with the reagent if it is intended to maintain a constant pH value.

The complex-forming tendency between the reagent and trivalent iron is greater if the acid is neutralized by quaternary sodium pyrophosphate instead of NaOH; so the velocity of demineralization of iron containing tissue (stains) is notably increased.

The velocity of the demineralization increases notably if the treated material is suspended near the upper surface of a high column of reagent. The complex-containing solution, having a higher density, sinks down and so an automatic circulation and renovation of the reagent takes place. A mother-of-pearl button will be completely demineralized in 8–14 days.

Although the mother-of-pearl is almost floating in the solution after demineralization, its characteristic iridescence remains unaffected; hence, contrary to general opinion, the light diffraction is not due to the aragonit layers of the material, but to the micellar structure of its organic component, easily recognizable under the microscope.

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The Detection and Isolation of Naturally Occurring Strains of Tobacco Mosaic Virus by Electrophoresis[1]

S. J. Singer,[2] J. G. Bald, S. G. Wildman,[3] and R. D. Owen

Gates and Crellin Chemistry Laboratories, California Institute of Technology, Pasadena, Plant Pathology Division, University of California, Los Angeles, and Kerckhoff Laboratories of Biology, California Institute of Technology

Previous work (*1*) has demonstrated the utility of electrophoretic analysis of plant cytoplasmic proteins in following the development of tobacco mosaic virus (TMV) in an infected tobacco plant. The virus component is distinguishable from the native cytoplasmic protein by its different electrophoretic mobility. A more extensive electrophoretic investigation has now revealed the presence of two virus components, which differ in electrophoretic mobility, in a naturally occurring mixture in the infected cytoplasms we have examined.

These two virus components exhibit symptomatic behavior closely similar to that of the Mild and Severe strains isolated by Johnson (*2*) by biological means from a similar source. We have utilized electrophoresis in separating the components, and have further distinguished them by serological methods. The following is an account of the results of this study, which will be reported in greater detail elsewhere.

The sample of TMV used in these studies was kindly supplied, in the form of an infected dry leaf, by James Johnson, of the University of Wisconsin. Electrophoresis experiments were performed in a 0.1 ionic strength buffer, pH 6.93, containing 0.08 *M* NaCl, 0.02 *M* Na cacodylate, and 0.0033 *M* cacodylic acid. Electrophoretic scanning patterns of the total cytoplasmic proteins extracted directly from infected leaves of Turkish tobacco are shown in Fig. 1. In addition to the fastest-moving virus component represented by the large, spikelike peak (*a*) and the more diffuse mound (*b*) representing the normal cytoplasmic proteins of intermediate mobility, there is also present a small amount of a third component producing a spikelike peak (*c*) on the slow side of the cytoplasmic protein boundary. The main virus component constitutes about 40% of the total protein, whereas the third minor component, which was later proved also to be virus protein, represents only about 5% of the total cytoplasmic protein. A fourth, still slower-moving component (*d*) of unknown nature is also present.[4]

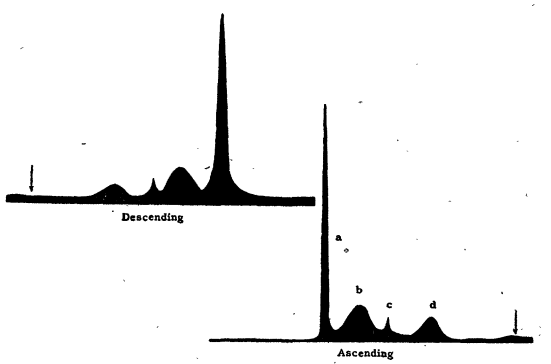

FIG. 1. Electrophoretic scanning patterns obtained in the Swingle Tiselius apparatus (*5*) of whole cytoplasmic proteins extracted from Turkish tobacco plants infected with tobacco mosaic virus; 18,000-sec migration in 0.1 μ NaCl-cacodylic acid buffer, pH 6.93, at a potential gradient of 5.10 v/cm; 1% total protein. Arrows indicate positions of starting boundaries.

In order to have enough material available for the electrophoretic isolation of the minor component, another group of Turkish tobacco plants was infected with TMV, and about 2 weeks after infection the virus was isolated according to previously described procedures (*3*). The virus preparation, freed from the lower molecular weight normal proteins and the com-

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[2] Gates and Crellin Chemistry Laboratories, California Institute of Technology, Pasadena, Contribution No. 1564.

[3] Present address: Botany Division, University of California, Los Angeles.

[4] This sample of cytoplasm was extracted from a mature leaf 16 days after it was inoculated with the virus, and it may be that the extra component arises from degenerative processes that occur in the leaf protoplasm as the result of virus infection. This component does not appear in cytoplasms extracted from healthy leaves.

ponent (*d*) mentioned above, was recycled by the centrifugal method of Stanley (*4*), except that the cacodylic acid buffer described above was used as a solvent instead of water. When examined electrophoretically in this buffer, the preparation was resolved into three spikelike peaks, the two slower-moving components constituting less than 10% of the total virus preparation, as shown in Fig. 2*A*. The

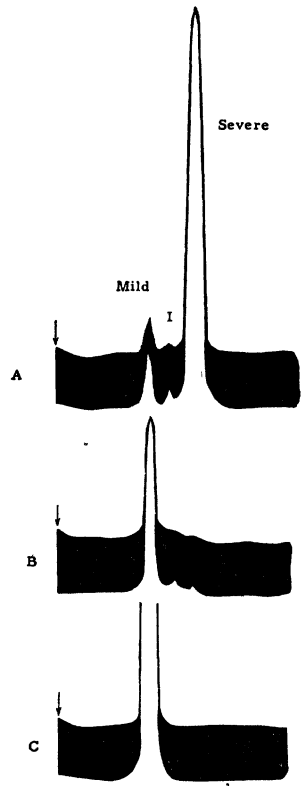

FIG. 2. Scanning patterns from the descending limb of the Perkin-Elmer electrophoresis cell containing various samples of tobacco mosaic virus; 5,280-sec migration time at a potential gradient of 5.84 v/cm. NaCl-cacodylic acid buffer, pH = 6.93, $\mu = 0.1$. Arrows indicate positions of starting boundaries. *A*, Original mixture isolated from *N. tabacum*. *B*, The Mild-component (component *c* in Fig. 1) after isolation by electrophoretic means. *C*, The Mild-component after passage through a single lesion produced on *N. glutinosa* and then propagated and recovered from *N. tabacum*.

component labeled *Severe* corresponds to component *a*, and *Mild* to component *c*, in Fig. 1. The component of intermediate mobility (*I*), which is obscured by the normal protein (component *b* in Fig. 1) in diagrams of infected whole cytoplasm, has repeatedly appeared in preparations of isolated virus obtained from plants infected with the original inoculum of TMV. The nature of this component is discussed below. The mobilities of the Severe, *I*, and Mild components in the descending limb of the electrophoresis cell are -7.8, -6.2, and -4.8×10^{-5} cm/sec/v/cm, respectively.

In all, about 2 g of the virus mixture was prepared and used for the electrophoretic isolation of the minor component of smallest mobility. The preparation was concentrated to about 3% total protein, divided into several samples, and each sample was subjected to prolonged electrophoresis in the Tiselius apparatus designed by Swingle (*5*). When sufficient separation of the virus components had occurred, a capillary tube was lowered into the descending limb of the electrophoresis cell, and the tip of the tube was accurately positioned by means of the optical system in such a way that the slowest-moving component (Mild) could be withdrawn from the mixture. Six individual analyses produced enough of the Mild-component so that it could be subjected to electrophoretic analysis in a Perkin-Elmer Model 38 apparatus. The results are shown in Fig. 2*B*. It is evident that the isolated component is nearly homogeneous, since only traces of the major (Severe) and intermediate minor (*I*) component can be identified in the pattern.[5]

Further examination of the isolated component in the analytical ultracentrifuge showed it to be indistinguishable in sedimentation rate from the other virus components of the mixture. Electron micrographs[6] revealed the Mild component to consist of rodlike particles very similar to ordinary TMV. The Mild component did not differ from ordinary TMV in either nitrogen or phosphorus content. Thus, apart from their difference in electrophoretic mobility, the two virus components have not yet otherwise been differentiated on physical and chemical grounds. However, biological studies readily proved the major (Severe) and the minor (Mild) component to yield different symptoms as evidence of viral activity.

The original mixture, containing largely Severe-type virus, was compared with electrophoretically isolated Mild-type by inoculation to Turkish tobacco. Whereas the mixture uniformly induced severe mosaic symptoms, the Mild isolate induced a variety of mosaic patterns. The most severe were like symptoms of the original mixture; the least severe, a mild mottle with little rugosity. The latter symptoms were obtained when the Mild inoculum was diluted to a level that reduced the chance of including the Severe-type, which was known to be present from previous electrophoretic analysis, among the infecting virus particles.

The Mild-type virus was readily separated from all traces of Severe-type by inoculation from a single lesion produced on *Nicotiana glutinosa* at high dilution of the Mild preparation isolated by electrophoresis. Tobacco plants inoculated from this single lesion isolate had only mild symptoms. Virus extracted from the infected plants migrated under a single boundary, as shown in Fig. 2*C*, and the electrophoretic mobility of the virus in the descending limb of the electrophoresis cell was the same (-4.8×10^{-5} cm/sec/

[5] It is of interest to note that in contrast to the behavior of the sedimentation constant of TMV, which is highly concentration-dependent (*6*), there is remarkably little variation in the electrophoretic mobilities of these components in spite of large changes in their concentrations.

[6] We are indebted to R. F. Baker, of the School of Medicine of the University of Southern California, for performing these experiments.

v/cm) as that of the slowest-moving component of the original mixture.

The Severe-type virus was readily obtained in an electrophoretically homogeneous condition by applying the original mixture to *N. glutinosa* at high dilution and then transferring from a single lesion to *N. tabacum*. The severe symptoms induced by the virus were not changed by this selective treatment, but the virus, when isolated from infected plants, migrated under a single peak in the electrophoresis apparatus and had a mobility identical with that of the prominent component of the original mixture (-7.8×10^{-5} cm/sec/v/cm).

The electrophoretically homogeneous preparations of Severe and Mild virus proteins, respectively, obtained after purification by the local-lesion technique and subsequent systemic infection of *N. tabacum*, were examined for their serological characteristics. Antisera produced in each of two rabbits, against the Mild virus, precipitated the Severe-type virus as they did the Mild-type, and there was little difference between the two virus preparations in terms of their qualitative reaction to these antisera. Quantitative analysis of the precipitate, however, showed that only part of the antibody to Mild-type was precipitated by the Severe-type; evidently the remainder was specific for antigenic components of Mild-type not found in Severe. This was confirmed, again in quantitative fashion, by absorbing, with the Severe-type, antisera to the Mild-type. When all the antibody capable of reacting with the Severe-type was removed by successive small additions of the antigen, and subsequent centrifuging of the precipitates, a measurable amount of antibody, specific for the Mild-type, remained in the supernatant. Excess antigen, added in the final absorption, was removed by centrifuging the absorbed serum for 1 hr at 30,000 rpm in the Size 40 head of a Spinco Model L centrifuge.

Similarly, antisera produced in three rabbits by injecting Severe-type virus showed both Severe-specific and cross-reactive antibody. In all cases, absorbing antiserum with the antigen against which it was produced removed all detectable antibody from it. It seems clear, therefore, that the Mild-type and the Severe-type virus proteins are serologically similar, but not identical, antigens.

We have not yet attempted the isolation of the component *I* (Fig. 2) from the original virus mixture. TMV is known to form aggregates under certain conditions, and it occurred to us that if aggregates were to form containing one molecule of the Severe and one molecule of the Mild strain, these dimers would migrate with the mobility of the *I*-component. In order to test this possibility, a mixture of roughly equal proportions of electrophoretically homogeneous preparations of the Mild and Severe strains was prepared at about 2% total protein in the cacodylic acid buffer, and was kept at 4° C for 1 week before it was subjected to electrophoresis. The diagram indicated the presence of only the Severe and Mild strains. Upon centrifuging down the TMV mixture and resuspending it in buffer, however, a significant amount of a peak with the mobility and appearance of the *I*-component appeared in the electrophoresis diagram. Whether all the *I*-component is simply the dimer of the Severe and Mild strains is at present undecided. The possibility exists that an independent strain with the mobility of the *I*-component is also present in the original mixture.

Some other strains of TMV, which have been isolated biologically, possess electrophoretic properties different from common TMV. The rib-grass strain (*7*) has a different isoelectric point (*8*) from that of the common variety, and a yellow-spot strain (*flavum*) studied by Friedrich-Freksa *et al.* (*9*) exhibits a pH-mobility relationship different from that of the common strain. Recently Oster (*10*), by means of a turbidometric method, has determined the apparent isoelectric points of several purified strains of TMV. In addition, we have recently examined a local strain of TMV which produced mosaic symptoms in *N. tabacum* differing in some respects from those produced by common TMV. Electrophoretic analysis demonstrated that the major component, constituting about 95% of the centrifugally isolated virus protein, had a mobility in the cacodylate buffer described above of -5.0×10^{-5} cm/sec/v/cm, markedly different from that of the Severe-type TMV. Mixed with this component was about 5% of another component with a mobility of -4.3×10^{-5}. Neither of these components has as yet been purified, but it is likely that both are strains of TMV. It may be of some significance that, of those few strains of TMV that have been discovered to have electrophoretic properties differing significantly from those of the common variety, all have been shown to have more alkaline isoelectric points or smaller anionic mobilities than common TMV.

Investigations are now in progress to elucidate the biological relationships and interactions among these strains of TMV, and to characterize the virus proteins more thoroughly by physical, chemical, and serological means.

The studies reported in this paper have further indicated the utility of electrophoresis in plant virus studies. Electrophoretic properties not only serve as precise characterization of different strains of viruses, but may also provide a means of recognizing the possible presence of strains otherwise difficult to detect, and of greatly facilitating their ultimate isolation and purification by biological means.

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