The separation of strains of tobacco mosaic virus by continuous filter-paper electrophoresis

Plant viruses frequently occur in their hosts as mixtures of viruses or virus strains. Centrifugal purification methods, as commonly applied to plant viruses do not separate individual components of these mixtures, but generally sediment viruses as a group, separating them from the host's protein constituents. The recent development of continuous filter-paper electrophoresis[1] suggested that this method could be utilized in the physical separation of mixtures of plant virus-proteins, and as will be shown below, with this method the individual components of a mixture of two tobacco mosaic virus (TMV) strains have been obtained in milligram quantities, such that each strain after separation is biologically distinct.

To date, moving-boundary electrophoresis is the only physical method which has been applied in the separation of mixtures of plant virus strains. By means of a moving-boundary cell individual virus strains have been isolated from mixtures of TMV strains by KAHLER AND WOODS[2], and by SINGER *et al.*[3]; in both instances however, the isolated strains were not biologically pure, but represented only an enrichment over the concentration of the strain in the original mixture. Removal of a component from a moving-boundary cell is tedious, and unless the components migrate at significantly different rates the separation may not be a complete one. Furthermore, with mixtures of more than two substances it is not possible to obtain all of the individual components.

Continuous filter-paper electrophoresis overcomes disadvantages encountered when moving-boundary electrophoresis is used as a preparative method. The use of filter paper as a supporting medium to overcome convection, combined with the large area of the paper over which the migration of the mixture takes place, enables the separation of mixtures of proteins even when the components of the mixtures possess almost identical electrophoretic mobilities. In addition, with highly active biological materials such as viruses it is of significance that the components often separate into individual containers, which obviates having to collect a component from a cell—with the inherent danger of accidental mixing.

The technique to be described was developed by using two strains of TMV which were known to differ in electrophoretic mobility. Only the work which deals with the development of the method, using artificial mixtures of the two TMV strains, will be reported here. Studies with this technique as applied to the separation of naturally occurring virus mixtures are continuing and will be published in detail later.

Inoculum from which the TMV was prepared was obtained from Dr. S. G. WILDMAN. The origins and purification method applied to the two strains, U-1 and U-2, with electrophoretic mobilities in 0.1 ionic strength cacodylic acid buffer, pH 6.9, of $-8.9 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sec^{-1} and $-4.9 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sec^{-1} respectively, are described by SIEGEL AND WILDMAN[4]. Before use, the virus-proteins were dialyzed against *M*/90 phosphate buffer, pH 6.9. Virus-protein concentrations were estimated by direct Nesslerization.

A "Continuous Electrophoresis" apparatus manufactured by Shandon Scientific Company, London, was employed in this work. The equipment was modified to provide for automatic application of the virus-protein to the paper by means of a synchronous motor-driven hypodermic syringe. A full-wave rectifier operated from a voltage regulator, provided an output potential regulated to $\pm$ 1%. Platinum-wire electrodes enclosed in dialysis tubing were clamped to the two vertical edges of the paper. A stream of buffer was circulated through the dialysis tubing to remove the products of electrolysis at the electrodes. The distance between the electrodes was approximately 40 cm. *M*/90 phosphate buffer, pH 6.9, circulated through the electrode tubing and also moved down the paper (Whatman No. 54) as the buffer curtain.

The conditions for a typical experiment were as follows:

The filter paper was placed in the apparatus, the buffer curtain was started, and a potential of 460 V was applied. After several hours, when equilibrium conditions were established, a 50:50 mixture of the purified virus strains, each at a concentration of 2.5 mg protein per ml, was applied to the top of the paper, 13.5 cm from the cathode, at the rate of approximately 0.2 ml per hour. The apparatus was run at room temperature for 48 hours; 45 mg of virus-protein was applied during the first 42 hours of the migration. The potential was continued, and the buffer was allowed to flow down the paper for an additional six hours to elute more virus from the paper. The buffer which flows down the paper collects in a series of 30 tubes placed at drip points cut into the bottom edge. During the 48 hours of the run an average of 4.0 ml of buffer collected in each of the 30 tubes. During the migration the current varied between 6 and 9 milliamperes.

Those tubes which contain the virus may be readily identified, either by serological test, by means of a spectrophotometer (extinction at 260 mμ), or by staining for the protein which remains on the paper with bromophenol blue, after KUNKEL AND TISELIUS[5]. Tubes 13–17, and 26–28 gave positive serological reactions with TMV (rabbit) antiserum. That virus had reached the lips of these tubes was confirmed by the staining of the paper, as shown in Fig. 1.

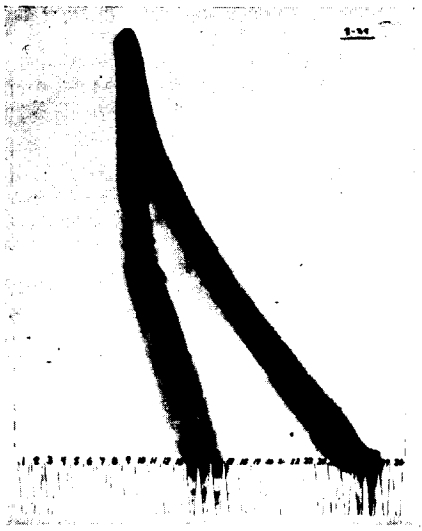

Fig. 1. Continuous electrophoretic separation of two strains of TMV. Strain U-1 migrated to tubes 26–28; strain U-2 migrated to tubes 13–17.

The solutions which collected in tubes 13–18 were pooled, as were the solutions which collected in tubes 25–29. The two solutions were dialyzed against *M*/90, pH 6.9, phosphate buffer for at least 72 hours to remove a nitrogen-containing substance of unknown composition which comes from the paper. After centrifugation at 2500 r.p.m. for 20 minutes in a clinical centrifuge, the amount of protein-nitrogen in each supernatant solution was determined. Tubes 13–18 contained a total of 10.5 mg of virus-protein, and tubes 25–29 contained 3.4 mg. Although equal amounts of each strain were applied to the paper it was commonly observed that strain U-2 moved to the bottom of the paper more rapidly than strain U-1; thus relatively more of strain U-2 collected in the tubes. Consequently, less U-2 remained on the paper, which accounts for the lighter staining of the U-2 band in Fig. 1.

In other experiments it was shown that the total yield of virus could be greatly increased if the process of elution of the virus from the paper was allowed to continue for several days.

An aliquot of each pooled solution (before dialysis) was tested to determine whether a biological separation of the two strains had been achieved. A few ml of each pooled solution was mixed with a small amount of celite and each was rubbed onto the leaves of several *Nicotiana rustica* (var. Olsen 68) plants. After 7 days the symptoms which appeared on the plants indicated that the two isolates were biologically distinct. The *N. rustica* plants which were rubbed with the solution from tubes 13–18 developed symptoms which were typical of those obtained with pure strain U-2; *i.e.*, small necrotic lesions developed on the inoculated leaves with no subsequent systemic infection. The plants which were rubbed with the isolate from tubes 25–29 showed markedly different symptoms, typical of those obtained with purified strain U-1 when it is inoculated into *N. rustica*. Local lesions appeared at the point of inoculation, but these spread rapidly and a systemic infection developed which, after 21 days, had killed the apex of the plant.

In the light of current experiments it is envisaged that the technique of continuous filter-paper electrophoresis can have wide application in the separation of mixtures of plant viruses and of virus strains, and in the separation of plant viruses from their accompanying host-proteins.

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N-terminal residues of serum lipoproteins

The shifts in lipoprotein pattern observed during the clearing reaction have led to the suggestion that low-density lipoproteins are interconvertible *in vivo*[1]. It has also been reported that α-lipoprotein concentrations may rise at the expense of low-density lipoprotein concentrations during *in vitro* clearing[2–3]. If interconversion occurs directly, through stripping down of lower density material by delipidation, the protein moieties of source and product should be identical. The amino acid composition of several lipoprotein fractions has been reported to be almost identical[4]