The Inhibition of Virus Increase by Malachite Green

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Since the reproduction of plant viruses has not been demonstrated outside of the living cell and purified plant viruses have not been proved to possess enzymic activity, it would appear that the processes involved in the reproduction of viruses are intimately associated with the metabolism of the host cell. Physical conditions and chemical compounds that affect host metabolism are likely to influence virus formation. There is a possibility that, by the use of enzyme blocks or inhibitors, enzyme systems indispensable for virus formation may be identified. Since the inhibition must take place *in vivo*, an inhibitor must not only possess a certain degree of specificity but also be active at a concentration nonlethal to the living tissue. Malachite green, a diaminotriphenylmethane dye, seems to possess these requirements. The inhibition of dehydrogenase activity (oxidation of glucose, lactate, succinate, and formate) of *B. coli* by malachite green was noted by Quastel and Wheatley (*4*); the ability of malachite green to penetrate living cells was shown by Albach (*1*); and Caldwell and Meiklejohn (*2*) found that this dye affected the respiration of tobacco stem tissue up to a dilution of 3.3 ppm.

By using a detached leaf tissue technic (Takahashi, *5*), the effect of low concentrations of malachite green on the increase of tobacco mosaic virus in leaf tissue has been studied. In these experiments 1-gm strips from infected half-leaf pairs were used, one set floated on a solution of malachite green and the other on distilled water. The experiments were conducted in the dark and terminated after 8 days of culture. In order to determine the extent of virus formation under these conditions, the tissues were homogenized, diluted 1 part in 20 with distilled water, and inoculated on 20 opposite halves of *Nicotiana glutinosa* leaves (Holmes, *3*).

Considerable inhibition of virus increase in this cultured leaf tissue was shown at concentrations of malachite green as low as 2 ppm, and more at 4 ppm. The total number of local lesions on 20 half-leaves of *Nicotiana glutinosa* for leaf tissue on 2 ppm was 70, and for the control on distilled water, 312. At 4 ppm the totals were 46 for treated and 1,068 for control. At the concentrations used in the above experiments malachite green has practically no effect on the extracted virus *in vitro*. A treated suspension of virus (4 ppm of malachite green) gave 1,890 local lesions, and the control, 2,060.

The inhibition of virus increase in living cells by low concentrations of malachite green appears to be due to its action as a block in enzyme reactions leading to virus formation. By the use of a series of more specific inhibitors there is a possibility of revealing some of the steps involved in virus formation. Meanwhile, the possibility that malachite green may be of some value in the chemotherapy of virus diseases must not be overlooked.

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Stability of Solutions of Pure Ascorbic Acid and of Dehydroascorbic Acid

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Results obtained in our laboratories, as well as those of other investigators (*4*, *7*), have indicated a lack of agreement between the 2,6-dichlorophenol-indophenol method of Bessey, *et al.* (*1*, *6*) and the 2,4-dinitrophenylhydrazine method of Roe, *et al.* (*9*, *10*) in the estimation of ascorbic acid in foods. Therefore, these two methods have been compared as to their ability to measure the ascorbic or dehydroascorbic acid content of freshly prepared and aged solutions. A study has also been made of the effects of oxalic and metaphosphoric acids on the stability of ascorbic and dehydroascorbic acids in solution.

TABLE 1

STABILITY OF ASCORBIC ACID AND DEHYDROASCORBIC ACID IN 0.5% OXALIC ACID AS MEASURED BY THE ROE AND BESSEY METHODS

Days stored at 4° C	Ascorbic acid solution μg/ml		Dehydroascorbic acid solution μg/ml	
	Roe	Bessey	Roe	Bessey
0	5.0	5.0	5.0	5.0
2			5.0	3.5
4	4.9		5.1	
6				2.4
8	5.1		5.4	
10	4.8		5.2	1.9
14		4.9		1.2
15	4.6		5.2	
22				0.8
24			5.1	
32		4.8		0.8

A stock solution containing 1,000 μg of pure ascorbic acid/ml of 0.5% oxalic acid was prepared. A working standard solution containing 100 μg of ascorbic acid/ml was obtained by diluting 10 ml of this solution with 10 ml of acetic acid (as required by the Roe method, *9*) and making up to a volume of 100 ml with 0.5% oxalic acid. A portion of this solution was treated with Norite to oxidize the ascorbic acid to dehydroascorbic acid (*9*). Both this dehydroascorbic acid solution and the remaining untreated ascorbic acid solution were stored at 4° C.

At intervals of one or more days, 5 ml of each was diluted to 100 ml with oxalic acid, and the resulting solutions were analyzed immediately by both the Roe and the Bessey methods. The results are shown in Table 1.

This procedure was repeated using similar solutions of ascorbic acid and dehydroascorbic acid in 5% metaphosphoric acid as stabilizing medium (Table 2).

TABLE 2

STABILITY OF ASCORBIC ACID AND DEHYDROASCORBIC ACID IN 5% METAPHOSPHORIC ACID AS MEASURED BY THE ROE AND BESSEY METHODS

Days stored at 4° C	Ascorbic acid solution μg/ml		Dehydroascorbic acid solution μg/ml	
	Roe	Bessey	Roe	Bessey
0	5.0	5.0	5.0	4.3
4	5.0	5.0	5.7	1.5
8	4.8	5.2	5.5	1.2
12	4.8	4.5	5.3	
15				0.4

Another stock solution containing 1,000 μg of dehydroascorbic acid/ml was prepared by oxidizing ascorbic acid, dissolved in water, with the theoretical amount of iodine, diluting to volume, and storing at room temperature. This was essentially a neutral solution, even though the small amount of hydriodic acid formed in the reaction was not neutralized. Aliquots of this solution were diluted 1:200 just before analysis (Table 3). Borsook

TABLE 3

STABILITY OF DEHYDROASCORBIC ACID IN WATER AS MEASURED BY THE ROE AND BESSEY METHODS

Days stored at room temperature	Dehydroascorbic acid solution μg/ml	
	Roe	Bessey
0	5.0	4.8
2	5.4	3.5
11	4.5	1.7
19	4.1	1.3

and his co-workers (*3*) have reported that one of the products of secondary oxidation of ascorbic acid is oxalic acid; however, tests on this solution when it was 10 weeks old failed to show any oxalic acid present.

These data indicate that:

(1) Ascorbic acid is stable in 0.5% oxalic acid–10% acetic acid solution at 4° C for at least 12 days (±2.5% for 12 days when measured by the Roe method and ±2.0% for 14 days when measured by the Bessey method). In 5% metaphosphoric acid–10% acetic acid solution, at 4° C, ascorbic acid is stable for at least 8 days (±2.8% for 12 days when measured by the Roe method and ±2.3% for 8 days when measured by the Bessey method). Oxalic acid is preferable as a preservative for ascorbic acid because it is more effective, more stable, less expensive, and more convenient. Ponting (*8*) and Gillam (*4*) have previously expressed this preference.

(2) Dehydroascorbic acid in oxalic or metaphosphoric acid solutions containing 10% acetic acid appears to be stable when measured by the Roe method, but is unstable when measured by the Bessey method. Since the indophenol method gives values which closely approximate the biologically active ascorbic acids content (*2, 3*), it is evident that the Roe method measures more than the biologically active forms. This corroborates the reports of Gillam (*4*) and Pijoan and Gerjovich (*7*). The latter authors stated that the phenylhydrazine reagent reacts with transformation products of ascorbic acid other than dehydroascorbic acid, which are biologically inactive.

(3) Dehydroascorbic acid is unstable in neutral solution at room temperature when assayed by either method. As with the acid solutions, the Roe method gives higher values than the Bessey method. However, in this case it appears that the chemical change of the original ascorbic acid had proceeded so far that substances had been formed which did not react with the phenylhydrazine reagent, but not far enough to produce oxalic acid. That such intermediary products are probable was suggested by Borsook, *et al.* (*3*).

(4) Both metaphosphoric acid and oxalic acid exert a stabilizing effect on ascorbic acid, but neither prevents the transformation of dehydroascorbic acid into derivatives which cannot be converted back to ascorbic acid by hydrogen sulfide treatment.

(5) The method of Roe apparently measures ascorbic acid (by oxidation), dehydroascorbic acid, and some other derivative(s) of ascorbic acid. This method may be useful for estimating the original ascorbic acid content of samples of fresh food which have been slurried with oxalic acid and stored for some time, or shipped long distances, before analysis. Metaphosphoric acid is probably too unstable for the preparation of such slurries. Additional data relating to this application of the Roe procedure in ascorbic acid studies will be presented at a later date (*5*). The probability that the Roe method measures the ascorbic acid originally present in the fresh sample, while the Bessey method measures only what is biologically active at the time of analysis may explain much of the lack of agreement in analytical results in the literature.

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