In the field of the enzymes, as was recently shown in collaboration with Rohdewald (*6*), a number of enzymes can be located on powder columns by painting a longitudinal streak with a brush carrying the solution of the corresponding substrate; after a brief incubation period a second brush applies a color reagent for the enzymatic cleavage product and thus indicates the borderlines of the zone. In any system of paper disks such operations will be carried out, of course, in the manner of spot tests as proposed earlier by Feigl and others for certain enzymes (*7*).

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Grasshopper Transmission of Three Plant Viruses[1, 2]

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The tobacco mosaic virus, potato virus X (latent potato virus), and tobacco ringspot virus have been studied intensively, but heretofore the satisfactory transmission of these viruses by insects has not been clearly shown. Investigations carried out in the greenhouse show that the differential grasshopper, *Melanoplus differentialis* (Thos.), can transmit these viruses from tobacco to tobacco.

Allard (*1, 2*) reported transmission of a virus thought to be tobacco mosaic, from tobacco to tobacco with the aphids *Myzus persicae* (Sulz.) and *Macrosiphum tabaci* Perg. Hoggan (*3*) indicated that *M. persicae* does not transmit the tobacco mosaic virus and suggested that Allard was working with the cucumber mosaic virus, which *M. persicae* does transmit. Hoggan (*4–6*) demonstrated that *M. persicae, M. pseudosolani* (Theob.), and *Macrosiphum solanifolii* (Ashm.) do not transmit the tobacco mosaic virus from tobacco to tobacco, although they will transmit it from tomato to tobacco and other solanaceous hosts. Gigante (*7*) has reported transmission of the tobacco mosaic virus by *Macrosiphum gei* Koch (=*M. solanifolii*) from tobacco to tobacco, tomato, pepper, and eggplant. No insect vector has been discovered for potato virus X and tobacco ringspot virus.

Single adult grasshoppers were used for all tests. The insects were reared on young corn plants and starved 4–6 hr before feeding on the virus source. Infected tobacco (*Nicotiana tabacum* L.) served as the source for the 3 viruses. A 1–2-minute feeding on the virus source was allowed for transmission tests made immediately after the feeding. For the 2- and 4-hr waiting periods[3] a 15-min feeding time was allowed, and for the 12- and 24-hr tests, a 30-min feeding time. Each insect was transferred by hand. It was caught by a hind leg with the thumb and forefinger and placed upon a leaf of the test plant. This method of handling was used to prevent transfer of the viruses by hand. Precautions were also taken to avoid infection from other sources. Unless otherwise stated, each grasshopper was moved to a new location on the test plant after feeding a few bites or making a hole 1–6 mm in diameter in the leaf. Except as noted, each grasshopper was allowed only 2 feedings at different locations on the test plant in the tests made immediately after feeding and after the 2- and 4-hr waiting periods. Those individuals tested after a 12-hr waiting period were allowed to feed in approximately 10 different locations on the test plant. Sixty-four per cent of the insects tested after a 24-hr waiting period were allowed to feed in 2 locations, and 36% at 10 locations.

A hybrid plant, *N. tabacum* × *N. glutinosa* L., which develops local lesions at the point of infection, or necrotic streaks if the virus is introduced into the veins, was used for the transmission tests with the tobacco mosaic virus. Single grasshoppers were tested for infectivity on individual plants, and the local lesions which developed were counted. Seventy-five grasshoppers were tested immediately after feeding on the virus source. Twenty-eight local lesions developed on 22 plants, which indicates that 22 (29.3%) of the 75 insects tested transmitted the virus. Seven (46.6%) of 15 insects tested after the 2-hr waiting period transmitted the virus. After the 4-hr waiting period, 10 (29.4%) of the 34 grasshoppers tested brought about the production of 12 local lesions. Only 2 (9.5%) of the 21 individuals tested after the 12-hr waiting period transmitted the virus. Tests with 58 insects following the 24-hr waiting periods resulted in no transmission.

In another experiment, individual grasshoppers were allowed to feed for a series of 18 times, one on each half of 9 leaves, after feeding on infected plants. Forty (83.3%) of 48 insects tested transmitted the virus, producing a total of 96 local lesions. The greatest

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[2] This paper is based upon work done for the Biological Department Chemical Corps, Camp Detrick, Frederick, Md., under Contract No. W-18-064-CM-226 with the University of Nebraska.

[3] "Waiting period" refers to the time between the feedings on the virus source and the test plants, during which time no food was consumed.

number of lesions occurred adjacent to feeding areas numbered from one to six. The occurrence of local lesions did not follow a definite pattern. In some cases they were consecutive, and in others as many as 16 feeding areas occurred between those producing lesions.

Another experiment consisted of feeding single grasshoppers on the virus source for 5 min and then immediately transferring them to individual hybrid plants to feed at will from 4:00 P.M. until 9:00 A.M. the following day. The amount of feeding ranged from single bites to almost complete consumption of the leaves. Twenty grasshoppers were tested, and 50% transmitted the virus, producing a total of 15 local lesions.

Local infection of the hybrid plants was not always adjacent to a feeding area. Approximately 3% of the total local lesions that developed in all tests occurred elsewhere. This might be due to transfer of the virus by the feet of the insects; however, grasshoppers do scar or make depressions in the leaf tissue with their mandibles while in search of a suitable feeding site. The latter seems to be the more plausible explanation. The feeding on a vein, or the development of a local lesion adjacent to one of the larger veins, brought about systemic infection of the hybrid plants in several instances.

In one experiment, single grasshoppers were transferred to individual healthy tobacco (*N. tabacum*) plants immediately after feeding on the virus source and were allowed to feed approximately 10 times in different locations. Fifty-seven (54.8%) of the 104 insects tested transmitted the tobacco mosaic virus, causing systemic infection of the plants. After a waiting period of 2 hr, 9 (45%) of the 20 insects tested transmitted the virus; and 9 (42.9%) of the 21 individuals tested after a 4-hr waiting period brought about systemic infection of the test plants.

Tobacco was also used as the test plant for the transmission of potato virus X (potato ringspot type) and tobacco ringspot virus. Single grasshoppers were transferred to individual healthy plants immediately after feeding on the virus source and were allowed to feed 6–8 times in different locations. The insects were disturbed between feedings, making the leaf perforations appear as isolated holes. Local infection appeared within 5–6 days. For the potato virus X, 18 (18%) of the 100 grasshoppers tested transmitted the virus to tobacco plants, which developed local lesions and later became systemically infected. Four of the initial infections did not occur adjacent to apparent feeding areas. With tobacco ringspot virus only 6 (6%) of the 100 insects tested transmitted the virus. Initial infection, with one exception, occurred adjacent to known feeding areas. In a majority of the cases, with both viruses, initial infection developed adjacent to feeding areas near one of the larger veins.

These experiments show that tobacco mosaic virus, potato virus X, and tobacco ringspot virus can be transmitted with the differential grasshopper under conditions as described above. This is possibly a simple mechanical transmission and nonspecific. The importance of grasshoppers transmitting these viruses under field conditions has yet to be investigated. A more detailed account of the work relating to this problem will be published elsewhere.

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Selective Damage to Fibroblasts by Desoxycorticosterone in Cultures of Mixed Tissues

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The remarkable selectivity which adrenocortical steroids appear to show for mesenchymal tissue, as demonstrated in pathological and clinical studies (*1*), raises the question as to whether this represents a direct effect on the cells of connective tissues. In rheumatic fever the connective tissues of the heart are profoundly altered by necrosis and fibrosis, whereas the changes in endothelium and in muscular activity appear to be secondary. Desoxycorticosterone (DOC) induces similar changes, but not in hypophysectomized animals (*2*). In this study it is proposed to explore the possibility of comparable *in vitro* effects of adrenal steroids on heart tissues, particularly with a view toward establishing whether there is one target tissue.

Hearts were removed from newborn line C white mice, cut into fragments about a millimeter across, and cemented into roller tubes with chicken plasma. Such fragments continued to pulsate in the cultures and at the same time provided outgrowths of two kinds of cells. The most abundant cells were of the fibroblast type, elongate bipolar cells arranged in radiating strands or in irregular networks (Fig. 1, right). The cells believed to be endothelium grew as continuous sheets of polygonal cells (Fig. 1, left). The initial growth was in nutrient solution: balanced saline (Gey's) + serum + embryo extract. The nutrient was then replaced by balanced saline containing the experimental agent. Desoxycorticosterone was used in doses sufficient to produce cytological changes in 6–24 hr. At this time the experimental solution was replaced with nutrient solution. It was thus possible to follow the relative rapidity of response of the two types of

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