creased opportunity f eventual restoration of organ function should be afforded.

Cognizant of the import of the nonspecific toxicity of massive doses of irradiation and of uncombated infection as causal influences in irradiation mortality, both of which are also under study here, an examination of some

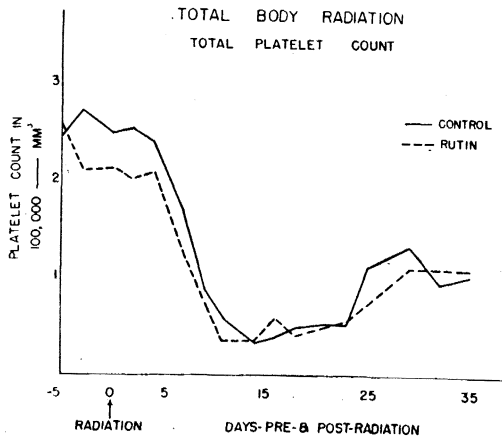

FIG. 1

protective and regulatory factors in maintaining the vascular integrity has been undertaken. The recent availability of the flavanol glycoside, rutin,[2] with which clinical instances of increased capillary fragility were controlled (*3, 4*), prompted its trial. In this preliminary report a summary of some of the data obtained with its use is presented. Details will be given in subsequent reports.

Fifty normal adult dogs similar in size were selected and divided into a control group of 25 dogs and a treated group of 25 dogs. The latter received 50 mg of the glycoside 3 times a day orally, commencing one week prior to irradiation and continuing throughout the course of the test. Except for the administration of, rutin, the two groups of dogs were treated identically.

A standard single dose of total-body X-irradiation of 350 r[3] (approximately the midlethal dose) was delivered to each dog used in these tests. Following the irradiation, 16 of the 25 (64%) untreated dogs succumbed in 13–30 days after X-irradiation, whereas only 3 of 25 (10%) rutin-treated dogs died 16, 28, and 31 days post-radiation.

Widespread premortem ecchymoses and intrapulmonary and intraintestinal hemorrhages were seen in all 16 untreated dogs which succumbed. Three of the surviving dogs of this group manifested subcutaneous ecchymoses and intestinal hemorrhages. Although characteristic widespread hemorrhage was seen in 2 of the 3 rutin-treated dogs which failed to survive, the 22 remaining exposed dogs were relatively free from petechiae and ecchymoses during the postradiation period of 40–60 days and at autopsy.

Studies of the peripheral blood of the two groups of dogs showed little or no difference in the postradiation depression of the hematological elements, especially the thrombocytes and leucocytes in the treated and control dogs. Illustrating this similarity, Fig. 1 shows the means of the platelets of the two groups of animals.

In the group given the glycoside several dogs were observed to develop a severe thrombocytopenia and leucopenia which persisted for 10–14 days. Recovery eventually ensued. In distinct contrast, recovery in untreated dogs with persistent severe depression of blood elements has rarely been observed in this laboratory.

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[2] A crystalline glycoside of quercetin. Furnished by the Eastern Regional Research Laboratory, Philadelphia, through the courtesy of J. F. Couch, and also by the Abbott Laboratories, North Chicago, Illinois.

[3] Radiation was administered from a Picker Industrial X-ray machine of 250 KVP, 15 ma, 37" t.s.d., and 14.22 mm parabolic aluminum and 0.53 copper filters with a half-value layer for copper of 2.15 mm.

A Rapid Chemical Test for Some Plant Virus Diseases[1]

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In a search for possible chemical reactions that might be of aid in the diagnosis of virus diseases of fruit trees, it was found than an alkaline extract of certain virus-infected peach or sweet cherry leaves produced, under certain conditions, a brilliant red coloration. A procedure was developed whereby the reaction could be used as a quantitative measure for some plant virus diseases. Although several thousand tests have been made during the past few months, this report can be of only a preliminary nature pending a more exhaustive study. Nevertheless, it seems desirable to report the procedure at this time because of its potential usefulness.

Thus far most of the studies have been confined to virus diseases of cherry and peach trees, and the discussion that follows is based on work with these plants. Leaf tissue was used as the source for all analytical material. An ordinary paper punch, with a diameter of approximately 6 mm, was used to obtain disks of leaf tissue as samples of standard size. For routine work, only one disk was taken from each leaf, midway between the base and tip and midway between the midrib and margin of the leaf.

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[2] The writer wishes to express his appreciation to E. L. Reeves, Earle Blodgett, Folke Johnson, Leo Campbell, and E. R. Parker for their generous cooperation in supplying known virus material of known identity.

For any one sample from a tree, 5 leaves were used and the results averaged. Each leaf disk was placed in a ½" test tube (standardized for use in a photoelectric colorimeter), and 5 ml of reagent was added. The tube was then heated in a boiling water bath for 5–10 min, allowed to cool for about 10 min, and then shaken thoroughly before a reading was taken in the colorimeter. The disk of leaf tissue either settled to the bottom of the tube or remained at the top of the reagent and therefore did not interfere with the reading. A green filter was used in the results reported here. The color reached a maximum in about 10 min and remained constant for at least 30 min. Normal leaves yielded a blue-green to yellow-green color, while leaves from plants affected with certain virus diseases gave varying intensities of red. Spectrophotometric studies in the visible range showed that maximum absorption differences between normal and virus diseased material occurred in the range of 450–525 mμ. The colorimeter was used to give a more precise estimation of the color, but for rapid qualitative tests, visual observation alone could be used for the detection of some of the diseases.

The reagent is composed of 40 gm of sodium hydroxide, 0.3 gm of cupric sulfate, 3 gm of sodium citrate, and 1,000 ml of distilled water. The sodium hydroxide should be dissolved in one portion of the water, the cupric sulfate and sodium citrate in another portion, and the two mixed after they are dissolved. Copper sulfate seems to catalyze the formation of the red color. Sodium citrate is added to prevent the precipitation of cupric oxide when the reagent is allowed to stand for more than several days. The reagent has a blue color, and a reagent blank should be run on the colorimeter.

The sampling procedure is of the utmost importance, and it is necessary to use discrimination in the choice of leaf samples. Most diseases have distinctive leaf symptoms and, for any one sample, leaves should be chosen that have comparable symptoms. Moreover, leaves of the same "physiological" type should be used. On sweet cherry trees there appear to be two forms of leaves: juvenile leaves, as represented in rapidly growing twigs such as terminal growths and "water sprouts," and adult leaves, as represented on the fruiting spurs. The juvenile leaves tend to be thicker, longer, and narrower than the adult leaves and, even on apparently normal cherry trees, give a fairly strong reaction with the test. Thus, in choosing samples from sweet cherry trees only spur leaves should be used. On terminal branch growths, there usually is a gradation from typically juvenile leaves at the tip to typically adult leaves at the base. It may be possible, by using basal leaves, to obtain adequate samples from young trees that have not as yet attained sufficient spur growths, but further study of this point is needed. Midterminal leaves were used as test material for peach trees. Since all peach leaves appear to be of a single "physiological" type, sampling was not as difficult as in the case of cherry trees.

Leaf samples were normally taken from the midlamina portions of leaves for the reason that virus-infected leaves usually showed a gradient in color reaction from tip to base. Little variation in color reaction was found in the various portions of normal leaves. When midrib samples were taken, they usually gave a much higher reading than the rest of the leaf, particularly in the case of some virus-infected leaves. There is a possibility that if both midrib and midlamina samples were taken, a further distinction of certain viruses might be obtained. Tissue other than leaf might be used for the test, but uniform sampling for rapid work might be more difficult. Drying the leaf samples does not alter the effect of the reaction and thus permits the preparation of a large number of samples for analysis at a later date.

TABLE 1

COLOR REACTIONS OF SOME VIRUS DISEASES OF SWEET CHERRIES AND PEACHES

	Group	Color	Colorimeter reading
Sweet Cherries			
0	Normal	Blue-green	10–20
I	Ring spot Mottle leaf (mild form)	Yellow-green to yellow	25–50
II	Mottle leaf (severe form) Rasp leaf	Reddish-yellow	60–100
III	Rusty mottle Twisted leaf Little cherry	Red	150 and above
Peaches			
0	"Normal" Wart Calico Ring spot Cherry mottle leaf (mild form)	Blue-green	15–30
I	Cherry rusty mottle	Yellow-green	30–40
II	Western X-disease (mild form) Little peach	Yellow to reddish-yellow	50–100
III	Western X-disease (moderate and severe forms)	Red	150 and above

On the basis of the color test, the virus diseases of sweet cherry trees and peach trees, thus far tested, may be tentatively segregated into the various groups as shown in Table 1.

There was little variation in the colorimeter readings of samples taken from normal trees. The standard error on the readings from 5 leaves was always less than 2. In the case of samples from virus-diseased trees, on the other hand, the variation increased in direct proportion to the rise in colorimeter reading. The average readings for any one virus disease, however, were always within a certain range, and the differences between the groups were statistically significant.

With this test it seems possible to distinguish not only between different virus diseases of the same host but also between different forms of the same disease. On cherries, for example, the mild form of mottle leaf gives colorimeter readings ranging from 25 to 35, while the severe

form gives readings of 60–80. The mild form of rusty mottle gives an average colorimeter reading of around 200, while the severe form averages nearly 400. On peaches, the mild form of Western X-disease gives an average reading of around 60; the moderate form, 175; and the severe form, 250.

As shown in Table 1, there seems to be a group of "mild" virus diseases of peaches, the leaves of which do not differ significantly in color reaction from that of "normal" peach foliage. There is a possibility that some of the trees from which the "normal" samples were taken may have contained a "latent" virus. Truly virus-free material might have given a consistently lower reading. On sweet cherries, it is also difficult to find a bearing tree that is completely free from all viruses, but with the spur type of growth there seems to be a certain amount of isolation for some spurs. Thus, it was possible to find "normal" spurs even on trees affected with a "mild" virus such as ring spot.

At the present time it is possible to characterize a given virus disease only by a certain colorimeter reading (or percentage transmission) because the exact chemical compound or compounds that give the color have not yet been determined. Tests on pure chemicals indicate that the color reaction is probably due to polyhydroxy phenols, possibly of the tannin group. Other workers (*1*) have shown that some virus diseases cause an increase in tannin content in the affected plants. Since tannins are recognized as protein precipitants, it seems possible that a virus infection may initiate a defense mechanism within the host plant, leading to the production of tannins.

The only factor known at present to interfere with the test is girdling. Leaves from a girdled branch of a virus-free tree give a red coloration similar to that obtained from some virus-infected leaves. Thus, the mechanism of the test may depend upon the virus causing some disturbance in the phloem of the host plant.

Preliminary studies indicate that the test will probably work on virus diseases of other trees such as apples and apricots, as well as on some virus diseases of berries, including raspberries, strawberries, and blueberries. Time has not permitted a study of the virus diseases of annual plants. Some plants may not be suitable for the particular test described here. Leaf samples of quick decline and psorosis of oranges, for example, do not give a color test. Whether tissue other than the leaf could be used in such cases is not known.

A color test for plant virus diseases would seem to have many potential uses. It should be of great aid in establishing sources of virus-free plant material for propagation purposes, and it should aid materially in the diagnosis of cases of some virus diseases where symptom expression is meager or atypical. It might also serve as a tool in physiological studies of the interaction between virus and host.

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Inhibition of Gastric Ulceration in the Rat by *o*-Hydroxybenzoic (Salicylic) Acid

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It has been shown (*2*) that extensive ulceration develops in the rumen of the stomach of the rat following ligation of the pylorus if the animals have been previously fasted for a length of time which depends upon their age. This ulceration may be inhibited and in some cases entirely prevented by the administration of certain substances. In examining the activity of monohydroxybenzoic acids, one of them was found to have a striking anti-

TABLE 1

Influence of Monohydroxybenzoic Acids on Gastric Ulceration in the Rat*

Exp. No.	Acid administered	Dose/rat† (mg)	Avg. body weight‡ (gm)	Gastric juice (ml)	Ulceration: % clear	Ulceration: Average ulceration	Ulceration: Index
1	Saline (controls)		130–109	7.6	0	3.0	3.0
	p-hydroxybenzoic	27.4	129–107	5.3	17	2.2	1.8
	m- " "	27.4	128–108	5.8	0	2.8	2.8
	o- " " (salicylic)	27.4	127–108	3.0	100	0	0
2	Saline (controls)		139–117	5.5	0	3.7	3.7
	Salicylic (*o*-hydroxybenzoic)	9.6	140–115	4.6	0	2.5	2.5
	Salicylic (*o*-hydroxybenzoic)	27.4	141–119	6.5	100	0	0
3	Sodium chloride (controls)	25	136–116	6.1	17	2.2	1.9
	Salicylic	41.4	134–112	2.8	87	0.2	0
	Acetyl salicylic (aspirin)	54.0	133–112	5.6	67	0.3	0.1

* Exp. 1 and 2 female and Exp. 3 male rats fasted 48 hr. before pylorus ligation when the trial substance was administered, and fasting continued an additional 9 hr. The acids were given as their sodium salts at pH 7.2.

† The test doses were administered intraperitoneally in Exp. 1, intravenously in Exp. 2, and *per os* in Exp. 3.

‡ There were 6 rats in each group. The first body-weight average was at the beginning of fasting, and the second, preoperative.

ulcer effect (Table 1). The sodium salts were used and the therapeutic effect obtained whether the compound was administered intraperitoneally, subcutaneously or intravenously. The latter route is usually the best for purposes of comparison. Administration of the active compound by mouth some little time preceding the ligation of the pylorus showed that it is effective when given in this manner.

Data on the antiulcer actvitiy of *o*-hydroxybenzoic (salicylic), *m*-hydroxybenzoic, and *p*-hydroxybenzoic acids