

notype of T4, but the genotype of T2. The properties of this "latent T2" virus would seem to merit investigation.

#### References

1. DELBRUCK, M., and BAILEY, W. T., JR. *Cold Spring Harbor Symposia*, **11**, 33 (1946).
2. LURIA, S. Private communication (1947).

## Paper Disk Columns in Glass Chromatographic Tubes

L. Zechmeister

*Gates and Crellin Laboratories of Chemistry,<sup>1</sup>  
California Institute of Technology, Pasadena*

The use of filter paper in selective adsorption experiments, probably first described by Schönbein (1), has become a powerful tool in research, especially since the studies of Consden, Gordon, and Martin (2). Although in most modifications of paper chromatography only very small amounts of material can be resolved, Mitchell and his colleagues must be credited with preparative applications; recently these authors carried out successful enzyme separations in their chromatopile (3, 4).

Up to the present time no use has been made of the classical Tswett glass tube packed with filter paper disks, for the following reason. Although a homogeneous, satisfactory column may readily be prepared from a powder, on the contrary, channeling and gross irregularities in the flow of solutions become manifest when paper disks are stamped into a cylindrical tube.

It was found that this difficulty can be overcome if the size of the disks is adapted to the average diameter of a good cylindrical tube by cutting the paper with unusually high precision. For this purpose a stainless steel die and a corresponding punch must be available by means of which a supply of precision-cut paper can be obtained. About 15 paper disks were cut simultaneously with our die when Eaton and Dikeman No. 615 filter paper was used. The same cutting device can, of course, be used to prepare paper for several glass tubes,<sup>2</sup> represented in Fig. 1, which possess similar diameters.

For example, the diameters of 2 tubes (measured in different directions and at various levels inside the tube) varied, respectively, between 1.716–1.730 in. (4.359–4.394 cm) and 1.730–1.736 in. (4.394–4.409 cm). The diameter of the die was 1.73064 in. (4.396 cm), and that of the punch was 1.7299 in. (4.3938 cm). If the average diameter of the glass tube was, for example, 1.68 in. (4.27 cm), the even flow of the solvent was markedly distorted.

In order to pack the chromatographic tube, the pro-

<sup>1</sup> Contribution No. 1436.

<sup>2</sup> The model used is the same as proposed earlier (5) and is manufactured by the Scientific Glass Apparatus Co., Bloomfield, N. J.

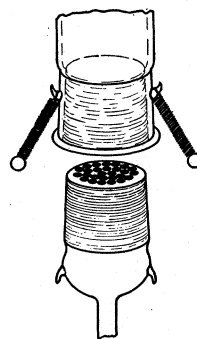


FIG. 1.

cedure shown in Fig. 2, upper part, is recommended. Each portion (20 paper disks, for example) is somewhat loosened up by ruffling the edges with the thumb, inserted a few centimeters deep into the horizontal glass tube and pushed in further with a smooth, cylindrical pestle (diameter, about 1 mm smaller than that of the tube), while the other hand is holding firm both parts of the glass tube (cf. Fig. 1) and exerting pressure. When the paper has reached its proper location, considerable pressure is exerted with the pestle for a moment while it is rotated by about a quarter turn. A column 20–25 cm high can be built up in about 15 min.

When suction of an ordinary water aspirator is applied, either aqueous or nonaqueous solutions flow evenly through a paper column which has been packed in the described way. The duration of the chromatographic experiment will then not exceed that required for a powder column of similar dimensions.

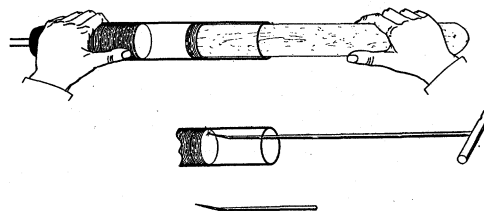


FIG. 2. Packing and removing a filter disk column. Left hand holds the ground glass section (cf. Fig. 1); dark area on left indicates position of the rubber stopper by means of which the tube will be attached to a suction flask.

Because of the tight contact between glass and paper, only short and broad columns can be extruded with a pestle or removed by means of a long-stem corkscrew; in general, however, the procedure as indicated in Fig. 2 (lower part) is recommended. About 1-cm sections of the column are rapidly taken out by using a sharply pointed and slightly bent steel pick which has a tempered end about 5 cm long.

The proposed technique shares the advantages of ordinary powder column experiments—i.e. those of a reasonably closed system—but has the disadvantage that the basic equipment requires the services of a precision mechanical shop.<sup>3</sup>

<sup>3</sup> We are indebted to J. H. Sturdivant for the facilities of the workshops under his direction.

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).

#### References

1. SCHÖNBEIN, CH. F. *Verhandl. naturforsch. Ges. Basel*, **III**, 249 (1861); Poggendorff's *Ann.* **114**, 275 (1861); *Z. anal. Chem.*, **1**, 212 (1862).
2. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, **38**, 224 (1944).
3. MITCHELL, H. K., and HASKINS, F. A. *Science*, **110**, 278 (1949).
4. MITCHELL, H. K., GORDON, M., and HASKINS, F. A. *J. Biol. Chem.*, **180**, 1071 (1949).
5. ZECHMEISTER, L., and CHOLNOKY, L. *Principles and Practice of Chromatography*. New York: Wiley; London: Chapman and Hall (1943), p. 63; ZECHMEISTER, L. *Progress in Chromatography 1938-1947*. New York: Wiley; London: Chapman and Hall (1950), p. 31.
6. ZECHMEISTER, L., and ROHDWALD, M. *Enzymologia*, **13**, 388 (1949).
7. FEIGL, F. *Qualitative Analysis by Spot Tests*. New York and Amsterdam: Elsevier (1946), p. 421; SASTRI, N. B., and SREENIVASAYA, M. *Mikrochimie*, **14**, 159 (1933-4); NOSAKA, K. *Mikrochim. Acta*, **1**, 788 (1937).

## Grasshopper Transmission of Three Plant Viruses<sup>1, 2</sup>

H. J. Walters

Department of Plant Pathology,  
University of Nebraska, Lincoln

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

<sup>1</sup>Published with the approval of the Director as Paper No. 502, Journal Series, Nebraska Agricultural Experiment Station.

<sup>2</sup>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.

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<sup>3</sup> 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

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