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Deleterious Effects of High Fat Diets on Survival Time of X-Irradiated Mice.* (26318)

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Considerable data are available indicating that sensitivity to total body x-irradiation is enhanced in animals fed diets deficient in fat. Cheng *et al.*(1,2) reported that the average survival time of rats fed a fat-free diet was significantly less following multiple sublethal doses of total body x-irradiation than that of animals fed a similar diet supplemented with cottonseed oil or methyl linoleate. Similar findings have been reported for the mouse(3). It has also been observed that whereas a diet containing 10% cottonseed oil resulted in longer survival following multiple sublethal doses of total body x-irradiation than occurred in mice fed a diet deficient in fat, higher levels of fat (*i.e.*, 20% or 30% cottonseed oil in the ration) decreased survival time to that of mice on the fat-free diet.† These studies were confirmed and extended in the present investigation.

Procedure. The basal fat-free ration employed in these studies consisted of cerelose, 69%; casein,‡ 24%; salt mixture,§ 5%; cellulose,‖ 2%; and the following vitamins per kilogram of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; Vit. B_{12}, 150 μg; 2-methyl-1, 4-naphthoquinone, 5 mg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of cerelose. Tests were conducted with mice fed the basal fat-free ration or the basal fat-free ration supplemented with 2%, 10%, 20% or 30% of the following fats: cottonseed oil, margarine fat¶ or butter fat,¶

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† Ershoff, B. H., unpublished data.

‡ Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, O.

§ Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, O.

‖ Solka Floc, Brown and Co., Boston, Mass.

¶ Margarine fat and butter fat were prepared by the procedure of Deuel *et al*(5). Margarine fat was prepared from Nucoa (Best Foods, Inc., New York, N. Y.); butter fat from butter (Arden Farms Co., Los Angeles, Calif.).

TABLE I. Comparative Effects of Graded Levels of Cottonseed Oil, Margarine Fat and Butter Fat on Survival Time of Mice Exposed to Multiple Sublethal Doses of Total Body X-irradiation.

Dietary group	No. of animals	Day after 1st x-irrad. when 50% of animals in group were dead	% survival*	Avg survival time, days†‡
Basal fat-free diet	25	42	0	46.3 ± 3.0
Idem + following supplements:				
2% cottonseed oil	24	54	33.3 (8)	66.0 +
10% " "	24	>103	75.0 (18)	95.2 +
20% " "	26	41	19.2 (5)	55.5 +
30% " "	26	32	15.4 (4)	45.5 +
2% margarine fat	30	56	30.0 (9)	68.7 +
10% " "	28	93	46.4 (13)	76.2 +
20% " "	25	51	20.0 (5)	59.6 +
30% " "	27	41	22.2 (6)	53.1 +
2% butter fat	28	39	0	40.3 ± 1.1
10% " "	28	39	3.6 (1)	41.3 +
20% " "	28	46	3.6 (1)	50.9 +
30% " "	26	50	30.8 (8)	62.6 +

* Experiment was terminated 103 days after first x-ray exposure. Values in parentheses indicate No. of animals which survived.

† Data were calculated on basis of a 103-day survival time for animals alive at termination of experiment.

‡ Including stand. error of mean.

which were added in place of an equal amount of cerelose.

Male mice of the Webster strain, 11 to 14 g in body weight, were divided into comparable groups of 40 animals per group. These were placed in metal cages with raised screen bottoms (5 animals per cage) and were fed the test rations indicated above. Food and water were provided *ad libitum*. The animals were fed daily and all food not consumed 24 hours after feeding was discarded. After 6 weeks of feeding, 10 of the mice in each dietary group were selected at random to serve as non-irradiated controls. The remaining mice received an exposure of 200 r total body x-irradiation which was repeated once weekly until a total dose of 1200 r (6 exposures) had been administered. Animals were continued on their respective diets for 103 days after the first x-ray exposure or until death, whichever occurred sooner. Mice that died before the 4th x-ray exposure were not included in the tabulation of data. Animals were weighed weekly during the experiment and data were obtained as to the day after the first x-ray exposure when 50% of the animals in each group were dead, average length of survival and percentage of animals in each group still alive at termination of experiment.

The following radiation factors were employed: GE Model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic filter;** HVL, 2.15 mm Cu; target distance to top of box, 82 cm; and dose rate, 15.6 r/min (measured in air, at top of box). The animals to be irradiated were placed in a wooden box divided into 60 equal compartments 1¼″ wide, 3″ long, and 1½″ deep. The partitions and top were made of ⅛″ cellulose acetate sheeting; and the top and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically driven turntable to ensure equivalent irradiation.

Results. Findings indicate that average survival time of mice following exposure to multiple sublethal doses of total body x-irradiation is dependent on both the amount and source of dietary fat (Table I). In

** A nonuniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand(6). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

agreement with earlier findings(3) a supplement of 2% cottonseed oil markedly increased average survival time of x-irradiated mice over that of animals fed the basal fat-free ration alone. At the 10% level of supplementation this increase was even more marked. Whereas 50% of the mice in the fat-free group were dead on the 42nd day after the first x-ray exposure, at the 2% level of cottonseed oil supplementation 50% mortality did not occur until the 54th day; while at the 10% level of cottonseed oil supplementation 75% of the animals in the group were still alive at termination of the experiment 103 days after the first x-irradiation. At higher levels of cottonseed oil supplementation, however, (*i.e.*, 20% or 30% of the diet) the protective effect of cottonseed oil on survival of x-irradiated mice was no longer evident, with average survival time on such diets not differing significantly from that of mice on the basal fat-free ration. A similar trend was observed in animals fed the various levels of margarine fat. In contrast to the results obtained with the cottonseed oil and margarine fat supplements, butter fat at levels of 2% or 10% of the diet did not increase survival following x-irradiation over that on the basal fat-free ration alone (a finding that may have been due to its low content of essential fatty acids). In addition butter fat at levels of 20% or 30% of the diet prolonged survival over that obtained at the 10% level of supplementation, a finding also in contrast to that obtained with the cottonseed oil and margarine fat supplements. All non-irradiated mice fed the cottonseed oil and margarine fat supplements survived the experimental period of 145 days (103 + 42 days). From 70 to 90% of the non-irradiated mice fed butter fat at levels of 10% of the diet or higher also survived. However, only 3 of the 10 non-irradiated mice fed butter fat at a 2% level in the diet and only 2 of the non-irradiated mice on the basal fat-free ration were alive at termination of the experiment, average survival time of the decedents being 126 and 123 days respectively (*i.e.*, equivalent to 84 and 81 days respectively after the first x-irradiation).

The cause for the deleterious effect of high levels of cottonseed oil and margarine fat on survival of x-irradiated mice is not readily apparent. It has been proposed that the deleterious effects of x-irradiation are due, at least in part, to excessive formation of free radicals(4). It is possible that the ingestion of unsaturated fatty acids in excess of body requirements for the essential fatty acids might result in the x-irradiated animal in the formation of more free radicals than might otherwise occur on diets containing less of these fats. If such were the case, survival time of x-irradiated mice on a fat-free diet supplemented with an unsaturated fat containing essential fatty acids might be expected to increase with increasing amounts of fat until a level is reached which provides an optimum amount of essential fatty acids; and thereafter decrease due to formation of excessive amounts of peroxides and free radicals. This suggestion might account for the effects obtained in the present experiment with cottonseed oil (which contains a high level of unsaturated fatty acids) and butter fat (which has a minimal amount of such acids); but the similarity of results obtained with cottonseed oil and margarine fat, despite their difference in unsaturated fatty acid content, is not consistent with this hypothesis. Since margarine fat is made from vegetable oils, it is possible that such oils contain a factor (or factors) other than unsaturated fatty acids which is toxic when fed at high levels to the x-irradiated mouse or results in formation of a toxic substance(s) or metabolite as a consequence of x-irradiation in the animal's tissues.

Summary. The effects of multiple sublethal doses of total body x-irradiation were determined on survival time of male mice fed either a purified fat-free ration or a similar diet supplemented with 2%, 10%, 20% or 30% cottonseed oil, margarine fat or butter fat. The effects obtained were dependent on the amount and source of dietary fat. At levels of 2% or 10% of the diet cottonseed oil and margarine fat increased survival time over that on the fat-free ration. When these fats were fed at higher levels (*i.e.*, 20% or 30% of the diet), however, survival time was

decreased below that obtained at the lower levels of supplementation. In contrast to the results obtained with the cottonseed oil or margarine fat supplements, butter fat at levels of 2% or 10% of the diet did not prolong survival over that on the fat-free ration; nor did it decrease survival time when fed at higher levels in the diet.

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Histochemical Localization of L-Gulonolactone Oxidase Activity in Tissues of Several Species.* (26319)

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Oxidation of L-gulonolactone by a specific oxidase is the final step in the pathway of synthesis of L-ascorbic acid from glucose(1). Coenzymes I and II do not participate in the reaction. The transfer of electrons is probably mediated by a flavoprotein the exact nature of which has not been defined. L-gulonolactone oxidase is confined to the liver of mammals, and to the kidneys of amphibia and reptiles(1). According to some investigators it is present only in microsomes(2) while others claim to find some activity in mitochondria(3). It is the absence of L-gulonolactone oxidase and consequent inability to synthesize Vit. C which results in the susceptibility of humans, monkeys, guinea pigs and a few other species to scurvy. In this paper we describe a histochemical method which demonstrates the distribution of the enzyme in tissue slices.

Materials and methods. Thirty adult white rats (250 g) and 10 adult guinea pigs (350 g) on standard laboratory diets were killed by a blow on the head and the tissues immediately removed, blocks 5 mm by 1 cm made, and frozen on dry ice. In addition kidney and liver tissue were obtained from 6 frogs (*Rana pipiens*) and treated similarly. Within 30 minutes of freezing the blocks were cut in a cryostat (−18°) at 20 to 34 μ thickness. Sections were put on coverslips and allowed to rinse for 5-10 minutes in cold (4°C) 0.03 M KCl solution. Then they were removed to an incubating mixture which consisted of 15 mg L-gulonolactone, 2.5 mg of the paranitrophenyl substituted ditetrazolium salt (Nitro-BT) in 4.75 cc of 0.1 M phosphate buffer pH 7.4. The solution was brought up to 5 cc by addition of 0.25 cc of acetone in which was dissolved 0.5 mg of menadione (Vit. K). The incubating mixture was warmed to 37°C before introduction of the sections. The tissues were then incubated at 37°C for 20 to 40 minutes. At termination of the incubation, the medium was drained off and replaced by 10% neutral formalin in which the sections were allowed to fix for 1 hour at room temperature. Sections were then placed on slides using glycerogel, and examined under the light microscope.

Control sections were incubated in solutions which individually excluded substrate and other components of the incubating mixture.

In some experiments sodium pyrophosphate or ethylene diamine tetra-acetic acid (EDTA) at varying concentrations were included in the incubating mixture.

Results. Rat liver was the only mammalian organ studied in which L-gulonolac-

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