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ARGINASE

ARGINASE as compared with the starting material—a watery extract from ground beef liver—was purified 50 times, using the common protein separation methods. The ferment solution obtained showed absence of catalase, amylase and proteolytic enzymes. Spectroscopic examination revealed the absence of hemoglobin, myoglobin and cytochrome C.

10% of this protein solution hydrolyzed 30 to 35 per cent. of 17.4 mg arginine within 10 minutes under the condition of the experiment.

While the addition of Mn^{++} -ions¹ to a crude liver extract is frequently without any effect upon the activity of the enzyme, it was found that in the further stages of purification Mn^{++} -ions are essential for obtaining maximum activity of the enzyme. The activation of arginase by metallic ions, for instance, Mn^{++} , is a time reaction requiring about 15 minutes under the condition of the experiment for obtaining optimum activation. The optimum pH for the purified enzyme (with or without Mn^{++} -ions) is about 9.5. Other ions, such as Fe^{++} , Ni^{++} and Co^{++} also activate the enzyme but to a less extent than Mn^{++} . Here again, however, the optimum pH for these ions was found to be about 9.5.

Arginase (no addition)	.35
“ + Mn^{++}	.71
“ + Mn^{++} —vitamin C	.71
“ + Co^{++}	.53
“ + Co^{++} —vitamin C	.56
“ + Ni^{++}	.50
“ + Fe^{++}	.48
“ + Fe^{++} —cysteine	.59

10% arginase; 17.4 mg arginine; heavy metal salts 0.5%; vitamin C or cysteine 1%; total volume 1.5 cc adjusted to pH 9.5; incubated for 10 minutes at 37.5° C. Numbers are n/20 KOH. Method of determination was the Linderstroem-Lang method² for titration of ornithine, modified to semi-micro.

In the purified state arginase is quite stable, tolerating dialysis for 48 hours at 4° C. without loss of activity. The ferment solution is also stable at this temperature for weeks and its activity not altered by evaporating from the frozen state to dryness and subsequent redissolving.

The isoelectric point of the enzyme was found by electrophoresis to be at pH 5.7. The investigation of the ash content of the purified enzyme gave in one case an Mn-content of 0.08 per cent.

¹ G. Klein and W. Ziese, *Klin. Woch.*, 14: 205, 1935.

² K. Linderstroem-Lang, L. Weil and H. Holter, *Z. physiol. Chem.*, 233: 174, 1935.

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THE INDUCTION OF FERTILITY IN GENETICALLY SELF-STERILE PLANTS

In hybridization experiments designed for the production of new colors in the various classes of commercially important Petunias, a strain of Golden Rose was discovered that has been found to be completely self-sterile under natural conditions. The self-sterility in this strain of Petunia behaves in inheritance as a simple Mendelian recessive character. By means of cuttings the strain has been maintained in the Botanical Laboratory of Bucknell University for the past five years. The plants are unusually floriferous and everblooming, due, in part at least, to their inability to produce seeds unless they receive pollen from plants which are not homozygous recessive for the self-sterility gene. Although many thousands of flowers were produced during this period and although repeated attempts were made to self-fertilize the plants, not a single seed capsule was ever produced until recently when self-fertility was induced by the techniques described below.

Dr. H. Clyde Eyster, of the Botany Department of the University of South Dakota, made microscopic observations of the pollen tubes in the stigmas and styles of the self-pollinated self-sterile plants and found that the pollen grains germinate well and develop into tubes which extend into the neck of the style but rarely if ever grow as far as one half the distance from the stigma to the ovary. In styles that had been cut three days after they had been self-pollinated, Dr. Eyster found that most of the pollen tubes grow only about one tenth of the distance from the stigma to the ovary, while an occasional tube grows somewhat less than one half of the distance down the style. Before any of the tubes reach the ovary, the style is cut off from the top of the ovary by the formation of an abscission layer. From these observations it appears that the self-sterility in the Golden Rose Petunia studied is caused by the very slow rate of growth of the pollen tubes and the formation of an abscission layer which severs the style from the ovary before any of the tubes enter the latter.

The pollen of the self-sterile plants was placed on the stigmas of normal self-fertile plants and allowed to develop for forty-eight hours. At the end of this time the styles were cut, preserved and sent to Dr. Eyster for microscopic examination. Approximately 75 per cent. of the pollen grains were found to have developed pollen tubes of varying lengths, including many which extended all the way down the style and,

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presumably, into the ovary. This result was to be expected since the pollen of the self-sterile plants causes normal seed pods with viable seeds to be produced on plants which are not homozygous recessive for the gene for self-sterility involved. So also the pollen of plants which are not homozygous recessive for the self-sterility gene develops normally in styles of the self-sterile plants. Observations of styles made forty-eight hours after they had been pollinated showed that from 75 to 95 per cent. of the pollen had germinated and had formed tubes of varying lengths, including many which extended all the way down the style.

Plants of the self-sterile strain of Golden Rose *Petunia* can be self-fertilized by either of two methods described below. If flower buds which are beginning to develop anthocyanin in the petals are opened and pollinated with pollen from fully opened flowers from the same plant, seed capsules containing viable seeds are produced. Similar results were found in *Petunia violacea* by Yasuda,¹ who refers to this method of self-fertilization as homo-pollination. In a more recent study Yasuda² found that the placenta in the ovary of *Petunia violacea* secretes a "special substance" which diffuses into the style and retards or completely inhibits the germination of the pollen and the development of pollen tubes. Preliminary studies with the self-sterile Golden Rose strain of *Petunia* in my laboratory indicate that when the sap expressed from the ovary of self-sterile plants is placed on the stigma and style of strains which are not homozygous recessive for the self-sterility gene, the latter strains are rendered cross-sterile with the self-sterile plants. This result tends to show that the ovarian secretion

which renders the plant self-sterile can be transferred to other plants and renders them cross-sterile with pollen from self-sterile plants.

The self-sterile plants can also be made self-fertile in a more simple and more remarkable manner. This may be accomplished by spraying the flowering plants with a solution composed of ten parts of alpha naphthalene acetamide dissolved in one million parts of water.³ Flowers which are sprayed with this solution immediately before or shortly after they have been self-pollinated produce seed capsules filled with viable seeds in exactly the same way that normal self-fertile plants of other strains produce seeds. Obviously alpha naphthalene acetamide neutralizes the effects of the ovarian secretion which diffuses into the style and inhibits or greatly retards the growth of the pollen tubes. Seeds from such self-fertilized self-sterile plants were found, when planted, to grow into normal seedlings and the per cent. of germination in all trials was unusually high.

Preliminary experiments indicate that the alpha naphthalene acetamide greatly increases the self-fertility or self-compatibility of highly inbred and highly sterile strains of *Tagetes erecta* (African marigolds), *Brassica oleracea* (cabbage) and *Trifolium pratense* (red clover). These results suggest that a great variety of economically important plants which are normally partly or completely self-sterile or self-incompatible may be made self-fertile by the use of a suitable solution of alpha naphthalene acetamide as indicated in the preliminary report given in this paper.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW PROCEDURE FOR ADSORPTION ANALYSIS

If a liquid containing one or more dissolved substances is allowed to pass slowly through a layer of a finely divided adsorbent, the different solutes, dependent upon the degree of adsorption, will become more or less retarded as compared to the liquid. If the solution flows upwards through the adsorbent and emerges into a vessel suitable for optical observation (for example, by the Toepler Schlieren method, as used in observations of electrophoretic migration¹) a number of boundaries will be observed, corresponding to the number of differently adsorbable components

present, and the volume of liquid between each boundary and the meniscus ("the retardation volume") will equal the ratio between the amount adsorbed and the concentration of the corresponding component.

If the mutual adsorption displacement effects are negligible the concentrations of the components in the observation tube should have the same value as in the original solution. A new method for qualitative and quantitative analysis, with a very wide field of application, may be based upon the principle described. A theoretical treatment and a description of the experimental arrangement has been given in two recent communications.² The method has been tried on mixtures

¹ Sadawo Yasuda, *Proc. Crop. Sci. Soc. Japan*, 2 (2): 122-126, 1930.

² Sadawo Yasuda, *Bot. Mag.* (Tokyo), 46 (548): 510-517, 1932.

³ A. Tiselius, *Trans. Farad. Soc.*, 33: 524, 1937. See also The Harvey Lectures, 35: 37, 1939-40.

³ The alpha naphthalene acetamide was used in the form of the commercial preparation known as Fruitone.

² A. Tiselius, *Arkiv för Kemi* (Royal Swedish Academy of Sciences), 14 B, No. 22 and No. 32. A detailed description of the method and some of its applications is to appear this year in "Advances in Colloid Science," edited