THE PHYSICAL AND CHEMICAL PROPERTIES OF A DISTINCTIVE STRAIN OF TOBACCO MOSAIC VIRUS

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It has been apparent for some time in both plant and animal virus diseases that the extent and type of the disease produced in a particular host may vary widely according to the strain of virus involved, and that members of the same virus family may range in pathogenicity from a strain which is quite innocuous to one which is definitely lethal. These facts make it of unusual interest to elucidate the nature of the chemical differences in the structure of a virus which are responsible for such variations in the biological properties. The nucleoproteins of the tobacco mosaic virus family are particularly suitable for such a study, for in many cases they can be obtained in comparatively large amounts and in a degree of purity superior to that of most other plant and animal viruses.

At this stage in the investigation of chemical differences between strains, the variants which differ most widely from the type strain with respect to biological properties seem to offer the best opportunity for demonstrating structural differences. Recently, Dr. Holmes isolated from rib-grass (Plantago lanceolata L.) a strain of tobacco mosaic virus which possesses unique properties and which can be easily distinguished from previously known strains by its ability to form necrotic ring lesions in Turkish tobacco, by its adaptation to rib-grass, and by its failure to produce local lesions in bean plants (Phaseolus vulgaris L.) (1). This unusual variant was shown beyond reasonable doubt to be a strain of tobacco mosaic virus by its ability to withstand heat and desiccation, by its inability to infect tobacco plants diseased with typical tobacco mosaic virus, by its precipitation with tobacco mosaic virus antiserum, and by the nature of its response to the genic constitution of tobacco plants. The present communication deals with the isolation and purification of the new virus and with the examination of some of its physical, chemical, and serological properties.

Preparation of the Virus—Young Turkish tobacco plants were inoculated with the rib-grass virus by rubbing one or two leaves on each plant with a gauze pad saturated with infective juice. This juice was obtained from a tobacco plant showing the typical symptoms of disease described by Holmes (1). About a month after inoculation, the plants were harvested and frozen. The virus was subsequently extracted from the macerated plant.
tissue, filtered through celite, and purified by differential centrifugation. The isolation and purification procedures corresponded in essential details with those commonly employed in this laboratory and recently used in the preparation of cucumber virus 4 (2).

The yields of purified virus obtained from diseased Turkish tobacco, while definitely smaller than those customarily obtained with ordinary tobacco mosaic virus, were nevertheless good. From 0.4 to 0.8 gm. of highly purified virus was obtained per liter of expressed juice as compared with 2 to 2.5 gm. per liter of the type strain obtained under similar conditions.

The rib-grass virus was tested for specific activity on *Nicotiana glutinosa* L. by the half leaf method. Various dilutions of the highly purified virus in 0.1 M phosphate buffer were compared with an arbitrary standard containing $10^{-4}$ gm. of virus per ml. The number of lesions obtained under these conditions was roughly proportional to the virus concentration in the range $10^{-4}$ to $10^{-6}$ gm. per ml. Concentrations below and above the latter range yielded fewer and more lesions, respectively, than would be expected from the virus concentration. This result is commonly obtained with other strains of tobacco mosaic virus. An almost identical number of lesions was obtained when the rib-grass virus was compared with ordinary tobacco mosaic virus on *Nicotiana glutinosa* at concentrations of $10^{-4}$ gm. per ml. However, as noted by Holmes (1), the lesions produced by the rib-grass strain were considerably smaller than those produced by ordinary tobacco mosaic virus and were also slightly different in character. The maximum infective dilution of the rib-grass virus appeared to be in the neighborhood of $10^{-10}$ gm. per ml. This value is comparable to that obtained with the ordinary strain.

**Crystallization of the Virus**—The distinctive properties of the rib-grass strain made it seem desirable to obtain the virus in crystalline form for the purpose of comparing it with ordinary tobacco mosaic virus. Crystallization was accomplished by adding slowly, with stirring, a 1:20 mixture of glacial acetic acid and 0.5 saturated ammonium sulfate to a 0.6 per cent solution of highly purified virus to which enough ammonium sulfate had been added to cause a faint turbidity (3). When the solution became very turbid, a few drops of saturated ammonium sulfate were added with vigorous stirring. Crystallization occurred almost immediately, to yield needles which appeared to be of the same type as the paracrystals of ordinary tobacco mosaic virus.

**Elementary and Carbohydrate Analyses**—Virus preparations obtained by differential centrifugation were further purified by electrodialysis in the type of cell described by Albancace (4). Dialysis was discontinued when the current dropped to a constant level. The drop in current was almost
always accompanied by a partial or complete precipitation of the salt-free virus. A suspension of the virus was then frozen and dried *in vacuo*. The white fluffy material thus obtained was further dried to constant weight at 110° in a drying oven and used for analyses. That little or no disintegration of the virus occurred during electrodialysis was demonstrated by the fact that virus precipitated by electrodialysis, when re-dissolved in dilute phosphate buffer, produced fully as many lesions on *Nicotiana glutinosa* as equivalent amounts of the highly active undialyzed virus.

Most of the analyses, with the exception of those for carbohydrate and phosphorus, were made by Dr. A. Elek, of this Institute, using customary micromethods. Carbohydrate and phosphorus were determined by standard colorimetric procedures as described recently for cucumber virus 4 (2, 5, 6). The average of duplicate analyses on five preparations of the virus indicated the presence of 50.28 per cent carbon, 6.98 per cent hydrogen, 15.69 per cent nitrogen, 0.64 per cent sulfur, 0.54 per cent phosphorus, 2.27 per cent ash, and 2.35 per cent carbohydrate. These values, with the exception of that for sulfur, agree fairly well with analyses on ultracentrifugally prepared tobacco mosaic virus of the ordinary type. However, the rib-grass strain appears to contain about 3 times as much sulfur as the type strain. The nature of this comparatively large amount of sulfur is at present under investigation. Preliminary tests have indicated that at least part of the sulfur is probably present as —SH, since even mildly denatured virus gave a strongly positive nitroprusside test.

**Nucleic Acid**—Nucleic acid was readily separated from the protein part of the virus by treatment with alkali, as described by Johnson and Harkins (7). The protein-free nucleic acid, obtained in the form of a dry white powder, was found to contain 34.10 per cent carbon, 3.93 per cent hydrogen, 15.80 per cent nitrogen, and 9.00 per cent phosphorus. It gave a strongly positive test for pentose with orcinol in hydrochloric acid, and a negative test for desoxypentose with diphenylamine in acetic acid. From these tests it may be concluded that the nucleic acid of the rib-grass strain, like that of ordinary tobacco mosaic virus, is of the ribonucleic acid or yeast type.

**Size and Shape of the Virus**—Solutions of the rib-grass virus were found to exhibit anisotropy of flow. Moreover, when a solution of the virus was caused to flow, the entire stream was doubly refracting and the double refraction persisted for a time after the stream left a pipette. These facts indicated that the rib-grass virus possessed the peculiar rod shape which is so characteristic of tobacco mosaic virus and its strains. It was, therefore, of considerable interest to compare the average size of these rods with that of ordinary tobacco mosaic virus.
Preparations of the virus were examined in the analytical ultracentrifuge by Dr. Max A. Lauffer. At dilutions of 1.0, 2.1, and 3.5 mg of virus per ml., sedimentation constants of $187 \times 10^{-13}$, $187 \times 10^{-13}$, and $183 \times 10^{-13}$, respectively, were obtained. These values correspond closely to those obtained for ordinary tobacco mosaic virus at corresponding dilutions (8).

Electron micrographs of the virus were made by Dr. T. F. Anderson, RCA Fellow of the National Research Council, using an electron microscope made available by the Radio Corporation of America, Camden, New Jersey. Like other strains of tobacco mosaic virus, the particles observed in micrographs of the rib-grass strain showed a considerable variation in size. A comparison of micrographs of ordinary tobacco mosaic virus and the rib-grass strain revealed no obvious difference in the average size of the particles. X-ray measurements have indicated an average diameter of about 15 m\(\mu\) for particles of the rib-grass virus (1), which is the same value obtained for three other strains of tobacco mosaic virus, including the ordinary form (9).

It may be concluded from the combined sedimentation, electron microscope, and x-ray data that the particles of tobacco mosaic virus and the rib-grass strain possess no readily demonstrable differences in size and shape. A particle size of about 15 m\(\mu\) in width and 280 m\(\mu\) in length and a molecular weight of about $4 \times 10^7$ have been assigned to ordinary tobacco mosaic virus (10, 11).

**Ultraviolet Absorption Spectra**—In order to characterize further the rib-grass virus, its ultraviolet absorption was compared with that of ordinary tobacco mosaic virus. Preparations of the intact viruses, their protein components, and their nucleic acids were examined by Dr. G. I. Lavin, using the techniques described in previous publications (12, 13). The protein components were those used in other experiments (14), and the nucleic acids were prepared by the method of Johnson and Harkins (7). The absorption coefficient, \(\alpha\), was calculated in each case from the concentration of material expressed as mg per ml. The absorptions of the two viruses and of their protein components were similar and those of their nucleic acid components were almost indistinguishable. The intact viruses showed a maximum absorption at about 2675 \(\AA\), and the proteins showed a maximum absorption at about 2800 \(\AA\). The relative positions of maxima and minima and the shapes of the curves are those to be expected from nucleoproteins and simple proteins, respectively (13). The absorption maxima of the nucleic acid components of the two viruses were at about 2600 \(\AA\), which is comparable to the values obtained for a number of other nucleic acids from various sources (13, 15, 16). While there appeared to be little difference between the absorption curves for tobacco mosaic and
the rib-grass viruses, and of their protein components, there were definite differences in the band structures as obtained with the continuous light of the hydrogen discharge tube. These differences were most pronounced in the tyrosine-tryptophane region, and in this respect are in accord with data dealing with the chemical composition of the two viruses (17).

Serological Tests—Precipitin tests with six strains of tobacco mosaic virus and cucumber viruses 3 and 4 as antigens and tobacco mosaic virus antiserum demonstrated a strong serological relationship between tobacco mosaic virus and all of the viruses tested, with the exception of the rib-grass strain and cucumber viruses 3 and 4 (17). A comparatively weak relationship was observed between the latter viruses and ordinary tobacco mosaic virus. As a corollary to these experiments, tests were made in the present study with antiserum to the rib-grass virus. This antiserum was obtained from the blood of a rabbit 8 to 10 days after the last of five spaced intravenous injections of a total of about 40 mg. of virus. Precipitin tests were made with a constant dilution of serum and with various dilutions of antigen according to the technique described in a previous communication (2). All of the viruses used as antigens were highly purified preparations obtained by differential centrifugation. Instead of the expected weak precipitation of strains against the rib-grass virus antiserum, precipitates were obtained which were qualitatively indistinguishable from those of the rib-grass virus itself. Subsequent repetition of the tests with sera obtained from two other rabbits yielded the same results.

Experiments were performed next in which separate portions of tobacco mosaic virus antiserum were absorbed with tobacco mosaic and rib-grass viruses. Portions of antiserum to the rib-grass virus were treated in a similar fashion. The total precipitate obtained in each absorption was suspended in water and analyzed for nitrogen by the Kjeldahl method. The results of the latter analyses indicated that about 0.6 as much precipitate was obtained when the rib-grass virus reacted with tobacco mosaic virus antiserum as when tobacco mosaic virus reacted with an equal portion of the same antiserum. On the other hand, more than 0.8 as much precipitate was obtained when tobacco mosaic virus reacted with rib-grass virus antiserum as was obtained in the homologous reaction. Thus, the quantitative reactions confirmed the qualitative tests which had shown unequal cross-precipitation of the two strains with the appropriate antisera.

Precipitin tests were also made with absorbed sera and strains of tobacco mosaic virus, as shown in Table I. The dilutions and other conditions of these tests were exactly the same as used for previous precipitin reactions with unabsorbed sera. The results obtained agreed with and extended
those reported by Chester (18) and by Bawden and Pirie (19) in showing that strains of tobacco mosaic virus possess both distinctive and common antigens.

In general, the serological reactions observed indicate that considerable information regarding strains of tobacco mosaic virus, and possibly the nature of antigen-antibody reactions as well, could be obtained by quantitative serological studies, perhaps of the Heidelberger type (20), par-

**Table I**

*Precipitation of Strains of Tobacco Mosaic Virus with Absorbed Antisera*

The signs indicate the degree of precipitation.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dilution of antigen (1:1 = 1 mg. per ml.)</th>
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<td>1:1</td>
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<tr>
<td>Tobacco mosaic virus antiserum absorbed with rib-grass virus</td>
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<tr>
<td>Holmes' rib-grass</td>
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<tr>
<td>Tobacco mosaic</td>
<td>++</td>
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<tr>
<td>Yellow aucuba</td>
<td>-</td>
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<tr>
<td>Green</td>
<td>-</td>
</tr>
<tr>
<td>Holmes' masked</td>
<td>+</td>
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<tr>
<td>J14D1</td>
<td>++</td>
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<tr>
<td>Cucumber virus 4</td>
<td>-</td>
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</table>

<table>
<thead>
<tr>
<th>Rib-grass virus antiserum absorbed with tobacco mosaic virus</th>
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<tbody>
<tr>
<td>Holmes' rib-grass</td>
<td>+++</td>
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<td>Tobacco mosaic</td>
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ticularly if such data were correlated with evidence now being obtained regarding the chemical composition of these unusual antigens.

**DISCUSSION**

The general composition, physicochemical properties, and size and shape of the rib-grass virus were found to agree in almost every respect with the similar properties of ordinary tobacco mosaic virus. These results may not, at first, appear surprising, because the rib-grass virus completely satisfies every important criterion for classification as a strain of tobacco mosaic virus, and hence would be expected to resemble closely the type strain. However, when it is recalled that the rib-grass virus has
been found to contain about 3 times as much sulfur as the type strain and
to differ strikingly from the latter virus in content of aromatic amino acids
(17), the coincidence of properties of the two viruses becomes remarkable.

The author wishes to express his appreciation to Dr. F. O. Holmes for
the original supply of the rib-grass virus and to Dr. W. M. Stanley for
encouragement and helpful suggestions during the course of this investiga-
tion.

SUMMARY

A distinctive strain of tobacco mosaic virus, originally discovered in
rib-grass, has been isolated from diseased Turkish tobacco and obtained
in a highly purified state by differential centrifugation. The new virus,
like ordinary tobacco mosaic virus, could be obtained in the form of needle-
like paracrystals. Elementary and carbohydrate analyses gave values
similar to those obtained with ultracentrifugally prepared tobacco mosaic
virus with one exception. The rib-grass virus contained about 0.64 per
cent sulfur in comparison to only 0.2 per cent sulfur reported for ordinary
tobacco mosaic virus. Nucleic acid was separated from the protein com-
ponent of the virus and found to be of the ribonucleic acid type.

Solutions of the virus were doubly refracting and examination in the
analytical ultracentrifuge and in the electron microscope indicated the pres-
ence of rod-like particles which in size and shape appeared indistinguish-
able from those of common tobacco mosaic virus.

Ultraviolet absorption curves were obtained for the rib-grass and tobacco
mosaic viruses, their protein components, and their nucleic acids. There
appeared to be little difference between the absorption curves of tobacco
mosaic and the rib-grass viruses and of their protein and nucleic acid com-
ponents. However, a significant difference between the two viruses was
apparent in the band structure as obtained with the continuous light of the
hydrogen discharge tube, particularly in the tyrosine-tryptophane region.
This result is in accordance with the chemical data previously reported from
this laboratory.

Serological tests indicated that the rib-grass virus and ordinary tobacco
mosaic virus contain common antigenic groups but, in addition, that each
possesses distinctive groups lacking in the other.

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