

Contamination is rare in plates which have been thoroughly washed. It is not necessary to remove solvents from extracts of samples because they are allowed to evaporate from the pads before the pads are placed on the assay plates. The method is free from interference due to non-antibiotic materials since these non-specific inhibitory materials in the feed extracts do not diffuse into the agar medium but apparently remain on the pads.

Using the single layer technic, it is essential that the plate be resting on a level surface when the agar is poured. Differences in agar depth in different areas of the plate can cause a 2-fold difference in assay values calculated from duplicate pads. In the 2-layer technic, the level surface is not as important since the agar is approximately 6 times as deep and the variations in depth will be small in relation to the total thickness of the agar.

Summary. A rapid simplified technic utilizing inexpensive equipment has been developed for the determination of penicillin in feeds and feed supplements. The method is useful to determine the quantities of penicillin added to feeds for nutritional purposes since it can be used to assay accurately as little as 0.5 g of procaine penicillin per ton of feed. Feeds which are not supplemented with antibiotics

do not give blank values. A sample of antibiotic-free feed of the same or similar composition as the samples to be assayed must be available. The test organism is *S. aureus* 209-P which is suspended in thin layers of agar medium upon which paper pads are placed containing methanol extracts of the feeds. The use of the paper pads to contain feed extracts and the use of Sulfasuxidine in the assay medium are the features of the method which permits the method to be used for the determination of the small amounts of penicillin added to feeds.

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Inability of Flavonoids to Modify Roentgen Ray Irradiation Mortality in Guinea Pigs.*† (19979)

THOMAS J. HALEY AND SAMUEL MANN.

From the Division of Pharmacology and Toxicology, Atomic Energy Project, School of Medicine, University of California at Los Angeles.

General disagreement exists regarding the usefulness of the flavonoid compounds in the prevention and treatment of Roentgen ray irradiation injury. Rekers and Field(1,2)

reported that rutin modified such damage in dogs but not in rats. However, Griffith *et al.* (3) found rutin beneficial in rats. On the other hand, Kohn, Robinette, and Capp(4) reported that rutin had no effect on Roentgen ray irradiation lethality in rats, and Cronkite *et al.*(5) obtained similar results in mice. Sokoloff *et al.*(6) reported that a citrus flavonoid mixture decreased such lethality in a British brown strain of rats, but Cronkite *et al.*(7) were unable to obtain any benefit in mice. Clark *et al.*(8) reported that "calcium

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TABLE I. Effect of Flavonoids on Radiation Mortality in 14 Groups of Guinea Pigs.

Medication and time		Total mortality—				
		10 days	15 days	20 days	25 days	30 days
Control	Rutin, 250 mg/kg	0/20	7/20	7/20	7/20	8/20
PM		2/20	6/20	9/20	9/20	9/20
PSM		6/20*	7/20	7/20	7/20	7/20
Control	HMC, 100 mg/kg	3/20	10/20	10/20	10/20	10/20
PM		0/20	7/20	8/20	11/20	11/20
PSM		6/20	13/20	13/20	13/20	13/20
Control	Hesperidin, 25 mg/kg	3/20	10/20	11/20	12/20	12/20
PM		5/20	12/20	14/20	14/20	14/20
PSM		4/20	9/20	11/20	12/20	13/20
Control	Naringin, 75 mg/kg	0/20	7/20	7/20	7/20	7/20
PM		2/20	5/20	6/20	6/20	6/20
PSM		3/20	7/20	7/20	7/20	7/20
PM	MG, 100 mg/kg	3/20	9/20	9/20	10/20	10/20
PSM		3/20	8/20	8/20	9/20	9/20

PM = Premedicated; PSM = Post-medicated; HMC = Hesperidin Methyl Chalcone;
MG = Methyl Glucamine.

* Significant at $P = .05$.

flavonate" reduced Roentgen ray irradiation mortality in guinea pigs. However, Clark and MacKay(9), upon the basis of their distribution studies of the various flavonoids in the intestinal tract, stated that intestinal decomposition of orally administered flavonoids made it highly unlikely that enough of the drug could be absorbed to produce the effects reported. Dauer and Coon(10) found various flavonoids of no benefit in irradiated rats, mice, and guinea pigs.

We have investigated the protectant action of several flavonoids in Roentgen ray irradiated guinea pigs. These animals were chosen for their high irradiation sensitivity (LD_{50} 30 days, 200 r) and because they are one of the few animal species which seem to require flavonoid compounds as an essential metabolite in their ascorbic acid metabolism(11). The results obtained indicate that the flavonoids used did not modify Roentgen ray irradiation mortality.

Experimental. Female guinea pigs, weighing 286 to 477 g (avg 393 g) were placed upon a high ascorbic acid diet for 4 weeks pre-irradiation and were maintained upon this diet until completion of the experiment. The flavonoids dissolved in saline containing 1% methylglucamine were administered intraperitoneally using alternating sides of the abdomen to reduce the incidence of trauma. The dosages used (rutin, 250 mg/kg; hes-

peridin, 25 mg/kg; hesperidin methyl chalcone, 100 mg/kg; naringin, 75 mg/kg) were based upon those shown to produce the greatest effect upon capillary permeability(12). Both pre- and post-irradiation medication for 10 days was used and the various treatments are shown in Table I. In all cases the control groups received injections of the same volume containing the same amount of methylglucamine as the medicated groups. The 200 r total irradiation dose was administered from above and below the animals with two 250 KVP Picker Industrial units operating simultaneously. The technical factors for both units were: 250 KVP; 15 MA; TSD 40 cm; filters 0.21 mm Cu inherent, 0.5 mm Cu parabolic and 1.0 mm Al; HVL 2.02 mm Cu; size of field—total body; r/minute measured in air 99.4-104.6. The animals were irradiated in groups of 2 or 3 animals each, depending upon the number of animal groups in the particular experiment. Both units were calibrated prior to each experiment with a Victoreen Thimble r meter.

Results. The results obtained with the various treatments are given in Table I. It is quite evident that none of the flavonoid compounds significantly decreased the total radiation mortality. Application of the χ^2 test showed that post-irradiation medication with rutin significantly increased the rate of mortality over that of the controls. The site of

injection, the solubilizer used and the handling of the animals, particularly after irradiation, were not involved in either the rate of mortality or the total mortality because if they had been, definite differences in both of these factors would have shown up in the pre- and post-irradiation methylglucamine groups. The non-irradiated control groups and the saline-methylglucamine and flavonoid injected control groups had no deaths during the experimental period or at any time thereafter up to 60 days. This indicates that the deaths obtained in the medicated irradiated groups were not due to the flavonoids themselves.

Discussion. The results herein reported concerning the ineffectiveness of parenterally administered rutin, hesperidin, hesperidin methyl chalcone, and naringin in modifying Roentgen ray irradiation mortality in guinea pigs confirm the observations of Dauer and Coon(10) who gave rutin, hesperidin methyl chalcone, and citrus vitamin P by the oral route. The results of previous investigators (2,4,7,10) as well as our own would definitely indicate that the flavonoids are incapable of modifying the radiation syndrome in mice, rats, guinea pigs, or dogs.

Summary. It has been shown that rutin, hesperidin, hesperidin methylchalcone, and naringin were ineffective in modifying Roent-

gen ray irradiation lethality in guinea pigs. There was an indication that post-irradiation medication with rutin may have increased the rate of mortality.

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Species Differences in Circulating 17-Hydroxycorticosteroid Concentrations.* (19980)

ALAN K. DONE, ROBERT S. ELY, RICHARD B. RAILE[†] AND VINCENT C. KELLEY.

From the Lockhart Memorial Laboratory, Department of Pediatrics, University of Utah College of Medicine, Salt Lake City, Utah.

Vogt(1) described a substance in adrenal vein plasma which protected adrenalectomized dogs from cold. Since then, bioassays of adrenal venous plasma have been accomplished by other workers(2,3) and various corticosteroids have been isolated from fresh adrenal

tissue(4,5). By the use of recently developed qualitative and quantitative technics, considerable species differences in the steroid content of adrenal venous blood have been demonstrated. By paper-partition chromatography, Bush(6) found that the dog and cat secreted mainly 17-hydroxycorticosterone and some corticosterone; the ferret secreted both compounds in approximately equal amounts; the rat and rabbit produced mainly, if not

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[†] Present address: Department of Pediatrics, University of Minnesota Hospitals, Minneapolis.