# Bioflavonoids and Survival Time of Mice Exposed to Multiple Sublethal Doses of X-Irradiation.* (26084)

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Disagreement exists regarding the effects of flavonoids on the mortality of animals following exposure to a single lethal dose of total body x-irradiation. Rekers and Field(1, 2) reported an improved survival rate for irradiated dogs administered rutin or other flavonoids. Clark *et al.*(3) obtained increased survival of guinea pigs treated with calcium flavonate. Sokoloff and co-workers (4,5) found that CVP, a complex mixture of flavonoids separated from citrus waste, significantly reduced the mortality of rats administered a single lethal dose of total body x-irradiation. Opposed to these findings are the negative results of other workers who reported that rutin(6-11), CVP(10) and other flavonoids(10,11) had no significant effect on radiation mortality following exposure to a single lethal dose of total body x-irradiation in mice, guinea pigs and rats. Since previous findings indicate that certain dietary factors which have little if any protective effect in animals exposed to a single lethal dose of total body x-irradiation prolong survival in animals administered multiple sublethal doses of total body x-irradiation(12,13), experiments were undertaken to determine the effects of flavonoids on length of survival following exposure to multiple sublethal doses of total body x-irradiation in the mouse.

*Procedure.* The basal ration employed in the present experiment consisted of cerelose, 59%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; cellulose,§ 2%; and the following vitamins per kg 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 $\mu$g; 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.

Male mice of the Webster strain were selected at 11 to 14 g in body weight and were divided into comparable groups consisting of 40 animals per group. These were placed in metal cages with raised screen bottoms (5 animals per cage) and were fed either the basal ration or the basal ration plus the supplements listed in Table I. The supplements were added to the basal ration in place of an equal amount of cerelose. 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 135 days after the first x-ray exposure or until death, whichever occurred sooner. All mice that died before the 4th x-ray exposure were not included in the tabulation of data. Data were obtained as to the day after first x-ray exposure on which 50% of the animals in each group were dead and average length of survival on each of the diets employed.

The following radiation factors were employed: GE Model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus

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† Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

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

TABLE I. Effects of Flavonoids 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 | No. surviving‡ | Avg length of survival after 1st x-irrad., days§‖ |
|---|---|---|---|---|
| Basal ration | 26 | 39 | 5 | 66.6 ± 8.2 |
| *Idem* + following supplements: | | | | |
| 2% hesperidin | 28 | 40 | 7 | 63.0 ± 9.0 |
| 4% " | 30 | 45 | 2 | 51.1 ± 4.4 |
| 2% lemon bioflavonoid complex | 30 | 54 | 4 | 63.7 ± 5.6 |
| 4% *idem* | 30 | 51 | 3 | 61.6 ± 5.5 |
| 2% naringen | 30 | 66 | 8 | 81.4 ± 6.8 |
| 4% " | 29 | 102 | 10 | 91.2 ± 8.0 |
| 2% hesperidin methyl chalcone | 27 | 52 | 7 | 72.3 ± 7.8 |
| 4% *idem* | 27 | >135 | 15 | 107.3 ± 7.5 |
| 2% rutin | 28 | 83 | 9 | 87.6 ± 7.4 |
| 4% " | 28 | 118 | 11 | 101.9 ± 7.1 |

* Each group initially consisted of 30 mice. Animals that died before the 4th x-irradiation were not included in the Table.

† Hesperidin (Hesperidin Purified, Product No. 165), lemon bioflavonoid complex, naringen, and hesperidin methyl chalcone employed in the present experiment were kindly provided by Mr. W. E. Baier and Dr. G. H. Joseph of Sunkist Growers, Ontario, Calif. Rutin (Rutin N.F.X.) was obtained from the Meer Corp., New York.

‡ The experiment was terminated 135 days after 1st x-irradiation.

§ Data were calculated on the basis of an avg survival time of 135 days for animals alive at termination of experiment.

‖ Including stand. error of mean calculated as follows: $\sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}} / \sqrt{n}$, where "x" equals length of survival after 1st x-irradiation and "n" is No. of observations.

a Cu parabolic filter‖; HVL, 2.15 mm Cu; target distance to top of box, 82 cm; and dose rate, 15.6 r per minute (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 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.* Significant differences were observed between the various dietary groups as to the day on which 50% mortality occurred and average length of survival following exposure to multiple sublethal doses of total body x-irradiation (Table I). Whereas hesperidin and lemon bioflavonoid complex at levels of 2% or 4% of the diet had little if any effect on length of survival, the day 50% mortality was attained or number of animals surviving, naringen, hesperidin methyl chalcone and rutin at levels of 2% or 4% of the diet resulted in increased survival over that of animals fed the basal ration alone. Findings were particularly significant in the case of animals fed the hesperidin methyl chalcone and rutin supplements at a 4% level in the diet. No significant differences were observed grossly among non-radiated mice in the various groups. Average body weight of radiated and non-radiated mice in the various dietary groups at different stages of the experiment is summarized in Table II.

*Discussion.* Present findings indicate that dietary supplements of naringen, hesperidin methyl chalcone and rutin significantly in-

‖ A nonuniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand (20). The center of the filter had a thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

TABLE II. Effects of Flavonoids on Body Weight of Radiated and Non-radiated Mice.*

| Dietary group | Avg body wt at time of 1st x-irrad. (g)† | Avg body wt of survivors‡ (g) | |
|---|---|---|---|
| | | Radiated | Non-radiated |
| Basal ration | 27.4 (39) | 27.7 ( 5) | 41.1 ( 9) |
| *Idem* + following supplements: | | | |
| 2% hesperidin | 30.0 (39) | 32.3 ( 7) | 42.5 (10) |
| 4% " | 30.4 (40) | 29.8 ( 2) | 38.3 ( 9) |
| 2% lemon bioflavonoid complex | 29.0 (39) | 28.1 ( 4) | 46.3 ( 9) |
| 4% *idem* | 29.1 (40) | 31.0 ( 3) | 41.6 (10) |
| 2% naringen | 29.7 (40) | 31.2 ( 8) | 41.5 (10) |
| 4% " | 26.9 (39) | 27.8 (10) | 37.7 (10) |
| 2% hesperidin methyl chalcone | 28.3 (37) | 29.3 ( 7) | 36.6 ( 9) |
| 4% *idem* | 30.2 (36) | 32.0 (15) | 39.9 ( 9) |
| 2% rutin | 27.7 (38) | 31.1 ( 9) | 46.1 ( 9) |
| 4% " | 28.7 (37) | 34.7 (11) | 41.9 ( 9) |

* Avg initial body wt of mice in the various groups ranged between 12.2 and 12.4 g. Originally 40 mice were employed in each group. After 6 wk of feeding, 10 mice in each group were selected at random to serve as non-irradiated controls; remainder were exposed to x-irradiation. Values in parentheses indicate No. of animals which survived and on which averages are based.

† First x-irradiation was administered after 42 days of feeding.

‡ Experiment was terminated 135 days after 1st x-irradiation. In the case of the non-irradiated mice, this was 177 days (135 + 42 days) after the start of feeding.

creased average survival time of mice exposed to multiple sublethal doses of total body x-irradiation but that other flavonoids were without activity in this regard. No data are available as to the factors responsible for these diverse results. It is of interest that whereas hesperidin methyl chalcone increased average survival time of mice exposed to multiple sublethal doses of total body x-irradiation, hesperidin *per se* was without activity in this regard. In view of the greater solubility of the hesperidin methyl chalcone(14), it is not unlikely that differences in solubility of the various flavonoids may have contributed to the diverse results. No data are available as to the *modus operandi* whereby the active flavonoids exerted their protective effect. It has been suggested that the deleterious effects of ionizing radiation in animals are due, at least in part, to excessive formation of peroxides(15,16). If such were the case, preventing or minimizing peroxide formation following radiation or decreasing the sensitivity of various tissues or enzyme systems to the peroxides thus formed might exert a protective effect. In view of the chelating and antioxidant properties of various flavonoids(17,18), it is not unlikely that one or more of these substances might have significant activity in preventing or minimizing peroxide formation following radiation exposure, thereby resulting in a radioprotective effect. This suggestion is in accord with the radioprotective effects recently obtained with the synthetic antioxidants, propyl gallate, DBH (2,5-di-tert-butyl-hydroquinone) and BHT (butylated hydroxy toluene)(19). It is possible that fat-soluble derivatives of flavonoids might be prepared that would be even more effective against radiation injury. Further studies to test these hypotheses are indicated.

*Summary.* Experiments were conducted to determine the effects of flavonoids on survival time of mice exposed to multiple sublethal doses of total body x-irradiation. Hesperidin and lemon bioflavonoid complex at levels of 2% or 4% in the diet had little if any protective effect. Naringen, hesperidin methyl chalcone and rutin at levels of 2% or 4% of the diet increased survival over that on the basal unsupplemented ration.

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## Estrogenic Effects of 18-Norestradiol -17$\beta$ and 18-Norestrone. (26085)

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Several workers have studied the effects of structural modification on biological actions and effectiveness of phenolic estrogens. Huggins and Jensen have discussed significance of carbon 17 hydroxyl groups of estradiol and of various modifications, related particularly to oxygenation at carbon 6 or carbon 16 (1,2); they showed that 17-desoxyestradiol was an active estrogen, albeit a material of low potency, and that 6 and 16 oxygenation resulted in estrogens that had shallow dose-response curves, *i.e.*, increment of uterine growth per unit increase in dose was less for these *impeded* estrogens than for estrone or estradiol. In mouse studies it was shown that there may be a whole series of possible dose-response curve slopes, extending from steep estrone slope, through intermediate slope of estradiol-17$\beta$, to shallow slope of estriol(3); similar results were obtained from spayed rats(4,5) and Drill, Cook and Edgren(6) have discussed several miscellaneous series of steroids; their finding most germane to present discussion was that 16-halogenation of 3-methoxyestrone does not produce an *impeded* or shallow slope. Most of these structural modifications have involved addition, removal or transposition of oxygenated functions. Recently synthesis of 18-norestrone and 18-norestradiol-17$\beta$ has been completed(7). The uterine growth stimulating effects of these materials will be discussed here.

*Materials and methods.* Technics employed here have been described previously. Uterine growth was determined from tests employing immature female mice that received test compound daily for 3 days and were sacrificed on fourth. At autopsy uteri were removed, cleaned of adherent tissues and weighed wet on a torsion balance(3).

*Results and discussion.* As discussed previously(3,4), estrone had steepest slope of compounds treated; remaining materials had slopes that were essentially similar (Fig. 1). Slopes for estradiol-17$\beta$ and estrone were appreciably more shallow than those cited earlier(3); this probably is result of change in mouse strain between the two studies (unknown strain from small breeder to Carworth mice from Small Animal Farms, Des Plaines, Ill.). The extremely high potency for estradiol-17$\beta$ is the result of slope of dose-response curve and low response level at which 2 materials were compared; at 50 mg uterine weight level estrone and estradiol-17$\beta$ would be equal in potency. Removal of angular methyl group at carbon 13 results in a sharp decrease in potency for each compound (Table I) with the estradiol derivative about

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