± 6.1 or 63 ± 7.1 days, respectively, as contrasted with a mean of 34 ± 3.3 days for those on the fat-free regimen. Corresponding averages for the female rats were 76 ± 7.0 days (15% oil), 69 ± 5.3 days (30% oil), and 39 ± 4.3 days (0% oil). In Series III, the variations were equally significant for the males; the survival times averaged 65 ± 3.0 days and 67 ± 3.2 days for the rats on the 2% and 30% cottonseed oil diets as contrasted with a value of 51 ± 3.1 days for the control male rats. On the other hand, although the averages for the females were similar to those for the males on the experimental diets (67 ± 2.6 days for 2% oil, 62 ± 3.4 days for 30% oil), they are not significantly different from the results on the control rats, which had a mean survival time of 61 ± 3.6 days.

The effectiveness of the fat diets in protecting the animals is further indicated by the fact that, coupled with the increased survival times, the average exposure to x-irradiation was considerably higher in the groups receiving fats than it was in the case of the animals on the fat-free diet. Although the hemoglobin level and leucocyte count decreased in all cases for animals exposed to x-rays, it was found that the results obtained by means of these data were too irregular to be satisfactory indices of the protective effect.

Because of the small amount of fat required to exert a protective action on irradiation damage, it seemed possible that the therapeutic agent in cottonseed oil might be the essential fatty acids. On the basis of an average food consumption of 10 g daily, this would represent an intake of 200 mg cottonseed oil/day. Since cottonseed oil contains about 50% linoleate (*2*), the daily ingestion of 100 mg of linoleate would thus be indicated. Although the optimum dose of linoleate for male rats probably exceeds 200 mg/day (*3*), definite growth responses can be demonstrated when amounts of linoleic acid as low as 5 mg/day are administered. In the case of female rats, the optimum daily dose probably approximates 50 mg (*4*). It is therefore evident that the linoleate intake of the rats receiving the 2% cottonseed-oil diet is within the physiological range. The present experiments were designed to demonstrate whether a protective action against x-irradiation, similar to that noted for cottonseed oil, would be exhibited by purified methyl linoleate. In order to obtain further evidence of the effectiveness of the essential acids, the dose chosen was 10 mg/day, which was high enough to produce an increase in body weight on a fat-free diet. but far below the optimum level for growth.

The tests were carried out on male and female rats from our stock colony which were 5–6 months old at the start of the present tests. They were placed on essential fatty acid-free diets at weaning and were continued on these diets until fat-deficiency symptoms were noted. The animals were considered to be depleted when a sustained drop in body weight was maintained over 2 weeks, or when the total weight gain over a 3-week period did not exceed 4 g. The time required for the deficiency symptoms to develop was 9–12 weeks. The fat depletion diet contained vitamin-test casein, 20%; sucrose, 71.64%; cellulose (Solka-Floc), 4%; Osborne-Mendel salt mixture, 4%; and synthetic B vitamins, 0.36%.[3] Hydrogenated coconut oil was added to this diet in amounts of 0, 5, 15, or 30%, replacing a corresponding amount of sucrose. A fat-soluble vitamin mixture was administered orally twice each week. The doses per day were as follows: α-tocopherol, 0.1 mg; vitamin A, 30 μg; and vitamin D, 100 IU.

After depletion, the rats were all continued on the fat-free basal diet for an 8-week assay period. At the termination of this interval, the animals were as-

[1] This investigation was supported in part by a research grant from the Atomic Energy Commission (No. AT(11-1)-113). Communication No. 319 from the Department of Biochemistry and Nutrition, University of Southern California.

[2] We wish to thank Merck & Co. for the B vitamins (except folic acid and biotin), the Lederle Laboratories for folic acid, and Hoffmann-LaRoche for the biotin.

[3] The composition of the water-soluble vitamin mixture in mg/100 g of the diet was as follows: thiamine chloride hydrochloride, 7.2; riboflavin, 2.74; pyridoxine, 2.7; calcium pantothenate, 6.0; nicotinic acid, 6.0; inositol, 50.0; *p*-aminobenzoic acid, 60.5; folic acid, 1.0; biotin, 0.25; choline chloride, 200; vitamin B_{12}, 0.005 (5γ); ascorbic acid, 20.0; and menadione, 0.5. The total weight of these supplements is 0.357 g.

TABLE 1

SURVIVAL OF MALE AND FEMALE RATS ON A FAT-FREE DIET AND SUBJECTED TO SUBLETHAL DOSES OF X-RAYS WITHOUT OR WITH SUPPLEMENTATION WITH METHYL LINOLEATE IN DOSES OF 10 MG DAILY

Category	Wks on experiments	Cumulative exposure to x-irradiation (r)	Male rats		Female rats	
			No linoleate	10 mg linoleate daily	No linoleate	10 mg linoleate daily
No. of rats at start	—	—	36	35	35	37
Av wt at start (g)	—	—	223.4	178.6	253.3	201.1
Mortality, %, and number (in parentheses)	1	300	0	0	0	0
	2	600	2.8(1)	0	0	0
	3	900	5.6(2)	0	0	0
	4	1200	22.2(8)	0	8.6(3)	0
	5	1200	30.6(10)	5.7(2)	20.0(7)	5.4(2)
	6	1200	36.1(13)	11.4(4)	28.5(10)	8.1(3)
	7	1500	47.2(17)	17.1(6)	39.9(14)	13.5(5)
	8	1500	55.6(20)	22.8(8)	45.6(16)	24.3(9)
	9	1800	61.1(22)	25.7(9)	59.9(21)	32.4(12)
	10	1800	69.4(25)	31.4(11)	62.7(22)	32.4(12)
	11	2100	80.5(29)	39.9(14)	71.3(25)	43.2(16)
	12	2100	86.1(31)	54.2(19)	74.1(26)	48.6(18)
	13	2400	88.9(32)	59.9(21)	85.5(30)	56.7(21)
	14	2400	91.6(33)	59.9(21)	85.5(30)	56.7(21)
Av survival time (days)*	—	—	53.4 ± 3.8	74.7 ± 3.3	58.5 ± 4.0	74.8 ± 3.3
MD:SEMD†	—	—	—	**4.16**	—	**3.42**
Av exposure (r)	—	—	1408	2040	1766	2059

* Including standard error of mean calculated as follows: $\sqrt{\varepsilon d^2/n-1}/\sqrt{n}$ where d is the deviation from the mean and n is the number of observations.

† Mean difference: standard error of mean difference when results on control and experimental group are compared. When this value exceeds 3, the differences are significant.

signed to the two groups employed in the current tests. Both groups received the same basal fat-free diet that had been used in the depletion and assay periods, except that the coconut oil was replaced by sucrose during the assay period. One group received 10 mg of methyl linoleate daily by mouth, by means of a tuberculin syringe with a blunted needle. The fat-soluble vitamin mixture was administered twice weekly. All rats were subjected to 300 r of x-irradiation weekly for 4 weeks, after which the same dose was given once in 3 weeks or on alternate weeks until a 2400 r exposure had been completed over a 14-week period. The rats were housed in individual cages and were weighed weekly. Food and water were given *ad lib.* The x-ray apparatus employed has been described earlier (*1, 5*). The results of the tests are summarized in Table 1.

The increased survival time of the male and female rats receiving 10 mg of methyl linoleate daily is significantly greater, from a statistical standpoint, than that of the rats receiving the basal fat-free diet without linoleate. Moreover, the survival time for the linoleate-supplemented rats was increased despite the fact that the average exposure to x-irradiation was 45% and 17% greater for the male and female rats, respectively, which were supplemented than for the unsupplemented controls. Had equal doses of x-irradiation been given to the two groups, wider variations in survival time would have been noted. Moreover, the method of calculation is such as to indicate minimum differences. Since the experiment was terminated after 14 weeks, the survival time of all rats still alive was calculated as 98 days. Had the experiment been allowed to continue without further irradiation, and if the actual survival time had been used for the calculations, the discrepancies in means between the two groups would have been greatly exaggerated. This is due to the fact that, at the termination of the experiment, only 8 rats (3 male, 5 female) of the unsupplemented group were still alive, whereas 30 rats (14 male, 16 female) of the group receiving 10 mg of methyl linoleate had survived.

The reason for the beneficial effect of methyl linoleate on irradiation injury is not immediately clear. It is known to be important, however, in the nutrition of the epidermis, since an eczematous condition of the skin and scaliness of the tail occur in essential fatty acid deficiency. It is of considerable interest that a number of substances that have proved efficacious in irradiation injury, among which are cystine, ascorbic acid, and vitamin P (*6*), are likewise substances of considerable importance in the metabolism of the epidermis.

References

1. CHENG, A. L. S., *et al. J. Nutrition,* **48**, 161 (1952).
2. DEUEL, H. J., JR. *The Lipids,* Vol. I. New York: Interscience (1951).
3. DEUEL, H. J., JR., *et al. J. Nutrition,* **45**, 535 (1951).
4. GREENBERG, S. M., *et al. Ibid.,* 521.
5. ERSHOFF, B. H. *Ibid.,* **47**, 289 (1952).
6. SELLE, W. A. Atomic Energy Project, Contract No. AT-04-1-GEN-12, Report No. U.C.L.A., **176** (Jan. 15, 1952).

Manuscript received August 6, 1952.