RESEARCH NOTES

ABSENCE OF HEMAGGLUTININS FROM BUFFER EXTRACTS OF VIRUS-INFECTED LEAVES

Dalzell, *et al.*[1] have reported positive hemagglutination reactions with virus-diseased plant tissues and suggest that there may be a correlation between the hemagglutinin and infective titers of the viruses.

Since the hemagglutination technique would be a very useful method in detecting plant viruses, attempts have been made in this laboratory to determine the agglutinability by six separate plant viruses for any one of five red blood cell species. The following viruses were separately inoculated to tobacco plants (*Nicotiana tabacum* var. Holmes Samsoun having the N localization factor from *N. glutinosa*): Cucumber virus I type strain, tobacco severe etch virus, tobacco ringspot virus, tobacco necrosis virus strain F, and the Johnson No. 1 ringspot strain of potato virus X. Cucumber virus I was also propagated on cucumber (*Cucumis sativus* var. Marketer) while the common type-distorting strain of tobacco mosaic virus was propagated on *N. tabacum* var. Virginia 12. Normal uninoculated plants of the species mentioned were also kept to be used as controls.

The red cell species used were: Chicken, guinea pig, rabbit, cow, pigeon, goat, and human type "O" cells. Freshly drawn blood was collected in 2-percent citrate solution and kept in the refrigerator for not more than 3 days before the tests were performed. Just before the test the cells were washed by centrifuging three times in 0.85-percent saline. A 2-percent suspension by volume of the red blood cells was finally made in saline.

Leaves showing good symptoms of viral infection were collected about 2 weeks after inoculation and ground up in mortars, a separate mortar being used for each virus. Extracts were made by adding approximately 4 cc. of 0.1-molar buffer per gram of leaf while grinding and the macerate was squeezed through two layers of gauze to obtain the extracts. Seven rows of serial twofold dilutions in saline were made from each extract in 0.5-ml. volumes. To each of the tubes of one row was added 0.5 ml. of chicken red cell suspension; to another row human type "O" cells, and so on.

Extracts from normal tobacco plants were similarly treated.

A control tube of red-cell suspension from each species plus saline was set also. All tubes were incubated at room temperature (±29°C.) and observed during a period of 2 hours at 15-minute intervals. The reaction was read by carefully observing the bottom of the tubes for the pattern of red-

[1] Dalzell, R. C., Boyle, J. S., and Reid, J. J., Hemagglutination as a test to determine virus infection of plants, *Soc. Amer. Bact. Proc.* **57** 25, 1957.

cell sedimentation. In no instance was definite agglutination observed. In a single case the cells of one rabbit were partly agglutinated nonspecifically by both the normal leaf extracts and most of the virus infected leaf extracts.

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