## GROWTH RESPONSE OF PLANTS TO RIBOFLAVIN AND ASCORBIC ACID

EXPERIMENTS at the University of Iowa on mineral nutrition of plants, involving the addition of riboflavin and ascorbic acid, have disclosed certain marked responses to these vitamins. Data are given for replicate pot cultures of eggplant (*Solanum melongea,* variety Black Beauty) and tobacco (*Nicotiana tobaccum,* variety Little Turkish) grown in silica gravel with Withrow's nutrient solution. Controls received only mineral salts while test cultures of the eggplant were supplemented with 2.5 P.P.M. synthetic (Merck) riboflavin, and those of tobacco were supplemented with 10 P.P.M. synthetic (Merck) ascorbic acid, dissolved directly in the aqueous solution. Pots were drained on alternate days and fresh nutrient solution added to maintain a uniform supply of mineral salts and vitamins.

Data (Table I A) show that riboflavin increased the growth of eggplant and (Table I B) that ascorbic acid increased the growth of tobacco.

TABLE I

A. GROWTH RESPONSE OF EGGPLANT IN NUTRIENT SOLUTION CONTAINING RIBOFLAVIN

| Age of plants in days | Stem length, cm. | | Fresh weight of tops in gms. | | Per cent. dry weight | | Fresh weight of roots in gms. | | Per cent. dry weight | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Test | Control | Test | Control | Test | Control | Test | Control | Test |
| 56 | 6.2 | 12.1 | 13.5 | 45.5 | 17.5 | 12.3 | 1.6 | 3.0 | 14.0 | 20.2 |
| 77 | 7.1 | 14.2 | 48.5 | 121.0 | 12.7 | 12.3 | 12.5 | 22.9 | 9.9 | 9.4 |
| B. GROWTH RESPONSE OF TOBACCO IN NUTRIENT SOLUTION CONTAINING ASCORBIC ACID | | | | | | | | | | |
| 56 | 25.1 | 34.2 | 38.2 | 82.8 | 8.2 | 8.1 | 3.0 | 5.9 | 12.1 | 16.3 |

The initial gain of eggplants receiving riboflavin was maintained throughout growth. The leaves were consistently coarser in texture, thicker, and darker in color than the controls. The roots were coarser and more extensive. Shoots of 56-day-old riboflavin plants were more highly hydrated (dry weight 12.3 per cent.) than controls (dry weight 17.5 per cent.), but the converse relationship existed in the roots (dry weight of riboflavin plants 20.2 per cent., controls 14.0 per cent.). These differences in percentage of dry weight tended to level out in both tops and roots with increasing age. At the age of 77 days the dry weight of tops of both test and control plants was approximately 12.5 per cent. and of both sets of roots about 9.5 per cent.

In most respects the tobacco plants receiving ascorbic acid showed gains in growth comparable to those of the eggplants receiving riboflavin. Hydrogen ion determinations of the press sap showed that riboflavin increased the acidity of the apices of the eggplant, while with the ascorbic acid there was a slight decrease in the acidity of the stem tips of the tobacco plants. The plants receiving the vitamins showed smaller pH variations of the different regions than in the controls. Initial gains due to the two vitamins were maintained throughout the experiment. Compositional differences persisted in mature plants even where gross external contrasts tended to disappear.

The data suggest definite species differences in response to riboflavin and ascorbic acid, as well as the marked stimulatory effect of riboflavin which has not been previously reported. The experiments are being continued to determine the exact character of the histological and metabolic differences in various species under the influence of riboflavin and ascorbic acid.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE USE OF PLASTIC AS A SUBSTITUTE FOR COVER GLASSES

As a result of the present conflict in Europe, the price of cover glasses used in the preparation of slides for the microscopic study of tissues has risen so much that the question as to how to meet this increase in price has become a serious problem for those laboratories in which the number of tissues studied in serial sections is great. There seems, accordingly, to be a widely felt need for a less expensive substitute for imported cover glasses. We have found that plastic serves for this purpose.

The use of plastic as a substitute for cover glasses in microscopic technique is not new, since Lentze[1] had recommended similar material about ten years ago. However, some difficulties are encountered in the covering of microscopic sections with plastic, which seem so far to have prevented the wider use of this material; but it is possible to overcome these difficulties to a very large extent and we wish therefore to describe the method which we have found to be satisfactory.

We are using cellulose acetate, trade name "Vue Pak,"[2] which can be obtained in sheets 0.005 inch or 0.127 mm thick, corresponding approximately to the thickness of cover glasses No. 1, which varies between 0.13 mm and 0.14 mm. The refractive index of this material is 1.49 to 1.50, which is very nearly that of glass. Resistance to heat is 140–180° F. Exposure of the mounted and dried slides first to a temperature of 110–115° F. for twelve hours, then keeping them at room temperature for another twelve hours, with a

[1] H. Lentze, *Klin. Wochenschr.*, 9: 1470, 1930.

[2] "Vue Pak" prepared by Monsanto Chemical Company.

repetition of this procedure on several days, left them unchanged. The effects of sunlight, weak acids and weak alkalies are very slight.

The plastic is obtained in sheets which can be cut in such a way that their size and shape are the same as those of cover glasses. These slips are kept covered on both sides with sheets of tissue paper. They are, as a rule, clean, but if cleaning should be necessary, they may be dipped in 50 per cent. alcohol, one at a time, and dried immediately. Thick, paper-filtered Canada balsam serves as mounting medium. The use of an excessive amount of xylol should be avoided.

The most objectionable feature in the use of plastic in the place of cover glasses is their tendency to "curl" during the process of drying. If the slips are kept in the oven overnight at a temperature of 37° C., from three to five per cent., sometimes even a larger number, of the cover slips may pull away at the edges. To overcome this difficulty as much as possible it is necessary, after mounting, to dry the slides slowly at room temperature for five to six days, in order that the Canada balsam may be well hardened before placing the slides into slide boxes. In this way as a rule a fairly satisfactory result is obtained, although the smoothness of the plastic is not always equal to that of cover glasses.

After the appearance of the article by H. O'Brien,[3] we substituted isobutyl methacrylate polymer for Canada balsam. The solution found suitable was approximately 1 part of isobutyl methacrylate polymer[4] to 2½ parts of xylol. These experiments are still in an early stage, but it seems that this change improves the results. Under these conditions the drying requires not more than one day and, so far, no curling of the plastic cover slips has occurred.

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## ERRATIC POTENTIALS OF ELECTRODES SEALED IN GLASS TUBING

OUR attention has been drawn to the erratic potentials that are observed in the potentiometric titration of dilute solutions in which platinum electrodes, sealed in glass tubing, are used. These erratic potentials were particularly obnoxious near the end point of a titration. We have noted them specifically in the potassium dichromate-stannous chloride and the thallous chloride-potassium iodate titrations. Complete elimination of this erratic behavior was obtained by removing the glass tubing from the electrode, or, by allowing only the wire (electrode) to touch the solution being titrated or the electrolyte of the cell being studied.

[3] Harold C. O'Brien, SCIENCE, 91: 412, 1940.

[4] The isobutyl methacrylate polymer prepared by du Pont Company.

A general investigation has indicated that the erratic behavior of electrodes sealed in glass tubing is much more prevalent than one would normally be led to believe is true. Apparently most observers have attributed the erratic potentials to some peculiarity of the reaction involved rather than to the physical structure of the electrode assembly. Particularly does this seem to be a source of trouble in the erratic behavior of certain E.M.F. cells built for special purposes. This phenomenon may be crucial in these cases, for not only are fluctuation or drifts introduced but the induced potentials may be large (300 mv) and irreproducible.

The cause of this trouble has not been entirely established. It may be due, partially or entirely, to strains in the electrodes as a result of the glass-metal seal. This may result in a variable junction potential as a result of the solution being in contact with the same metal in different standard states; this could be reduced to a constant value or eliminated by a very careful annealing process. The disturbance is definitely in the region of the glass-metal-solution interface. Elimination of this interface seems to eliminate the source of trouble. In potentiometric titrations, where the electrodes must be subject to frequent cleaning by burnishing in a flame or treatment with hot aqua regia, we have found it highly desirable to eliminate the glass casing for the electrodes or to construct the electrode in such a manner that the solution does not come into contact with the metal-glass interface. This may also be necessary for many other types of cell measurements. Our recent experiences with this phenomenon have been such that we are certain that it is of a general nature and requires consideration where cell data are being obtained.

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