concentrations of penicillin in the rabbit sera. Trials on man are being carried out by Dr. Peeney, and will be communicated by him. We are also indebted to Mr. G. A. Rowe for the histological sections, and to Glaxo Laboratories for presenting us with a generous supply of penicillin.

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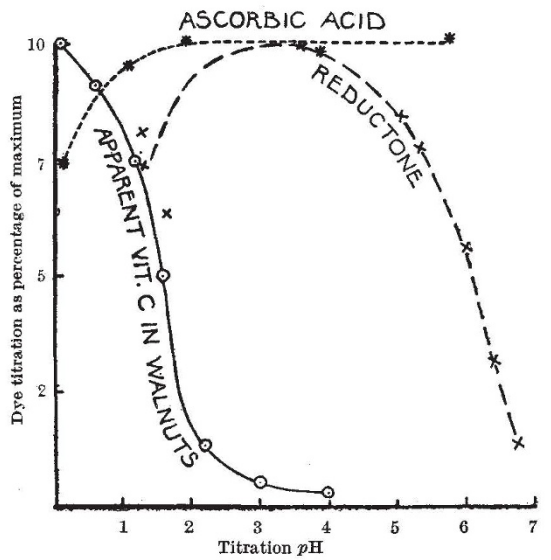
Effect of *p*H in the Dye Titration of Vitamin C in Certain Plant Materials

IN the estimation of vitamin C in plant materials by titration with 2:6 dichlorophenolindophenol, it has hitherto been generally assumed that the dye titration value is sufficiently independent of the titration *p*H to make it unnecessary to control the latter at all precisely. The usual procedure of preparing extracts with metaphosphoric or trichloracetic acid solutions to inhibit the action of ascorbic acid oxidase results in a *p*H which may range from 0·8 to 1·5, or even higher, according to the experimental conditions.

So far as ascorbic acid *per se* is concerned, this assumption may be justified by the findings by Martius and Euler[1] in 1934 that the dye titration value is practically constant from *p*H 4 down to *p*H 2, and then falls only slightly until the *p*H is reduced well below 1. When, however, ascorbic acid is being estimated in the presence of certain substances such as reductone, it has to be borne in mind that the dye titration value of the latter is not so constant as that of ascorbic acid. Martius and Euler found that under certain conditions the dye titration value of reductone falls rapidly as the *p*H is lowered below its optimum of 3–4, and that at *p*H 1 it might be decreased by 30 per cent. Their results for reductone and ascorbic acid are collated in the accompanying graph, and are in general agreement with results obtained here.

Recent work in these laboratories has shown the presence in different walnut extracts of a non-specific dye reductant clearly differentiated from ascorbic acid spectroscopically and by its reaction with formaldehyde and ascorbic acid oxidase and closely resembling reductone in its properties, especially in regard to its reaction with the above dye. It differs from reductone in the effect of *p*H on its dye titration value, which is at a maximum at a *p*H below 0·2–0·3 and falls as the *p*H rises until it reaches a minimum at *p*H 3·5–4·0. The graph includes a typical curve obtained with an extract of the mesocarp of *Juglans regia*, in which ascorbic acid had first been removed by treatment with formaldehyde at *p*H 4·5. (This treatment has no appreciable effect on the non-specific dye reductant, which is practically unattacked by formaldehyde during the time necessary for carrying out an estimation.) The results were obtained by visual and potentiometric methods previously described[2], and have been confirmed in numerous experiments on different walnut tissues in which the titration time ranged from 1 to 11 minutes. Taken in conjunction with the findings of Martius and Euler they indicate that:

(*a*) When ascorbic acid is being estimated by the dye titration in materials containing interfering substances such as have been encountered in walnuts, considerable errors may be caused by failure to allow for the effect of the titration *p*H, especially with a method employing titrations at two widely different *p*H values[3]. This may, in fact, explain certain discrepancies previously encountered[4] between results given by different methods.



EFFECT OF TITRATION *p*H ON DYE TITRATION VALUE OF ASCORBIC ACID AND REDUCTONE (TAKEN FROM DATA OF MARTIUS AND EULER) AND OF APPARENT VITAMIN C IN WALNUTS (OBTAINED BY DESTROYING TRUE VITAMIN C WITH FORMALDEHYDE AT *p*H 4·5)

(*b*) Walnuts may contain interfering dye reductants other than the glucoreductone and closely related compounds such as reductic acid and dihydroxymaleic acid, which are sometimes included in the generic term 'reductones'. In order to avoid premature conclusions, it would therefore seem advisable at present to avoid applying the name 'reductones' to substances in walnuts.

Full details of these results will be published elsewhere.

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Enzymic Oxidation of Ascorbic Acid by Apples

IN 1937, Johnson and Zilva[1] confirmed the fact that cabbage, cauliflower, cucumber and marrow contained an enzyme capable of oxidizing ascorbic acid directly, but reported that no such enzyme could be found in apple or potato. In respect of apples they pointed out that in the presence of catechol or of apple juice, the phenolases of apple oxidized ascorbic acid indirectly, but that ascorbic acid oxidase activity could not be demonstrated in the absence of phenolase activity, either in crude juice, filtered juice, or tissue extract.

Experiments carried out in this laboratory on the respiration of slices of apple tissue indicated that in Granny Smith apples ascorbic acid might be directly oxidized by an enzyme. The presence of this enzyme was confirmed by cutting Granny Smith apples into small pieces, freezing them, and expressing the juice by squeezing the tissue through muslin. The filtered juice oxidized ascorbic acid but showed no phenolase activity. Filtered juice which had been boiled for a few seconds showed very little activity towards ascorbic acid. The rate of oxidation was measured in a Warburg apparatus.

Attempts to isolate the enzyme responsible for the oxidation of ascorbic acid, using the method of Tauber, Kleiner and Mishkind[2], have so far yielded precipitates with little or no activity, but by the use of Szent-Györgyi's method[3] precipitates of relatively high activity were obtained. The yields were of the order of 1 milligram of dry precipitate per gram of fresh tissue. At *p*H 5·9 and 25° C., the specific activity of the preparation towards ascorbic acid,

$$W, \left(\frac{\text{mm.}^3\ O_2\ \text{taken up}}{\text{mgm. enzyme} \times \text{minutes}}\right),$$

varied from 0·02 to 0·1. The enzyme preparation had no phenolase activity; this indicates that ascorbic acid was oxidized directly.

Enzyme preparations showing similar behaviour have been obtained from Jonathan and from Cox's Orange Pippin apples.

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Thermally Evaporated Anti-Reflexion Films

IN a recent communication[1], J. Bannon has described a technique for hard-baking magnesium fluoride films on glass surfaces; this prompts us to discuss briefly our own experience.

These laboratories have been engaged in large-scale production of hard-bake magnesium fluoride since 1943. From this year until October 1946 both the quality of our films as well as the production efficiency has steadily improved. In the initial experiments we baked the films in an air oven at 390° C. for the harder glasses. For some reason not clearly understood, we were able to harden films deposited on the softer glasses at temperatures around 375° C. On the basis of our earlier attempts we believe that: (1) films baked in an air oven were not consistently satisfactory for mass production; (2) films were not so hard as the best which had been vacuum-baked; (3) the type of source, as well as the degassing procedure followed in conditioning the fluoride before coating, affects the hardness.

Although U.S. Army Specification No. 51-70-4B was used as a production check, it has been noticed that more than 90 per cent of our films are much more durable than the test would indicate. A really hard coating deposited on the hypotenuse of a 7 × 50 binocular prism, for example, when rubbed 20 cycles with the standard eraser at pressures of 10 lb. or more, should show no more hairlining than an uncoated prism treated in the same manner. The prism surface is viewed by internal reflexion under an inspection lamp.

The length of baking time does not influence the hardness. Indeed, in a well-degassed system, the glass can be coated immediately the working temperature (250°–300° C.) is reached. Longer baking may help to condition a contaminated system, but the hardness is not improved thereby. A factor contributing to soft coatings, even under adequate heating conditions, is the deposition of 'slow fluoride' on the glass as an undercoat. By the use of this term we indicate molecular rays, the components of which have been slowed down by collision (*a*) at the walls of the chamber, (*b*) with other molecules in space, (*c*) by energy absorption in the molten material of the fluoride source, or (*d*) by low-temperature evaporation. Such molecular rays, it is safe to assume, impinge on the glass surface at low velocity. As an undercoat they give rise to films having poor adhesion to the surface no