# SPECIAL ARTICLES

## INDUCTION BY FAST NEUTRONS OF MUTATIONS IN ANTIRRHINUM AND MYOSOTIS[1]

IN a recent paper[2] the author has shown that variations in germination as well as in the external morphology of some plants result from bombarding dry seeds with fast neutrons. It is now possible to record changes in flower color and flower form observed in the same plants.

To recapitulate briefly: seeds of six different genera with very small seeds were put into gelatin capsules, the latter were placed in turn in a lead box ($8 \times 8 \times 6$ cm) whose centimeter thick walls were lined inside with a 2 mm layer of paraffin. The box and its contents were laid on a metal shelf attached to the outside of the cyclotron "tank" so as to be close to the bombarding chamber, but at a distance of about 60 cm from the target. In this way the seeds could absorb only stray emanations from the cyclotron, whenever it was in operation, during the three months in which these experiments were conducted.

The most conspicuous results with respect to changes in flower color and flower morphology were seen in the cultures of *Antirrhinum* and *Myosotis*. The former showed more marked color changes, whereas in the latter morphological variations were more frequently found.

The seeds from which the *Antirrhinum* and *Myosotis* cultures were grown were exposed to the stray neutrons during part of the time through a period of six weeks. Other seeds in the same experiments (1) were subjected to the bombardment for periods ranging from one to nine weeks. In the case of the snapdragon cultures, controls and treated seeds were grown from the same seed capsule.

*Myosotis* flowers showed conspicuous alterations in their morphology. The various deformations and anomalies were as follows:

(1) Twisted petals
(2) Cleft petals
(3) Fused petals
(4) Increase in number of petals
(5) Decrease in number of petals
(6) Different-sized petals
(7) Decrease in number of sepals

The anomalies were often separate on different flowers or occurred in combination with one another, for example, (1) 8 petals, fused at the base, some of which were small and others very large; (2) 5 petals fused, 3 sepals; (3) 5 petals and 4 sepals; (4) 2 large, separate petals, 2 fused, and 3 sepals; or (5) 3 petals and 3 sepals.

"A Dictionary of Color"[3] was used to identify the flower colors and to record them for future reference. Colors given in parentheses indicate the names which the author used for identification in the greenhouse as they were often more descriptive than those given in the dictionary.

Variations in floral color in *Myosotis* were not marked, although in two plants there were "orchid" (pinkish-purple) flowers.

Three groups of *Antirrhinum* were grown from treated seed, and there were controls for each set as well. For Culture 1 the normal color was yellow L1 plate 10 (lemon yellow), for Culture 2 cerise (rose pink) and for Culture 3 was burnt orange (orange bronze).

In Culture 1 there were no observable differences from the controls except that the individual blossoms were slightly reduced in size.

The greatest color variations from the normal controls were found in Culture 2 and these variations with the percentage of plants having these differences were as follows:

| | | |
|---|---|---|
| (1) | cerise (rose pink)—normal color | 48.2 per cent. |
| (2) | pink "5T" (light pink) | 5.5 " " |
| (3) | pink "1T" (pink white) | .8 " " |
| (4) | magenta (lavender) | 1.6 " " |
| (5) | burnt orange (orange bronze) | 34.7 " " |
| (6) | bittersweet (light bronze) | 9.2 " " |

Of the total it is seen that 54.5 per cent. of the plants were either like the normal (cerise) or but slightly different from it (pink "5T" and pink "1T"), 43.9 per cent. were bronze-like, and only 1.6 per cent. were lavender. With the exception of the lavender flowers, which were smaller, the blossoms of this culture were about equal in size with the controls.

Culture 3 likewise showed marked variations from the normal color. The variants and the percentage of plants in the culture which were normal and which were different from the control are indicated as follows:

| | | |
|---|---|---|
| (1) | burnt orange (orange bronze)—normal color | 54.1 per cent. |
| (2) | bittersweet (light bronze) | 29.1 " " |
| (3) | yellow L1 plate 10 (canary yellow) | 4.1 " " |
| (4) | spinel rose (pink) | 12.7 " " |

[1] Papers from the Department of Botany of the University of Michigan, No. 663.
[2] R. M. Chatters, SCIENCE, 87: 262–263, 1938.
[3] A. Maerz and P. M. Rea, "Dictionary of Color," McGraw-Hill Company, New York, 1930.

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.

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## 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 β-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 β-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,[1] 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 ± 0.1.

The experiments reported herein were carried out at 35° C. and pH 9.0 using glycine or veronal as buffer. β-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 treatment 20 min. | Subsequent treatment 15 min. | Inorganic P released in 10 min. at 35° mg |
|---|---|---|---|
| (1) ..... | .. | .. | 0.765 |
| (2) ..... | $N_2$ | $N_2$ | 0.755 |
| (3) ..... | $O_2$ | $N_2$ | 0.780 |
| (4) ..... | $H_2$ | $N_2$ | 0.105 |
| (5) ..... | $H_2$ | $O_2$ | 0.740 |

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[1] The use of the Tiselius apparatus was made possible through the kindness of Dr. Ronald Ferry.