which measurements are usually made by solid counting techniques, this represents 20 mg.–2 grams. Sample 7, when removed from the counter, precipitated as $BaCO_3$ on a 2-cm. filter, and measured on a bell-type β-counter (*3*) with a 4-mg./cm.^{2} window, gave an observed counting rate, less background, of 50 c.p.m. This is 2.5 × background as against 250 × background on the sample when measured as a gas.

While there is no proof in the data that this is an absolute disintegration rate measured by these counters, the fact that the ionizing events registered are those that take place within the cylindrical volume defined by the cathode strongly suggests that the final corrected counting rate is indeed the absolute disintegration rate of the activity within the counter tube. If there were losses, one would expect, from the nature of the discharge avalanche that constitutes the pulse from the counter tube, that these would be greater in the tube of larger diameter. The data on counters Nos. 18 and 19 demonstrate that this is not the case. Thus, measurements on C^{14} made in this way, together with the mass spectrometer analysis of C^{14} activity preparations, will allow a determination of the presently quite uncertain decay constant of this isotope with an accuracy far greater than is possible by solid counting (*5*), although this will probably not approach that possible with modern ionization chamber methods after the mean energy of the β-ray is obtained from the spectrum.

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Effect of Flavonols on the Bacteriostatic Action of Dicoumarol

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Goth (*3*) reported that dicoumarol possessed bacteriostatic action toward certain bacteria which was not antagonized by 2-methyl-1,4-naphthoquinone (vitamin K). This would indicate that dicoumarol exerts its bacteriostatic activity through a mechanism different from that by which it induces hypoprothrombinemia and hemorrhage. In connection with some investigations in progress at this Laboratory (*2*), it was of interest to determine the effect of compounds containing the γ-pyrone structure on the antibacterial action of dicoumarol. For this purpose we have used the flavonol glycosides, rutin (*1*) and quercitrin, and the aglycone, quercetin. The effect of rutin was of especial interest, since it has pronounced physiological activity in diminishing the tendency to hemorrhage by restoring fragile capillaries to normal (*4*, *5*).

The tests were made in nutrient broth (peptone, 0.5 per cent; beef extract, 0.3 per cent; and sodium chloride, 0.5 per cent) adjusted to pH 6.95. Nutrient broth solutions containing desired concentrations of dicoumarol and flavonols were dispensed in 5-ml. quantities in test tubes, sterilized by autoclaving at 15 pounds for 15 minutes, inoculated with 0.01 cc. of a 16-hour broth culture of *Staphylococcus aureus* (F.D.A. 209P), and incubated at 37° C. The antagonistic effect of the flavonols on dicoumarol was determined by using a Klett-Summerson photoelectric colorimeter to measure the density of bacterial growth in the presence of increasing quantities of the flavonols.

TABLE 1

Antagonistic Effect of Rutin, Quercitrin, and Quercetin on the Bacteriostatic Activity of Dicoumarol Toward *Staph. aureus*

(Expressed as turbidity readings on Klett-Summerson colorimeter scale)

		Dicoumarol (mg./ml.)			
		0.0	0.02	0.04	0.08
Rutin (mg./ml.; 22 hrs. at 37°C.)	0.0	62	47	0	0
	0.01	66	50	0	0
	0.05	62	58	26	0
	0.5	62	55	59	42
Quercitrin (mg./ml.; 15 hrs. at 37°C.)	0.0	47	27	0	0
	0.05	48	48	35	0
	0.1	52	53	39	21
	0.5	44	38	30	29
	1.0	17	16	13	14
Quercetin (mg./ml.; 19 hrs. at 37°C.)	0.0	45	29	0	0
	0.01	48	42	16	0
	0.05	38	22	13	0
	0.10	0	0	0	0

The results in Table 1 show that all three flavonols were capable of neutralizing the bacteriostatic action of dicoumarol. The inhibitory effect of 0.04 mg./ml. of dicoumarol was overcome by 0.05 mg./ml. of rutin and completely neutralized by 0.5 mg./ml. Higher concentrations of dicoumarol required increased amounts of rutin to show proportional antagonism. Rutin *per se* does not appear to have any effect on the growth of *Staph. aureus*.

Quercitrin was somewhat less effective than rutin as an antagonist toward dicoumarol. This may be partly due to the fact that in high concentrations quercitrin exhibits toxicity toward *Staph. aureus*. In concentrations up to 0.1 mg./ml. it showed increasing antagonism toward dicoumarol; however, above this value the toxic effect began to show up, and at 1.0 mg./ml. there was a 64 per cent inhibition in the growth of *Staph. aureus*.

Quercetin was the least effective of the three flavonols tested. It did not overcome the bacteriostatic effect of 0.08 mg./ml. of dicoumarol, and showed only partial antagonism to the lower concentrations. It exhibited considerable toxicity toward *Staph. aureus*, completely inhibiting the growth in a concentration of 0.1 mg./ml.

The antibacterial action of quercitrin is probably due to the presence of some quercetin from the hydrolysis of the rhamno-

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side. Investigation of the sample of quercitrin by ultraviolet absorption revealed a small quantity of quercetin. As little as 4–5 per cent of quercetin would be sufficient to produce the degree of bacteriostasis observed in this experiment.

This is the first time that the flavonols have been shown to possess any antibiotic action. The discovery is especially timely, considering the present intensified interest in the subject of antibiotics derived from plants. The results also suggest the possibility of using rutin or the other flavonols to antagonize the hemorrhagic action of dicoumarol *in vivo*.

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The Production of Experimental Pellagra by Adenine[1]

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Adenine, linked with other substances, is a constituent of many enzyme systems essential for life and, as a component of the nucleoproteins, is part of the structure of the cell. Many physiological processes are governed by catalysts containing adenine. Presumably, the concentration of free and bound adenine in tissues and body fluids is regulated by a mechanism which determines its formation and utilization, and such a regulation must be of importance to the animal economy.

An attempt was made to disturb this regulation by increasing the concentration of adenine, thereby interfering with the normal metabolism of adenine-containing substances, and of vitamins (especially of the B group), hormones, and enzymes.

Adenine[2] in the form of the free base alone or mixed with monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) was given orally, in daily doses ranging from 400 to 500 mg. each, to three dogs over a period of from one to three weeks. The dogs (females) used for the experiments were fed before and during the experiment with K.F.S., Cero Meato (Kennel Food Supply Company, Fairfield, Connecticut), and with Red Heart, Diet B (John Morrell Company, Ottumwa, Iowa). Occasionally this diet was supplemented with milk.

The history of a dog which showed the characteristics common to all three animals given adenine supplemented with phosphate was as follows: Its weight was 11,260 grams; its age, about three years; time in the laboratory, seven months. The dog was given 400 mg. of adenine and 400 mg. of monobasic sodium phosphate daily in capsules by mouth. Loss of appetite was observed after the second day, and the dog became apathetic. On the fifth day the animal refused all food but continued to take water. The fur looked unkempt, and loss of hair was apparent. The systolic pressure was about 30–35 mm. Hg. above the values found during the control period. On the seventh day along the inner side of the cheeks and the inner upper lips areas of redness were present, stippled with false membrane and disseminated pustules. The tongue was pale grey, and the first quarter of the anterior portion had a brownish-grey appearance. A foul odor from the mouth was evident. The gums bled easily on slight pressure, especially near the alveolar arches of the teeth. Bilaterally, about two inches back from the tip of the tongue on the lateral inferior portion, inflamed areas, about one-half inch long and one-quarter inch wide, were present. The dog vomited occasionally. The systolic pressure was 25 mm. Hg. above the average value for the normal period. At the time of the last dose, given on the ninth day, a marked skin rash was evident, the mouth had a very strong odor, and the entire mouth and pharynx were inflamed. Areas of one-fourth to one-half inch in diameter, covered by a yellowish or yellowish-grey exudate, were present on the inner lips and cheeks, especially at the angle and on the floor of the mouth. Slight pressure on the gums, inner lips, and cheeks produced bleeding. The red line extending along the alveolar arches of the teeth had become a deeper red, and the inflamed areas on the lateral inferior portion of the tongue were covered with an exudate. The tongue had darkened, and the brownish-grey appearance of the anterior-superior portion had extended posteriorly to about two-thirds of its length. The remainder of the tongue was dark grey. The portion close to the tip was almost black and markedly atrophied. Drooling from the mouth was continuous, and the saliva contained some blood. The weakness of the hind legs was pronounced, and the dog staggered when it walked. The cornea of the eye was opaque, giving the iris a spotted, cloudy appearance. The sclera showed marked vascularization. On the margins of the eyelids a yellowish, thick secretion accumulated, causing the lids to adhere.

During the following days the weakness of the animal increased rapidly, and the blood pressure dropped below normal. Voluntary control of the tongue seemed to be almost completely lost. There was an increase in the number and severity of the inflammatory lesions in the mouth and pharynx. Occasionally the dog vomited small amounts of a bloody fluid. The day before the animal died 50 cc. of warm milk was given by stomach tube but was not retained. Death occurred on the 14th day of the experiment, at which time the animal weighed 7,170 grams. The total amount each of adenine and monobasic sodium phosphate given was 3.6 grams. Gross autopsy findings included: alopecia, emaciation, glossitis, gingivitis, stomatitis, congested liver, marked submucosal hemorrhages in the duodenum and marked congestion in the jejunum and ileum, yellowish-grey mottling of the entire surface of the kidney, hemorrhages in the medulla, and bulging of the cut surface.

Two dogs were given a daily dose of 300 mg. of adenine (free base) alone for a period of 21 days. The above-described

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