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Failure of Rutin and Related Flavonoids to Influence Mortality Following Acute Whole Body X-Irradiation.* (19491)

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The flavonoids were the first compounds reported to reduce the mortality in animals following whole body x-irradiation. The rationale for the use of the flavonoids in the attempt to prevent or treat radiation injury has been based upon the claim that these compounds reduce the hemorrhage resulting from increased capillary fragility(1). Rekers and Field(2) observed a reduced hemorrhagic tendency and obtained improved survival rates among irradiated dogs pretreated with rutin or other flavonoids. Clark, Uncapher, and Jordon(3) obtained increased survival of guinea pigs treated with calcium flavonate. Sokoloff, Redd, and Dutcher(4) found that CVP, a citrus "vitamin P" compound, significantly reduced the mortality of rats exposed to 800 r of x-radiation. Opposed to these findings are the negative results of other workers. Kaplan(5) and Cronkite *et al.*(6) used rutin in mice, and Kohn and coworkers (7), and Patt and his associates(8) used rutin in rats without observing any effect on radiation mortality. Similarly CVP had no beneficial effect in mice(9), and rutin and hesperidin failed to influence the mortality or hemorrhagic changes in irradiated young chicks(10).

In view of the conflicting reports regarding the value of the flavonoids in reducing the mortality of animals exposed to acute whole body x-radiation, the present investigation was undertaken in order to explore further the possible usefulness of these compounds.

Materials and methods. The flavonoids used include: rutin in pure form, rutin in de-ionized form, rutin solubilized with piperazine hexahydrate, quercitrin, quercetin, hesperidin, dihydroquercetin, naringen, calcium flavonate, hesperidin methyl chalcone, xanthorhamnin, hemoeriodictyol, morin (de-ionized), citrus vit. P (CVP), and lemon juice infusion (LPI). CVP is reported(9) to contain 4% of a quercitrin-like substance, 15% eriodictin, 80% hesperidin-glucose, and 0.38% calcium phosphorus ash. Lemon peel infusion contains eriodictyol glycoside, hesperidin, perhaps other flavonone glycosides, i-inositol, some fractions of the vit. B complex, and some natural soluble carbohydrates. A total of 1132 CF_1 female mice and 232 female Sprague-Dawley rats were used. The mice were 7 to 9 weeks in age and weighed 18 to 24 g prior to irradiation. The rats were 10 to 18 weeks old and weighed 116 to 246 g at the time they were started on the flavonoid treatment. In addition, 70 female guinea pigs with a weight range of 205 to 400 g were included in the studies. The mice and rats were fed Wayne Dog Blox. The guinea pigs were given Purina Rabbit Chow Meal supplemented daily by fresh cabbage and timothy hay. Animal weights, deaths, and food and drinking water consumed, if containing a flavonoid, were recorded daily. In all tests the administration of the compounds was con-

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tinued post-irradiation until there were no survivors or until the 30th day post-irradiation.

Rutin was solubilized by the addition of an aqueous solution of piperazine hexahydrate to a suspension of rutin in water. To solubilize 25 mg of rutin in 1 ml of water, 20 mg of piperazine were required. The pH of the resulting solution was 9.1-9.3. Clark and MacKay(11) have reported that in the body rutin splits off from the piperazine hexahydrate. The other insoluble flavonoids were dissolved in propylene glycol, while the soluble flavonoids were dissolved in water. An x-ray unit operated at 250 kvp and 15 ma was used for all exposures. Inherent filtration of the tube was 0.25 mm Cu, and a filter of 0.25 mm Cu + 1.0 mm Al was added. The radiation intensity was measured with a Victoreen condenser r-meter corrected for temperature and pressure. For the mice the target distance was 60 cm and the dose rate 48-50 r/min.; for the rats and guinea pigs the distance was 75 cm and the dose rate 31-36 r/min. The animals were exposed to a revolving cage divided into concentrically arranged compartments. Each flavonoid-treated group of animals, except where specified, was irradiated simultaneously with an equal number of untreated control animals. The effect of each treatment itself was tested in groups of unirradiated animals, with normal (untreated and unirradiated) animals serving as controls.

Results. Table I shows the schedule of flavonoid treatment and the x-ray doses received by each group of animals, along with the 30-day mortality results obtained.

Rutin administered by various methods in mice. As demonstrated by the data in the table the 30-day mortality among irradiated mice was not diminished by treatment with rutin given by intraperitoneal injection, by single daily administration by stomach tube, or by incorporating it in the drinking water or in the food. Furthermore, no significant difference was noted between the treated and untreated animals with respect to weight loss or time of death after irradiation. Of these rutin treatments the only one which adversely affected the control unirradiated mice was the 5% rutin diet. This caused a 4.3% weight loss of 12 mice which consumed it for 7 days. For this reason the group of mice receiving this diet before being irradiated was placed on the 2.5% rutin diet following irradiation.

Other flavonoids orally or in diet of mice. To determine whether other flavonoids might provide some protection different compounds were given orally or in the diet according to the schedules outlined in the table. All the mice thus treated appeared healthy and active and maintained a body weight equal to that of the untreated controls. In these tests none of the flavonoids exerted any favorable influence on 30-day mortality, on time of onset of morbidity or mortality, or on weight loss.

Comparison of the 30-day LD_{50} of x-ray in rutin treated and untreated mice. The mortality data for all rutin treated mice exposed to 700, 600 and 500 r were pooled together and compared with those of the untreated irradiated controls. This involved a total of 224 mice treated with the various forms of rutin by different routes of administration and 294 mice which served as untreated irradiated controls. When the percent mortality for each group at 700, 600, and 500 r was plotted by the log-probit method the 30-day LD_{50} was estimated to be 550 r for the rutin treated animals and 560 r for the controls. The close approximation of the slopes of the two curves was a further indication that the rutin had no influence on the lethal effects of whole body x-irradiation at the x-ray doses used.

Flavonoids in rats. Solubilized rutin, hesperidin methyl chalcone, and CVP were administered to rats through their drinking water according to the tabulated schedule. Half of each group of 18-week-old rats (see table) was given daily for 7 days post-irradiation, via stomach tube, 40 mg of flavonoid in 2 ml of water in addition to the flavonoid they were taking in the drinking water. This treatment was supplemented by daily subcutaneous injections of 100 mg of sodium ascorbate in 0.5 ml of distilled water during the 7-day post-irradiation period. Two groups of younger rats, 11 and 10 weeks old, respectively, received no treatment other than CVP in the drinking water. The results of these

tests showed no evidence of protection against injury by x-rays in rats.

Flavonoids in guinea pigs. Seventy young female guinea pigs were divided into 7 groups

TABLE I. Mortalities Among Various Species of X-irradiated Animals Untreated and Treated with Flavonoid Compounds.

Flavonoid	Procedure	Species and No. of animals Control	Treated	Dose whole body X-ray (r)	% mortality, 30 days Untreated	Treated
		CF_1 mice				
Solubilized rutin	125 mg/kg intraper. daily 7 days pre-irrad. and continued post-irrad.	12	12	800	100	100
		12	12	700	91.7	100
		12	12	600	83.4	75
		12	12	500	25	25
Solubilized rutin	Orally by stomach tube 5 mg in .2 ml H_2O daily 7 days pre-irrad. and continued post-irrad.	12	11	800	100	100
		11	10	700	100	100
		10	12	600	80	83.4
		12	12	500	41.6	33.4
Solubilized rutin	.1% in drinking water 14 days pre-irrad. and continued post-irrad.; mean daily intake 5 mg per mouse.	24	24	800	100	100
		24	24	700	95.8	100
		24	24	600	85.4	79.2
		24	24	500	25	29.2
Powdered rutin in pure form	5%, 2.5% or 1% in food for 7 days pre-irrad. and continued post-irrad. at conc. of 2.5% or 1%					
	Mean daily doses					
	5 % 177.4 mg	12	12	700	100	100
	2.5 113.2	12	12	700	91.7	83.4
	1 42.7	12	12	700	100	100
	2.5 126.8	12	12	600	58.4	66.7
	1 39.2	12	12	600	75	75
Hesperidin methyl chalcone in H_2O	Orally: 7 days pre-irrad. and continued post-irrad. Daily doses, 10 mg in .2 ml		12	700	*	100
Citrus vit. P (CVP) in H_2O			12	700	*	91.7
Xanthorhamnin in H_2O			12	700	*	91.7
Dihydroquercetin in PG	(PG = propylene glycol)		10	700	*	100
Morin (de-ionized) in PG			10	700	*	100
Homoeriodictyol in PG			12	700	*	100
Rutin (pure) in PG			11	700	*	100
Rutin (de-ionized) in PG			11	700	*	100
	2.5% in diet for 14 days pre-irrad. and continued post-irrad. Mean daily dose, mg:					
Quercitrin	89.5		12	700	†	100
	101		12	600	‡	66.7
Quercetin	166		12	700	†	100
	111.5		12	600	‡	75
Dihydroquercetin	112.2		12	700	†	100
	100		11	600	‡	63.6
Naringen	120.2		12	700	†	91.7
	195		12	600	‡	66.7
Calcium flavonate	94		12	700	†	100
	207		10	600	‡	60
Hesperidin	105.2		12	700	†	83.4
	101		12	600	‡	66.7
Hesperidin methyl chalcone	92.2		12	700	†	91.7
	104		12	600	‡	75
Citrus vit. P (CVP)	180		12	700	†	100
	201		12	600	‡	75
Lemon peel infusion (LPI)	123.2		12	700	†	100
	111.5		10	600	‡	70

TABLE I. (Continued.)

Flavonoid	Procedure	Species and No. of animals Control	Treated	Dose whole body X-ray (r)	% mortality, 30 days Untreated	Treated
		Rats 18 wk old				
Solubilized rutin	.1% in drinking water 21	22	22	700	100	90.9
Hesperidin methyl chalcone	days pre-irrad., plus 100	24	24	700	100	100
Citrus vit. P (CVP)	mg of sodium ascorbate subcut., and 40 mg of flavonoid by stomach tube daily post-irrad.	24	24	700	83.3	100
		Rats 11 wk old				
CVP 32.2 mg daily	14 days pre-irrad. in drinking water, and continued post-irrad.	26	26	600	46.1	68.4
" 29.3 " "		Rats 10 wk old				
		20	20	500	45	40
Mean daily doses (mg)		Guinea pigs				
Solubilized rutin 73.5	.1% in drinking water 21		10	250	§	50
Solubilized rutin 76.2 + vit. C 100	days pre-irrad. and continued post-irrad. supplemented with subcut. inj. of		10	250	§	70
CVP 82.3			10	250	§	90
Hesperidin methyl chalcone 84.1	100 mg sodium ascorbate daily post-irrad. for 7 days		10	250	§	100

* Pooled 30-day mortality of 93 untreated mice at 700 r = 94.3%.
† " " " "119 " " at 700 r = 97.5%.
‡ " " " "118 " " at 600 r = 66.9%.
§ " " " " 30 " guinea pigs at 250 r = 43.4%.
Mean daily doses in food and drinking water were calculated on basis of pre-irradiation intake.

of 10 each; 40 were given flavonoids in the drinking water and 30 served as controls. Sodium ascorbate was added daily to the drinking water of one of the two groups receiving the solubilized rutin. Each animal in this group received approximately 100 mg of vitamin C per day. These flavonoids, with or without vit. C, did not provide any protection against the lethal effects of x-ray nor against the weight loss of those animals which survived for 30 days. Indeed, the mortality and weight losses shown by the treated animals were consistently greater than those shown by the untreated ones.

Discussion. The most prominent clinical manifestation of exposure to x-radiation is described by Warren and Draeger(12) as the hemorrhagic tendency reflected in moderately severe cases by hemorrhagic gingivitis, petechiae, epistaxis, hematemesis, and bloody diarrhea. These changes have also been described in animals(13). Thus it was logical for investigators(2-4) to administer rutin and related flavonoids in the hope that the prevention of such effects of ionizing radiation might favorably affect mortality in animals. Rutin had become the flavonoid of choice for the control of bleeding believed due to increased capillary fragility. However, any agent effective in offsetting the bleeding tendency induced by x-irradiation would be expected to influence mortality only to the extent to which the hemorrhagic syndrome contributes to death. The possibility that bleeding may play a more important role in some species than in others in causing radiation death should perhaps not be neglected in the attempt to explain the discordant results of various investigators. It is of interest to note that in the findings reported in the literature on the influence of the flavonoids on radiation mortality in animals there are no direct contradictions. Of the 3 important experimental variables, namely animal species, specific flavonoid tested, and route of administration, no 2 groups of workers used the same combination. Those reporting positive results used some manner of oral administration of a flavonoid agent in dogs(2), guinea pigs(3), and rats(4), while those describing negative findings used a parenteral route in mice(6,9), rats(7,8), and chicks(10), or the oral route in mice(5). In 2 of these studies, one negative(7), and the other positive(4), rats were treated orally in

both, but the flavonoids tested were different.

The results of the present study introduce only one questionable direct contradiction to the work of others who report a protective action of a flavonoid against the lethal effect of radiation. Negative results were obtained by us when rats were treated orally with CVP, contrary to the results obtained by Sokoloff *et al.*(4) under the same general conditions. These workers, however, used a British brown breed of rat treated by repeated single oral doses of the flavonoid, while we used Sprague-Dawley rats treated by the CVP in the drinking water. The total daily dose of CVP administered was much larger in our tests and the pre-irradiation treatment period was longer. In the present studies on mice, rats and guinea pigs, there was no evidence that the flavonoids offer any protection to these species against the lethal effects of acute whole body x-irradiation. These results provide general confirmation of the negative findings of others(5-10) in this regard. More direct confirmation of the negative findings reported by others is seen in the results of the present tests in which rutin was given intraperitoneally to mice, simulating the conditions of Cronkite *et al.*(6), and in which rutin was given to mice in the drinking water or food, simulating the conditions of Kaplan(5).

The data of others have shown that the flavonoids have a very low toxicity(14,15). The parenteral injections of 50 mg/kg of rutin in rats and guinea pigs, and of 200 mg/kg in rabbits was harmless(14). In the present study, mice tolerated a daily intake of approximately 250 mg/kg of solubilized rutin in drinking water for 30 days. The daily oral administration, for 21 days, of rutin and the related flavonoids in doses as high as 500 mg/kg in distilled water or in propylene glycol was not harmful. Likewise, mice given 2.5% flavonoids in the diet for 14 days showed no significant weight losses. Intraperitoneal injection of 125 mg/kg of rutin for 7 days in mice, and large amounts of the soluble flavonoids orally in rats had no adverse effects. The possibility may exist, however, that the amounts of CVP and hesperidin methyl chalcone consumed by the guinea pigs had a deleterious effect since the mortality and weight loss in these tests was significantly greater in the treated than in the untreated animals. In this case, however, the supplemental vit. C should not be disregarded as a possible contributing factor.

The fact that workers reporting positive results in testing flavonoids against radiation lethality(2-4) all administered the compounds by the oral route is of interest in view of the studies of Clark and MacKay(11) on the metabolism of a number of flavonoids when given orally. These workers found in human experiments that urinary excretion was negligible following large oral doses (50 mg/kg) and that rutin is not recoverable from the stools and is destroyed when incubated in an aqueous stool suspension. In animals, the percentage of the oral doses excreted in the urine was negligible. Some of the fed compounds were almost completely recoverable from the gastrointestinal tract. They reported that the more soluble flavonoids were no better absorbed than the more insoluble ones such as rutin. The insoluble complexes of rutin dissociated to the typically insoluble rutin in the intestine. The work of Clark and MacKay(11) supports the cumulative evidence that it is unlikely that the flavonoids are vitamin-like and that they exert any significant physiologic effect.

Various authors(16-18) have suggested the possible value of the flavonoids under conditions of stress in which there is an increased demand for ascorbic acid. Allen(19) suggested that rutin and ascorbic acid together might be more effective in reducing the hemorrhagic state in the radiation syndrome. In the present study, however, this combination in guinea pigs, which are unable to synthesize vit. C, and in rats gave no indication of a beneficial effect on radiation mortality.

Summary. 1. Rutin, rutin complexed with piperazine hexahydrate, and de-ionized rutin administered to mice by different routes had no protective action against the lethal effects of whole body x-irradiation in doses of 500, 600, 700 or 800 r. 2. Quercitrin, quercetin, hesperidin, hesperidin methyl chalcone, dihydroquercetin, naringen, calcium flavonate, xanthorhamnin, morin (de-ionized), citrus vit. P (CVP), and lemon juice infusion (LPI),

administered in the diet, had no protective action on mice exposed to acute whole body x-radiation. 3. Rutin complex, hesperidin methyl chalcone, and citrus vit. P had no protective effect on rats and guinea pigs exposed to acute whole body x-irradiation. Supplemental vit. C did not enhance the action of the flavonoids or decrease mortality. 4. The LD_{50} of 250 kvp x-ray, based on the 30-day mortality, for the CF_1 mice used in this experiment, was approximately 550 r.

Rutin, de-ionized rutin, de-ionized morin, dihydroquercetin, homoeriodictyol and xanthorhamnin were supplied by Dr. S. H. Wender and Dr. T. B. Gage of the University of Oklahoma; rutin, quercitrin and quercetin by Dr. J. F. Couch of the United States Department of Agriculture; solubilized rutin in sterile ampules, rutin and piperazine hexahydrate by the Abbott Laboratories; hesperidin, hesperidin methyl chalcone, naringen, calcium flavonate, and lemon juice infusion (LPI) by Dr. A. J. Lorenz of the California Fruit Growers Exchange; and citrus vitamin P (CVP) by the Vitamerican Oil Co.

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Eosinopenia Produced by ACTH in Patients with Schizophrenia. (19492)

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The contention that adrenal cortical function is deranged in patients with schizophrenia, particularly those with long-standing disease, has been advanced for several years by Pincus, Hoagland, and their associates(1). Using changes in urinary excretion of sodium, potassium, uric acid, and steroids, and in the level of circulating blood lymphocytes as indices, they have reported that as few as 20% of schizophrenic subjects respond in normal fashion to the injection of 25 mg of pituitary adrenocorticotropic hormone. Despite the

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