

### A NEW METHOD FOR THE ESTIMATION OF VITAMIN-C

VITAMIN-C is the only vitamin for which reliable and simple chemical methods are found. The one generally employed is Tillman's 2:6-dichlorophenol indophenol dye titration method. But this method suffers from the disadvantage of lack of precision in the end-point with old solutions of the dye, while with fresh solutions the experimental procedure becomes tedious. Several alternative methods also are to be found, and of these excepting the method of Roe & his collaborators<sup>1-3</sup> in which the red colour produced by dehydro-ascorbic acid with 2:4-dinitro-phenylhydrazine is measured, all are based on the reducing property of vitamin-C on several reagents, e.g., methylene blue,<sup>4</sup> phospho-18-tungstic acid,<sup>5</sup> silicomolybdic acid<sup>6</sup> arsenotungstic—and arsenotungstomolybdic acids<sup>7</sup> and ferridipyridyl sulphate.<sup>8</sup> We tried Folin's phosphomolybdic acid reagent (containing tungstic acid) for sugar and found that it gives blue colour with vitamin-C at room temperature only very slowly, taking many hours to reach the maximum. However, the colour develops rapidly at 100° C., but under these conditions fructose also produces the blue colour.

Next our choice fell on the phosphomolybdic acid reagent of Polis and Sortwell<sup>9</sup>. We found that this reagent gives even in the cold (30° C) an intense blue colour with such small concentrations of the vitamin as M/1000. The colour is found to be quite stable for several hours.

A number of substances (citrate, oxalate, malonate, malate, etc.) and sugars present in fruits were tested and none is found to give any colour in the cold. On heating to 100° C. few sugars gave colour, fructose yielding comparatively more colour. Cysteine and glutathione (GSH) gave no colour in the cold even with M/100 solutions. On keeping, however, a faint greenish blue colour began to develop slowly. These thiol compounds produced greenish blue colours in high concentrations, e.g. M/10, but the colours took several hours to reach the maximum intensities.

The method we have worked out, for the present for pure aqueous solutions of ascorbic acid, is as follows.

5 ml. of vit-C (M/1000 to M/100) in 10 per cent. acetic acid are treated with 0.2 ml.

of the phosphomolybdic acid reagent and shaken well. A blue colour rapidly develops and reaches its maximum intensity in about 5 mins. The colour is found to be quite stable and proportional to the concentration of vit-C. The colour can be estimated colorimetrically, preferably on a photo-electric colorimeter. A further advantage of our method lies in the fact that the blue reduction compound can be estimated by titration against M/100 potassium permanganate from a micro-burette, till the blue colour disappears and there remains only a yellow colour due to the reagent. From the titre the vit-C content can be calculated.

Further work on the application of the method for foods and biological fluids is in progress.

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**OVERALL GROWTH-PROMOTING POTENCY OF NEERA (UNFERMENTED JUICE) AND TODDY (FERMENTED BEER) FROM PHOENIX SYLVESTRIS**

POWERFUL growth-promoting and therapeutic properties are popularly attributed to Neera or the sweet unfermented juice of the palmyra palm, *Phoenix sylvestris*. The problem of Neera—its preservation, nutritional value and its economic disposal—has, at the moment, attained great topical significance, and as part of a comprehensive scheme of investigation, the overall growth-promoting potency of the unfermented and fermented forms of Neera