

Here again the majority of the plants were like the controls in flower color (orange bronze) or a slight variation from it (light bronze).

Colored photographs of the floral variants and their controls have been made on "Kodachrom" film by Mr. Ralph Bennett, of the University of Michigan Department of Botany. Solutions containing the pigments from the flowers of the *Antirrhinum* cultures have been photographed in color by Dr. O. L. Inman, of the C. F. Kettering Foundation at Antioch College.

Seeds from a number of the anomalous plants have been collected and will be sown to obtain further data on the genetical effects of the bombardments on dry seeds.

All exposures were made with the University of Michigan cyclotron, which is supported by the Horace H. and Mary A. Rackham fund. The author wishes again to thank Professor J. M. Cork and Dr. R. L. Thornton for their assistance.

Summarizing: Some plants grown from dry seeds which had been exposed to stray neutrons from the cyclotron have given rise to flowers which are different in color and in morphology from their controls. It is hoped that the genetic relations of the new types may be determined through further experimentation.

ROY M. CHATTERS

DEPARTMENT OF BOTANY,  
UNIVERSITY OF MICHIGAN

### REVERSIBLE INACTIVATION OF PHOSPHATASE

A STUDY has been made of the effect of various oxidation and reduction systems on the activity of the enzyme phosphatase. It has been found that the activity of a potent enzyme preparation can be decreased to one tenth its initial value by reduction with hydrogen, using platinized or palladized asbestos as catalyst. The activity of the enzyme can be completely restored to its initial value by molecular oxygen in the presence of the same catalyst. The original enzyme preparation is as active in nitrogen as in air or oxygen, showing that oxygen is not essential for the activity of the enzyme. Treatment of the original enzyme preparation with oxygen in the presence of platinized asbestos was also without significant effect.

The enzyme, inactivated by hydrogen and the catalyst, remained inactive when the hydrogen was replaced by nitrogen, showing that the reduced enzyme, and not the hydrogen, was responsible for the inactivity.

Although it was found possible to inactivate the enzyme by reducing agents other than hydrogen, reactivation of the enzyme in these cases by oxidizing agents has not been achieved. Reducing agents, such as cysteine, cyanide and ascorbic acid, inhibited the

activity of the enzyme in the order named. The inhibiting action of ascorbic acid was slight and detectable only in concentrations above 0.01 molar. The effect of various reversible dyes on the activity of the enzyme was studied. Anthraquinone  $\beta$ -sulfonate, phenosafranine, indigo-tetrasulfonate and methylene blue were without effect in their oxidized states. In their reduced states, however, they caused irreversible inactivation of the enzyme, the degree of inactivation depending upon the oxidation-reduction potential of the dye. The more negative the  $E'_0$  of the dye, the greater was its inhibiting effect. The semiquinones of anthraquinone  $\beta$ -sulfonate and phenosafranine were slightly less active than the completely reduced dyes.

The phosphatase was prepared from beef kidneys by autolysis and repeated fractional precipitation, modified after the method of Albers. The enzyme exerted its greatest activity at pH 9.2. Though the enzyme preparations were of high activity, they were by no means pure. Tested by electrophoresis in the Tiselius apparatus,<sup>1</sup> the most active preparations contained at least three demonstrable protein components. The isoelectric point of the active component, measured in acetate buffer, ionic strength = 0.10, at 1° C., was pH  $4.5 \pm 0.1$ .

The experiments reported herein were carried out at 35° C. and pH 9.0 using glycine or veronal as buffer.  $\beta$ -glycerophosphate was used as substrate.  $MgSO_4$ , in a final concentration of 0.0007 molar, was present in all experiments. Phosphates were usually determined colorimetrically by the method of Fiske and Subbarow, and also, in some cases, gravimetrically by the method of von Lorenz.

The protocol of a typical experiment demonstrating the reversible inactivation with hydrogen and oxygen follows:

0.05 mg enzyme in 30 cc solution  
Substrate: glycerophosphate, 0.003 molar  
Buffer: glycocoll, 0.017 molar, pH 9.0  
 $MgSO_4$ : 0.0007 molar  
Pt-asbestos present as catalyst

|           | Initial<br>treatment<br>20 min. | Subsequent<br>treatment<br>15 min. | Inorganic P<br>released in<br>10 min. at 35°<br>mg |
|-----------|---------------------------------|------------------------------------|----------------------------------------------------|
| (1) ..... | N <sub>2</sub>                  | N <sub>2</sub>                     | 0.765                                              |
| (2) ..... | N <sub>2</sub>                  | N <sub>2</sub>                     | 0.755                                              |
| (3) ..... | O <sub>2</sub>                  | N <sub>2</sub>                     | 0.780                                              |
| (4) ..... | H <sub>2</sub>                  | N <sub>2</sub>                     | 0.105                                              |
| (5) ..... | H <sub>2</sub>                  | O <sub>2</sub>                     | 0.740                                              |

M. KIESE

A. B. HASTINGS

DEPARTMENT OF BIOLOGICAL CHEMISTRY,  
HARVARD MEDICAL SCHOOL

<sup>1</sup> The use of the Tiselius apparatus was made possible through the kindness of Dr. Ronald Ferry.