Preliminary experiments on the mechanism of this inhibition reaction, which is instantaneous, suggest that it is reversible and noncompetitive. Indications have been obtained that *Saccharomyces carlsbergensis* likewise contains a DNase inhibitor which, however, appears to behave somewhat differently.

A full account of this and related work on other cellular systems, including those of higher organisms, will appear at a later date. But attention may be drawn to the following sequence of autolytic reactions, possibly delicately balanced in the living cell: (1) activation of yeast protease (*2*); (2) digestion of DNase inhibitor; (3) liberation of active DNase; (4) depolymerization of DNA. If, as appears likely, the cleavage of the DNA macromolecules is of importance in the life of the cell, the evidence of the existence within the cell of a specific regulation of this process may be of more general biological interest.

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Effect of Flavonoids (Vitamin P) on Mortality From Total Body Roentgen Irradiation[1]

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In previous papers Clark and Geissman (*1, 2*) reported a test for flavonoids and related compounds (vitamin P-like substances) based on the potentiation of the epinephrine response of isolated mammalian smooth muscle. In a study of the relation of molecular structure to activity, some 70 pure compounds were examined, and the minimum structure essential for high activity was predicted, synthesized, tested, and found to confirm the prediction.

In attempts to extend these and other observations to the intact animal, among other things a study was included of the effects of these compounds on the hemorrhagic syndrome associated with total-body roentgen irradiation. This study was initiated in September 1947, and the purpose of the present communication is to demonstrate the potential usefulness of this approach in further experimental assessment of the antihemorrhagic effects of the flavonoid pigments.

Griffith, *et al.* were the first to demonstrate a beneficial effect of flavonoids in experimental roentgen-irradiation injury. They found a beneficial effect of rutin[2] therapy in accelerating the restorative processes following severe X-ray burns of rats' extremities (*8*) and in preventing the capillary fragility increase following intraperitoneal administration of radon ointment in rats (*7*). They also indicated that rutin probably had no beneficial effect in total-body roentgen-irradiated rats but gave no particulars (*7*).

Rekers and Field (*10*) later reported a decrease in mortality of dogs by rutin therapy before and after 350 r total-body roentgen irradiation.

The beneficial effect of rutin in the case of the X-ray burns of rats' extremities was limited to an acceleration of the restorative processes, since there was no effect on the time of onset or the severity of the lesions. This probably is related to the similar beneficial effect of rutin in the prevention of tissue loss in frostbite gangrene in rabbits' extremities, reported by Fuhrman and Crismon (*4, 5*).

We wish to report the effect of one of several flavonoid substances being studied in several species (rats, mice, guinea pigs) of small laboratory animals subjected to approximately median lethal doses of total-body roentgen irradiation.

Large, healthy guinea pigs of approximately 500-gm body weight were fed on Rockland guinea pig ration[3] and given supplementary ascorbic acid in 0.2% concentration fresh daily in the drinking fluid. One group served as controls and the other as flavonoid treated, for which the drinking water also contained 0.2% "calcium flavonate,"[4] prepared fresh daily. Previous experiments had indicated that single, daily, oral, large doses of flavonoids are not as effective as constant ingestion in the food or water.

After a week of such treatment the animals were given 220–225 r total-body irradiation[5] in a single dose, not including backscatter. They were placed in a multicompartment wooden box, controls and experimentals being arranged alternately in a checkerboard fashion, 4

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[2] Quercetin-3-rhamnoglucoside, a common flavonoid active in decreasing capillary fragility, as first shown by Sevin (*11*) and later by Griffith, Couch, and Lindauer (*9*).

[3] Arcady Farms Milling Company, Chicago, Illinois.

[4] A water-soluble preparation from lemon peel, essentially free of water, sugars, and hesperidin. The alcohol extract of the fresh peels is precipitated in alkaline medium with calcium, the precipitate suspended in water and adjusted to an acid pH, reprecipitated by the addition of alcohol, filtered, and the material obtained from the filtrate by evaporation. It gives a cyanidin test about half as intense as rutin on a weight basis. Prepared and supplied by the California Fruit Growers Exchange, Sunkist Building, Los Angeles, California.

[5] GE Model KX-3, 220-kv deep therapy unit. The factors were: 200 kv, 20 MA, ½ mm Cu + 1 mm Al added filtration (HVL, 1.05 mm Cu), 100-cm target distance, 8.5 r/min. The unit is calibrated semiannually by a registered X-ray physicist. The variation in output over the past year has been less than 3%.

controls and 4 experimentals in each exposure, in a manner described by Goldfeder, *et al.* (*6*) for mice and with backscatter made uniform by use of a rice phantom, in accordance with the technique of Ellinger (*3*).

Following irradiation, treatment was continued for the duration of the experiment. Animals dying were autopsied. Clinical symptoms were graded twice weekly with emphasis on visible manifestations of anemia and purpura. Albinism lent itself best for the assessment of such symptoms.

In several experiments involving a total of 230 animals it was observed that, under the conditions described, 220–225 r consistently killed 67% of the animals, with 50% dead within 13 days. With the exception of occasional secondary infections, recovery from radiation injury usually was complete within 30 days; hence the experiments were terminated, although the animals remained under observation for several weeks thereafter.

The data in Table 1 illustrate the effect of "calcium flavonate."

TABLE 1

	Total No. guinea pigs used	Total No. died	Died %	50% death time (days)
Controls	45	30	67	13
Treated	26	9	35	..

The hemorrhagic symptoms (petechial hemorrhages, ecchymoses, generalized purpura) of the treated animals were considerably less marked than those of the controls.

It is concluded that under the experimental conditions described, a flavonoid preparation derived from lemons, administered in the drinking water, reduces the mortality from total-body roentgen irradiation by about half. In our opinion this justifies the employment of smaller laboratory animals than the dog in further studies of this nature and may offer an intact animal assay for vitamin P-like substances. Studies are in progress in attempts to elucidate the molecular configurations necessary for higher activity of the flavonoids and related substances by this and similar techniques.

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Urinary Flow, Excretion of Solutes, and Osmotic Work During Diuresis of Solute Loading in Hydropenia in Man

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Interest in the study of the osmotic limitations of the kidney has been renewed in the last years after having lapsed since the early studies by Korányi and others (*5*). Gamble, in studies of man during water deprivation (*4*), came to the conclusion that 1,400 milliosmols/liter represented the maximum urinary concentration attainable. Given this fixed maximum value, a minimal urinary volume could then be calculated from any solute load. Studies by McCance and co-workers (*6*) added the information that forced osmotic diuresis in hydropenia produced by urea, sodium chloride or bicarbonate, and potassium chloride increased urinary flow while decreasing the level of urinary osmolarity. The conclusion was reached that for the substances studied the total solute concentration rather than the level of any one solute determined the maximum osmolarity of the urine.

Another aspect of the osmotic relationships of the kidney concerns the work involved to produce the observed concentration differences between urine and plasma. The concept of renal osmotic work was first applied by Dresser (*2*), Galeotti (*3*), and Rhorer (*8*). Later a general thermodynamic treatment of the minimal work necessary for the production of urine was given by Borsook and Winegarden (*1*). Recently Newburgh (*7*) refocused attention on the application of the concept and presented a discussion of the clinical implications of concentration changes which tend to reduce the renal work.

The studies to be reported were undertaken in order to determine in a broad manner, by the use of loading substances of various kinds, the pattern of urinary flow and excretion of solutes under the condition of water restriction. Furthermore, it was hoped to find out whether under such conditions a biologic maximum of renal osmotic work existed and, if so, whether it was the same or different for varying solutes. Such a maximum of work, if extant, would represent a measure of over-all work capacity of the kidney, with possible physiological and clinical implications.

The subjects were boys, 8–15 years of age, with normal renal function and without major disease. One group of young diabetic patients was included for the study of forced diuresis produced by glucose. The loading substances were administered as a rule in amounts of 500–2,000 milliosmols/1.73 m^2 of body surface, by the oral or, more often, the intravenous route, in concentrated

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