

Iwatake,⁷ Lyman, Schultze and King⁸ and others to protect vitamin C in solution against oxidation. This property, combined with the protein-precipitating ability of metaphosphoric acid, was made use of in the present work in preparing milk samples for titration. To eliminate the uncertain protein-precipitating power encountered with ordinary metaphosphoric acid solutions, the required volume of a stable sodium metaphosphate solution, prepared by the method of Briggs,⁹ was acidified just before each titration and added to the milk. Using 10 ml of 10 per cent. sodium metaphosphate solution, the addition of 0.6 ml of concentrated hydrochloric acid was found to bring the pH of the solution to a point (pH 2.5-3.0) where immediate and complete flocculation of protein resulted upon addition of the metaphosphate solution to 10 ml of milk.

Titration was made in the presence of precipitated milk protein. By repeated centrifuging and washing of the milk protein followed by separate titrations of combined centrifugate and protein residue, it was found that slightly higher ascorbic acid values were obtained in titrations of milk in the presence of precipitated protein. However, upon the addition of pure ascorbic acid to the protein residues and repetition of the centrifuging procedures and titrations, it was shown that the slightly higher apparent values obtained in the presence of milk protein were due to adsorption of negatively charged dye by positively charged protein. No adsorption of ascorbic acid by the milk protein could be demonstrated.

The new apparatus and improved technique provides a simple but reliable method of obtaining and determining vitamin C of milk in its naturally occurring form. The improvements mentioned should be of value in following fluctuations of vitamin C in milk at different stages of lactation, at various seasons of the years, during feeding experiments and under numerous other conditions.

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A PLANT GROWTH INHIBITOR

DURING the course of physiological investigations on the plant hormone relationships in radish, strain French Breakfast, an ether extraction was made of 4.935 grams fresh weight of cotyledons from seven-day-old seedlings. These plants had been grown in

⁷ A. Fujita and D. Iwatake, *Biochem. Zeits.*, 277: 293, 1935.

⁸ C. M. Lyman, M. O. Schultze and C. G. King, *Jour. Biol. Chem.*, 118: 757, 1937.

⁹ D. Briggs, *Proc. Soc. Exp. Biol. and Med.*, 37: 634, 1938.

the open in rich, loamy soil. The extraction was carried out according to the simplified auxin extraction method of Van Overbeek.¹ On testing the extract by the Avena test (Went and Thimann²) positive curvatures of from 17 to 23 degrees were found instead of the usual negative ones. (If the substance being tested is growth-promoting then the Avena plant will grow more rapidly on the side on which the substance is applied. Thus, because of this unsymmetrical growth, the plant will become curved in a direction away from the side on which the substance is applied. This is known as a negative curvature. If, however, the material causes an inhibition of growth then the plant will likewise grow unsymmetrically, but now the resulting curvature will be in a direction toward the side of application of the substance. This is known as a positive curvature.)

The relation between the concentration of the extract and degrees of positive curvature was investigated. In determining the amount of positive curvature 48 Avena plants were used at each dilution value. The inhibitor, extracted as above, was taken up in 1½ per cent. agar and cut into blocks 1.6 × 2 × 2 mm for application to the test plants. The standard Avena technique for auxin determination was used except that the curvature—positive in this case—was measured 150 minutes after applying the inhibitor instead of after 90 minutes, as is customary when testing growth-promoting substances. The results are seen in Fig. 1.

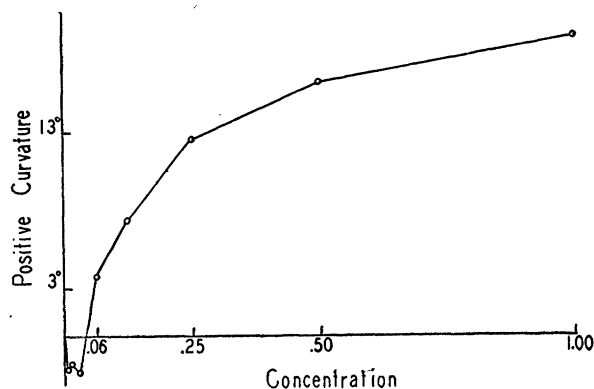


FIG. 1. Relation between positive curvature of Avena plants and two-fold dilutions of inhibitor substance.

This graph shows that positive curvatures between 3 to 13 degrees are proportional to the concentration of the inhibitor.

Using the method given by Schneider and Went³ a Photokymograph test was made of the reaction time of the coleoptile to growth inhibitor. The results are pre-

¹ J. Van Overbeek, *Proc. Nat. Acad. Sci.*, 24: 42, 1938.

² F. W. Went and K. V. Thimann, "Phytohormones," Macmillan Company, New York, 1937.

³ C. L. Schneider and F. W. Went, *Bot. Gaz.*, 99: 470, 1938.

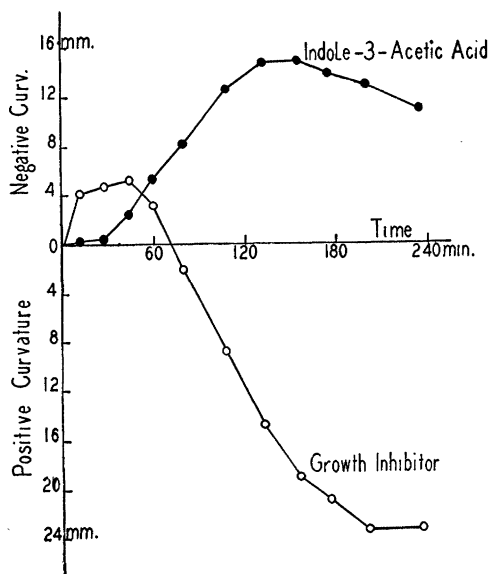


FIG. 2. Curvature rate of *Avena* plants upon application of: (a) .05 mg/liter, indole-3-acetic acid, and (b) growth inhibitor. (Ordinate values given as mm deviation of the extended coleoptile from the vertical position.)

sented in Fig. 2. It is observed that a negative curvature is initiated which rapidly changes between the first and second hour to a positive curvature, reaching its maximum three hours after the application of the inhibitor. It is interesting to note that the reaction rate for this negative curvature is different from the negative curvature caused by auxin. This is shown by the control run made at the same time as the inhibitor test but by using a growth-promoting substance, indole-3-acetic acid, .05 mg per liter. Each point on the graph is the average of twelve *Avena* test plants.

Inhibitor was found in the cotyledons of radish plants grown in the light or dark, but it was not found in the hypocotyl in either case.

The inhibitor substance is of neutral character. Accordingly as one would expect on the basis of Went's⁴ potential gradient theory of auxin transport, it should be transported acropetally as well as basipetally. Experiments prove this to be the case, as was shown by equal amounts of inhibitor passing through normal and inverted 4 mm long sections of *Avena* coleoptiles. Similar experiments show there is likewise no inhibitor transport polarity in radish hypocotyl sections.

In conclusion, it may be said that the positive curvatures resulting from the application of the inhibitor are not to be considered the same as the positive curvatures resulting from the retardation of the physiological tip regeneration in the *Avena* coleoptile because these are usually of slight magnitude, and furthermore they are

never preceded by a negative curvature during the first hour.

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⁴ F. W. Went, *Jahrb. wiss. Bot.*, 76: 582, 1932.