BIOCHEMICAL STUDIES ON A PLANT VIRUS

by

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BIOCHEMICAL STUDIES ON A PLANT VIRUS

INTRODUCTION

In 1950 a virus was isolated by Dr. Frank P. McWhorter of the Department of Plant Pathology at Oregon State College from infected peas found near Weston, Oregon. This appeared to be a new type of virus. The name, western ringspot virus, was proposed by McWhorter.

A taxonomic study of western ringspot virus has been made by McWhorter using protection tests and other standard methods of virus classification. Western ringspot has been found to occupy a position intermediate between the classical yellow bean mosaics and the classical tobacco ringspot virus. It seems to be somewhat more closely related to the yellow bean mosaics than to the ringspots.[1]

Western ringspot virus occurs naturally in potatoes and various legumes. It has been found to occur naturally only in combination with other viruses. In such combinations it is often extremely synergistic, that is, the combination of western ringspot virus and one or more other viruses has a much greater effect on the host plant than any of the components alone. In recent years these synergistic combinations have proved very damaging to the pea and bean crops in Oregon, (9, pp.453-457).

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1. Personal communication with Dr. McWhorter.

In the field, in combination with other viruses, western ringspot may be transmitted by the pea aphid; in a pure form it is rarely aphid transmitted, however, (1, pp.27-29; and 9, p.456).

Because of the economic importance of western ringspot virus, because of its peculiar synergistic relationship with other viruses, and because of the difficulty of assigning it a position in the present system of virus classification, a complete biochemical study of this virus is highly desirable. The work represented by this thesis is the first step in such a study. This work is concerned primarily with the purification of the virus to such a stage that it might be used in subsequent biochemical studies.

EXPERIMENTAL

General Considerations

The virus in the present study was supplied by Dr. McWhorter as a pure culture of western ringspot virus 906. Throughout this study the host plant was the Bountiful bush bean, (Phaseolus vulgaris L.). Plants were grown under greenhouse conditions.

The general procedure for concentration of the virus consisted of subjecting the sap from infected plants to various fractionating procedures then determining the virus activity and total protein in each fraction. An increase in the activity/protein ratio was interpreted as indicating purification.

To eliminate, insofar as possible, loss of virus activity due to ageing, all experiments described were performed in a coldroom maintained at 5° and were completed in as short a period of time as possible, but in every instance within a 48 hour period after harvesting the infected material.

Method of Protein Determination

Total protein was determined by Weichselbaum's method with modifications, (11, pp.16-20). A sample containing from 1 to 20 mg. of protein was used for each determination. If the sample was in solution, it was

precipitated with an equal volume of 9% trichloroacetic acid and collected by centrifugation. The precipitate was then dissolved in 1 ml. of 0.1 normal sodium hydoxide solution and made up to 5 ml. with the thymol-urea reagent of Weichselbaum. Subsequent steps were the same as described by Weichselbaum. Many of the samples were colored, therefore, suitable blanks were always used. Pure egg albumin prepared by the method of Kekwich and Cannan, (7, pp.232-233), was employed as the standard.

Methods of Virus Determination

Several standard methods of determining virus concentration were investigated in an attempt to find the one best suited to the present study. This work will be described briefly.

The serological method is one of the most rapid and accurate methods for determining virus concentration. The method has been described in detail by several authors, (2, pp.126-149, 161-166; 3, p.124; and 4, pp.909-911). An attempt was made to develop antiserum to the virus in two female flemish giant rabbits. Infected plant sap, which had been clarified by centrifuging 20 minutes at 20,000 X g, served as the source of antigen. The rabbits received 5 c.c. interperitoneal injections of the sap every 5 days for 45 days. After an additional 10 days, 15 c.c. of blood was taken from the ear of each

rabbit. Serial dilutions of the serum from this blood were prepared and mixed with constant amounts of sap, clarified by centrifuging 20 minutes at 20,000 X g. Sap from both healthy and diseased plants was used. No precipitate formed which could have represented a virus-antibody complex.

One of the most common methods of determining virus activity is the local lesion method, (2, pp.150-158; and 5, pp.39-55). Although western ringspot virus is known to form local lesions on some varieties of tobacco and on broad beans[1], it does so only sporadically so that this method is not suitable for quantitative work.

A variation of the local lesion method is the starch-iodine method (2, pp.158-161; and 6, pp.163-172). An attempt was made to adapt this method to the present study. Western ringspot virus was inoculated on young bean plants. Three days after inoculation a fraction of the plants were placed in the dark. At varying intervals of time leaves were removed from the plants, decolorized with 95% ethanol, and stained with iodine-potassium iodide solution. The procedure was repeated 6 days after inoculation. In no case was there any difference in staining between the leaves from the inoculated plants and those from uninoculated controls.

The only method for determination of virus concentration which gave successful results, and therefore the

method used throughout this study, was the systemic infection method. This method gives an approximation of the concentration of the virus in a sample relative to some standard. In all cases untreated sap from infected plants was used as the standard. The method, as used, consisted of inoculating test plants with a known dilution of the sample to be tested for virus activity. Test plants were also inoculated with the reference standard at several dilutions. After the disease had developed in the test plants, the fraction of plants becoming infected in the unknown group was compared with the fractions becoming infected in the standard groups to give an approximation of the amount of virus in the unknown relative to that in the standard.

To obtain a measure of uniformity in the determination of virus activity, a standard procedure was adhered to throughout the present work. Plants were inoculated about 2 weeks after planting at which time the primary leaves were 2 inches or more in length and the first trifoliate leaves had not yet appeared. Prior to inoculation the two primary leaves were dusted lightly with 400 grit carborundum. The virus, in solution in 0.1 molar phosphate buffer, was kept on ice until the time of inoculation. The virus solution was applied with the forefinger by dipping into the solution then rubbing across one of the primary leaves, 4 times from

the apex toward the base of the leaf and once from base to apex. The procedure was then repeated with the other primary leaf. About 30 plants were inoculated with each sample being tested for activity. After the inoculation of each group of 30 plants, the leaves were rinsed well with tap water to remove the carborundum and buffer salts.

The inoculated plants, which became infected, first showed disease symptoms from one to two weeks after inoculation. The first symptom which could be observed was a curling up of the first trifoliate leaflets. Later on these became distorted and often discolored. About a month after inoculation the primary leaves began to show discoloration and necrosis. The infected plants from virus activity determinations were saved and later used as a source of infected material.

Preparation of Starting Material

The starting material for all experimental work was the sap from infected plants. Small quantities of sap were conveniently obtained by grinding diseased plants in a mortar with a little sand, expressing the sap through several layers of cheesecloth, and centrifuging this crude sap for 10 minutes at 7000 X g to remove starch granules and other debris. Larger quantities of sap were obtained by grinding the infected plants in a

meat grinder equipped with a peanut butter attachment and again expressing the sap through cheesecloth and centrifuging to remove debris. About 2 ml. of sap could be obtained from each infected plant.

It was found that the virus content in the infected plants rose sharply for the first four weeks after inoculation then leveled off; this is indicated in Table I, which shows the result of inoculating test plants with sap from infected plants at varying intervals of time after inoculation. As also shown in Table I, the amount of protein in the sap did not vary appreciably with age. Because of the low virus level in plants which had only been infected for a short period of time, an attempt was made to use only plants which had been inoculated for four weeks or more.

Experiments were performed to determine whether the virus was localized in any particular part of the plant. These experiments, as summarized in Table II, indicated that the virus did occur in all the above ground parts of the plant. The roots were not investigated in these studies. All experimental work was done using sap obtained from the entire infected plant exclusive of the roots.

TABLE I[2]

Effect of length of time after inoculation on virus content in plants

Number of days after inoculation	Dilution	Activity	Mg. protein per ml. of sap
17	1:50	15/32	21.0
24	1:50	25/32	23.4
28	1:50	31/32	22.4
35	1:50	30/32	24.4
50	1:50	30/32	17.2

[2] The following conventions are followed in all tables in this paper:

1. Samples designated, "control," refer to infected plant sap. At the beginning of each experiment, a sample of the sap was set aside to be used as the reference standard for activity.
2. Dilutions were made with pH 6.0, 0.1 molar phosphate buffer. All dilutions were made with reference to undiluted sap. For example, if 100 ml. of sap had been subjected to fractionating procedures such that the volume of the fraction of interest was 20 ml., this fraction would first be diluted 5 times to the volume of the original sap, then further diluted as indicated in the table. Direct comparison may therefore be made between samples in any given experiment.
3. The figures given for activity refer to the number of plants becoming infected over the number inoculated.
4. In the tables under the subheading, "Purification Procedure," each protein value listed represents the total protein in the sample under consideration, extrapolated to the value which would have been obtained had the starting material consisted of 100 ml. of sap. Direct comparison may, therefore, be made between any of the protein values given in these tables.

TABLE II[2]

Activity and protein content of sap from different parts of infected plants

Part of plant	Dilution	Activity	Mg. protein per ml. of sap
		Test 1	
Primary leaves	1:10	0/36	26.4
Trifoliate leaves	1:10	4/36	35.4
Stems and petioles	1:10	3/36	18.8
		Test 2	
Primary leaves	1:10	7/26	----
Trifoliate leaves	1:10	1/26	----
Stems and petioles	1:10	4/26	----
		Test 3	
Primary leaves	1:20	47/55 (85%)	----
Lower trifoliate leaves	1:20	114/163 (71%)	----
Upper trifoliate leaves	1:20	40/69 (58%)	----

Purification Procedures

By application of techniques commonly used in the purification of plant viruses, (2, pp.167-183; 10, vol.2, pp.60-84), a scheme for the partial purification of western ringspot virus was worked out. The starting material for the purification consisted of the sap of bountiful bean plants infected with western ringspot virus 906. Sap was prepared by the methods previously described in this paper. The method of purification will be described giving the quantities of reagents used in processing a 100 ml. sample of infected sap.

The first step in the purification consists of precipitating the virus with alcohol then extracting the virus from the precipitate with phosphate buffer. To 100 ml. of sap are added over a 5 minute period and with rapid stirring 20 ml. of 90% ethanol. After standing for 15 minutes, the suspension is centrifuged 10 minutes at 10,000 X g. The supernatant liquid is discarded. The precipitate is suspended in 30 ml. of pH 6.0, 0.1 molar phosphate buffer (0.012 moles of disodium hydrogen phosphate and 0.088 moles of potassium dihydrogen phosphate per liter of solution). The suspension is centrifuged 15 minutes at 15,000 X g. The extraction is repeated one or two more times with 15 ml. portions of phosphate buffer. The supernatant liquids from the

extractions are combined and the residue is discarded.

Twenty ml. of 90% ethanol added to 100 ml. of sap was found to precipitate about two thirds of the total protein from the plant sap and all of the virus. Half this amount of alcohol precipitated less protein but left a considerable amount of virus in the supernatant liquid. This is shown in Table III. If more than 20 ml. of ethanol were added to 100 ml. of sap, it became difficult to recover the virus from the precipitate.

TABLE III[2]

Activity and protein content found in the supernatant liquid after adding 90% ethanol in varying amounts to sap from infected plants. Figures in sample column refer to number of ml. of ethanol per 100 ml. of sap

Sample	Dilution	Activity	Mg. of protein
Control	1:10	11/31	2660
Control	1:20	4/31	
10 ml.	1:10	5/31	1900
20 ml.	1:10	0/31	980
30 ml.	1:10	0/31	680
40 ml.	1:10	0/31	480

The amount of time which elapsed between the addition of ethanol to the sap and the addition of buffer to the precipitate thus formed was found to influence the amount

of total protein which redissolved in the buffer and also the amount of virus which could be extracted from the precipitate. An increase in the time interval resulted in a decrease in the amount of protein redissolved by the buffer and also a decrease in the amount of virus extracted from the precipitate. Table IV illustrates this. Thirty minutes was taken to be the optimum length of time for the ethanol to act.

TABLE IV[2]

Activity and protein concentration found in the supernatant buffer after extracting precipitates formed by the action of ethanol on plant sap for varying intervals of time. Figures in the sample column represent the number of minutes between the addition of the ethanol to the sap and the addition of the buffer to the precipitate thus formed. These figures include a 10 minute centrifugation time.

Sample	Dilution	Activity	Mg. of protein
Control	1:10	8/30	3080
15 min.	1:10	4/30	850
30 min.	1:10	3/30	640
75 min.	1:10	1/30	550

Several experiments were set up to determine the optimum pH for the buffer used to extract the virus from the precipitate. It was found that between the pH range of 5.0 to 8.0 the total amount of protein redissolved

from the precipitate increased as the pH of the buffer was increased. The amount of virus extracted reached a maximum for the buffers within the pH range of 5.5 to 7.0. This is illustrated in Table V. Distilled water proved only very slightly effective at removing the virus from the precipitate.

About one third of the total protein in the precipitate was redissolved by the treatment with phosphate buffer at pH 6.0. Although the purification procedure as thus far described was followed closely, and each operation was performed in as uniform a manner as possible from run to run, the amount of virus recovered was quite variable, ranging from less than one fifth of the total to almost the entire amount. The supernatant liquid remaining after precipitation of the virus with ethanol at a concentration of 20 ml. per 100 ml. of sap was never found to contain more than a trace of virus activity. The source of the wide variation in virus recovery must, therefore, lie in the extraction of the virus from the precipitate with buffer. The actual cause for this variation could not be determined. The results of four different extractions, as shown in Table VI, illustrate the points mentioned above.

The second step in the purification consists of removing a part of the inert material from the supernatant

TABLE V[2]

Activity and protein content found in the supernatant liquids resulting from the extraction of the precipitate by buffers at various pH values. pH 5.0 and 5.5 buffers were 0.1 molar acetate buffers; pH 6.0, 6.5, 7.0, and 8.0 were 0.1 molar phosphate buffers.

Sample	Dilution	Activity	Mg. of protein
	Test 1		
Control	1:10	34/34	3470
pH 5.0	1:10	12/34	220
pH 5.5	1:10	33/34	640
pH 6.0	1:10	34/34	1090
pH 6.5	1:10	34/34	1190
pH 7.0	1:10	33/34	1210
	Test 2		
Control	1:10	14/29	2480
Control	1:50	4/29	
pH 6.0	1:10	21/29	310
pH 8.0	1:10	2/29	480
	Test 3		
Control	1:10	28/31	2840
pH 6.0	1:10	26/31	865
H_2O (pH 6.5)	1:10	6/31	530

TABLE VI[2]

Activities and protein contents of the supernatant buffers, and protein contents of the residues resulting from the extraction of precipitates produced by action of ethanol on plant sap.

Sample	Dilution	Activity	Mg. of protein
	Trial 1		
Control	1:10	14/29	2480
Control	1:50	4/29	
Supernatant buffer	1:10	21/29	310
Residue	----	----	1230
	Trial 2		
Control	1:10	24/32	3300
Control	1:50	11/32	
Supernatant buffer	1:10	20/32	730
Residue	----	----	1400
	Trial 3		
Control	1:10	8/30	3080
Supernatant buffer	1:10	3/30	640
Residue	----	----	1440
	Trial 4		
Control	1:10	6/32	----
Control	1:50	2/32	----
Supernatant buffer	1:10	1/32	----

liquid from step 1 by adjusting the pH to 5.5. 0.5 molar acetic acid is added dropwise with stirring to the supernatant liquid from step 1 until the pH of the solution is 5.5. After standing for one hour the suspension is centrifuged for 10 minutes at 10,000 X g. The precipitate is discarded.

About two thirds of the protein in the supernatant liquid from step 1 was found to be precipitated in this manner. Less than one fifth of the remaining virus was precipitated and lost. Table VII illustrates the effect of this step. If the suspension at pH 5.5 was not allowed to stand for an hour, the amount of protein which precipitated was less, while the virus loss was about the same.

TABLE VII[2]

Activity and amount of protein in the supernatant liquid and in the precipitate resulting from adjustment of the supernatant buffer from step 1 to pH 5.5. The precipitate was redissolved in pH 8.0, 0.1 molar phosphate buffer in order to determine activity.

Sample	Dilution	Activity	Mg. of protein
Control	1:10	14/29	2480
Control	1:50	4/29	
Supernatant buffer from step 1	1:10	21/29	310
Supernatant liquid	1:10	16/29	106
Precipitate	1:10	2/29	195

The third step in the purification consists of precipitating the virus from the supernatant liquid of step 2 with ammonium sulfate. Over a 10 minute period are added with stirring 30 gm. of ammonium sulfate to every 100 ml. of supernatant liquid from step 2. After allowing the suspension to stand for an additional few minutes, the precipitate is collected by centrifuging for five minutes at 5000 X g and suspended in 20 ml. of pH 6.0, 0.1 molar phosphate buffer. The supernatant liquid is discarded.

It was found that the virus was not completely precipitated until 30 gm. of ammonium sulfate had been added to each 100 ml. of supernatant liquid from step 2. At this salt concentration nearly all of the dissolved protein precipitated. This is shown in Table VIII. In this instance, ammonium sulfate was added in increments of 6 gm. per each 100 ml. of supernatant liquid from step 2. The precipitate formed by the addition of each increment of salt was collected, dissolved in buffer, and assayed for virus activity and protein content.

The effect of step 3 is mainly to reduce the virus solution to a smaller volume and to insolubilize some of the normal plant proteins so they can be removed in the subsequent step.

TABLE VIII[2]

Activity and protein content of the precipitates formed by addition of ammonium sulfate in increments to the supernatant liquid from step 2. Activity and protein content of the supernatant liquid remaining after completing the addition of ammonium sulfate are also shown. Figures in the sample column refer to the gm. of salt added per 100 ml. of liquid to form the precipitate under consideration.

Sample	Dilution	Activity	Mg. of protein
Control	1:10	32/33	3200
Control	1:50	23/33	
Supernatant liquid from step 2	1:10	20/33	250
6 gm.	----	-----	0
12 gm.	----	-----	0
18 gm.	1:10	11/33	106
24 gm.	1:10	9/33	62
30 gm.	1:10	6/33	62
Supernatant liquid	1:10	0/33	19

The final step in the purification consists of sedimenting the virus by high speed centrifugation. The suspension from step 3 is first centrifuged for 20 minutes at 9000 X g to remove insoluble particles. The precipitate is discarded and the supernatant liquid is centrifuged for 60 minutes at 80,000 X g. In the present work a Spinco model L ultracentrifuge equipped with rotor

number 30 was used for this operation. The supernatant liquid from this high speed centrifugation is discarded and the glassy, green-brown pellet is suspended in 2 ml. of pH 6.0, 0.1 molar phosphate buffer. This suspension is centrifuged 10 minutes at 7000 X g. The residue is extracted twice more with 1.5 ml. portions of phosphate buffer. The supernatants from these three extractions are combined. After standing for about 16 hours, the small amount of insoluble green material that forms is centrifuged off.

About one third of the virus activity remaining from step 3 was lost by this procedure as shown in Table IX. The protein was reduced to its final value of 1 to 10 mg. protein per each 100 ml. of sap originally used.

TABLE IX[2]

Activity of the final virus preparation as compared with that in the suspension from step 3.

Sample	Dilution	Activity
Control	1:10	31/35
Control	1:40	19/35
Control	1:100	13/35
Suspension from step 3	1:10	19/35
Suspension from step 3	1:100	2/35
Final solution	undiluted	27/35
Final solution	1:10	1/35

It should be borne in mind that the entire procedure for purification of the virus was carried out at temperatures near 5° and in as short a period of time as possible. The time span between the harvesting of the infected plants and the inoculation to determine the activity of the preparation was usually on the order of 30 hours.

Table X shows some overall results of the purification procedure as described.

TABLE X[2]

Trial 1

Starting material: 1000 ml. of sap containing 26,800 mg. of protein. Final product: 10 ml. of solution containing 10 mg. of protein.

Sample	Dilution	Activity
Control	undiluted	32/32
Control	1:10	30/32
Final product	undiluted	32/32

Trial 2

Starting material: 200 ml. of sap containing 5520 mg. of protein. Final product: 10 ml. of solution 18.5 mg. of protein.

Sample	Dilution	Activity
Control	1:10	14/32
Control	1:20	9/32
Control	1:100	3/32
Control	1:500	0/32
Final product	undiluted	20/32
Final product	1:10	2/32

RESULTS AND DISCUSSION

Solutions of virus prepared by the method described above were perfectly clear and either colorless or pale yellow-green in color.

The yields of virus were somewhat variable. In general one tenth to one fifth of the virus present in the sap appeared in the final preparation as determined by activity measurements. This can be seen from Table X.

One hundred ml. of sap ordinarily contained from 2000 to 3000 mg. of protein as determined by the biuret method. The virus preparations from 100 ml. of sap contained from 1 to 10 mg. of protein. This is shown in Table X.

The ultraviolet absorption spectra for the virus preparations were similiar to those of other viruses, (8, pp.260-263). There was an absorption minimum at 2380-2420 Å and an absorption maximum at 2570-2580 Å. A typical set of absorption values is shown in Table XI.

Absorption curves for the protein and nucleic acid components of tobacco ringspot virus, a virus closely related to western ringspot, have been reported by Lavin, Loring, and Stanley, (8, pp.260-263). At 2600 Å the absorption coefficient for the protein component is given as 4.8 $cm.^2/mg.$; that for the nucleic acid component is given as 30.2 $cm.^2/mg.$ If the assumption is

TABLE XI

Absorption spectrum of a virus preparation diluted 1:30 with pH 6.0, 0.1 molar phosphate buffer.

Wave length (Å)	Optical density	Wave length (Å)	Optical density
2200	1.17	2750	.597
2250	.970	2800	.486
2300	.818	2850	.385
2350	.708	2900	.297
2400	.689	2950	.228
2450	.738	3000	.190
2500	.800	3050	.173
2550	.846	3100	.167
2600	.847	3200	.163
2650	.807	3300	.154
2700	.714	3400	.138

made that the absorption values for tobacco ringspot virus are the same as those for western ringspot, a nucleic acid - protein ratio can be calculated. The optical density values given in Table XI are for a 1:30 dilution of the virus preparation. At this dilution the protein content of the solution was 0.062 mg./ml. as found by the biuret determination. The contribution to the optical density by the protein of the virus solution can be calculated to be 0.129 at 2600 Å using the absorption coefficient for tobacco ringspot virus protein. Subtracting this value from 0.847, the optical density of the virus solution at 2600 Å, leaves 0.718 as the contribution to the optical density by the nucleic acid of the virus solution. Using the absorption coefficient for tobacco ringspot nucleic acid, the value 0.055 mg./ml. can be arrived at as the nucleic acid content of the diluted virus preparation. This gives a protein - nucleic acid ratio of 1.00:0.89. This may be compared with the protein - nucleic acid ratio of 1.00:0.66 for tobacco ringspot virus, (10, p.82). In these calculations the assumption is made that protein and nucleic acid are the only absorbing entities in the solution at 2600 Å.

Electron photomicrographs of a virus preparation were taken. A 1:100 dilution of the preparation was seen to consist primarily of two types of spherical particles. The smaller type was calculated to be

about 25 mμ in diameter and the larger type about 90 mμ. A minor portion of the material as seen under the electron microscope did not fall into either of these types. A typical field is shown in figure 1. In a 1:1000 dilution of the preparation the smaller particles were observed as in the 1:100 dilution but the larger type of particle was essentially absent as shown in figure 2. This might be interpreted as indicating that the larger particles are aggregates of the smaller ones. The smaller particles lie in the size range which has been found for other spherical plant viruses, (10, vol.2, pp.73-85).

Electrophoretic patterns of virus preparations in pH 7.4 phosphate buffer of ionic strength 0.1 and in pH 8.6 barbiturate buffer of ionic strength 0.1 revealed, in both instances, one major and two minor components. The combined concentration of the minor components was, approximately, one tenth that of the major component. One of the electrophoretic patterns obtained is shown in figure 3. As can be seen, the protein concentration was lower than is necessary for a good electrophoretic pattern. The amount of material which could be prepared at one time was not great enough to permit a proper electrophoretic analysis.

The above evidence indicates that, while the virus preparations are not pure, they do consist of only two or three significant components and thus approach purity.

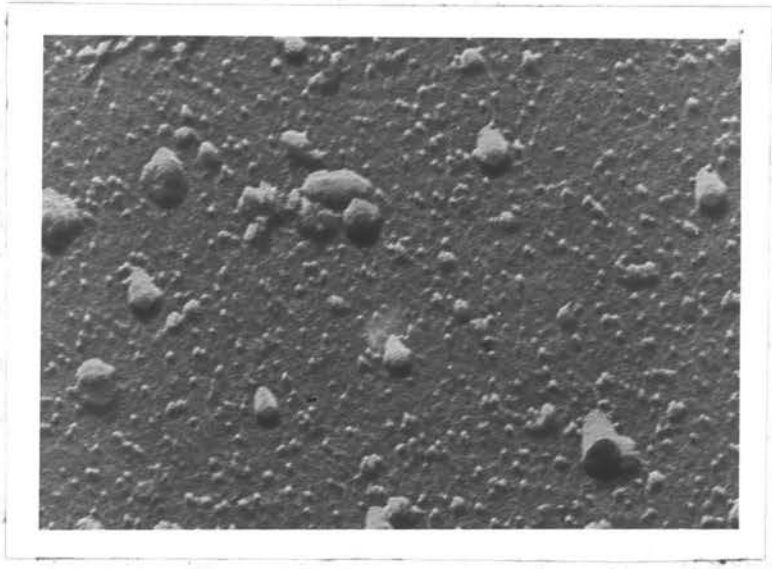

Figure 1. Electron photomicrograph of a virus preparation diluted 1:100 with distilled water; magnification 40,000 X; shadowcast with chromium at a 25° angle.

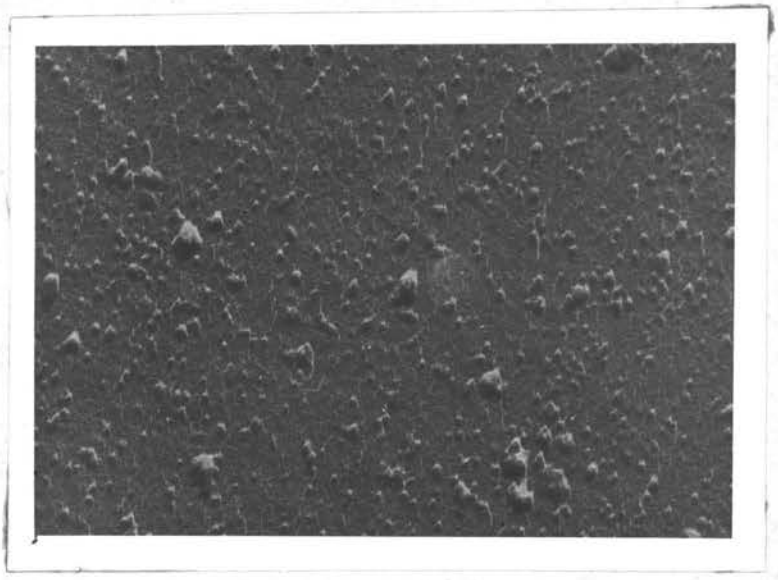

Figure 2. Electron photomicrograph of a virus preparation diluted 1:1000 with distilled water; magnification 40,000 X; shadowcast with chromium at a 25° angle.

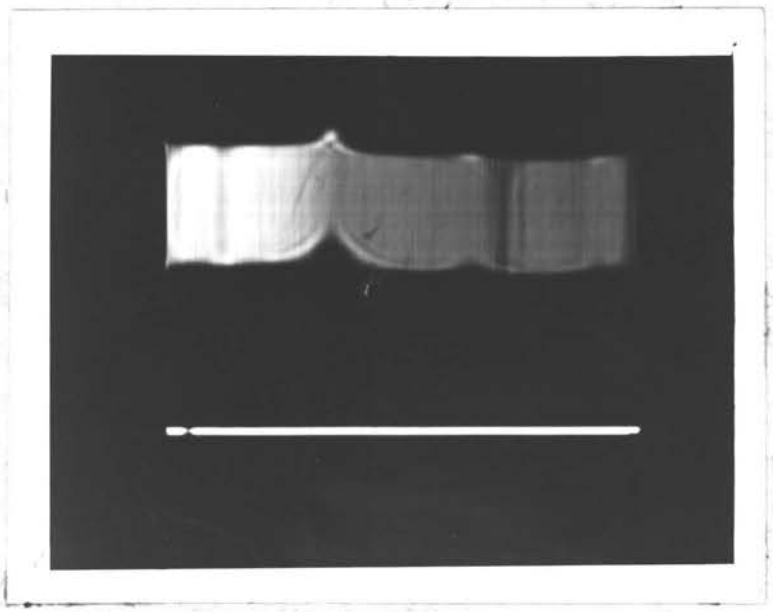

Figure 3. Electrophoretic pattern of a virus preparation in pH 7.4 phosphate buffer of ionic strength 0.1; time, 32 minutes; current, 0.013 ampere; potential, 150 volts; protein content, 1.8 mg./ml.

SUMMARY

An attempt has been made to isolate western ringspot virus 906 in a pure form. The host plant used in these studies was the Bountiful bush bean, (Phaseolus vulgaris L.). The general method of attack consisted of subjecting sap from infected plants to various fractionating procedures and testing each fraction for protein content and virus activity. An increase in the activity/protein ratio was interpreted as indicating purification. Protein determinations were made by a modified biuret procedure. Virus activity was determined by comparing the ratio, plants infected/plants inoculated, for the unknown samples with that for reference samples.

By application of techniques commonly used for purification of plant viruses, a procedure has been worked out for partial purification of western ringspot virus. The virus is precipitated from the sap of infected plants with ethanol then extracted from the precipitate with pH 6.0 phosphate buffer. Part of the inert material is removed from the resulting solution by adjusting the pH to 5.5. The virus is next precipitated with ammonium sulfate. The precipitate is redissolved in buffer and the resulting solution is centrifuged, first at low speed to remove insoluble material, then at high speed to sediment the virus. The virus is extracted with buffer from the

pellet formed by high speed centrifugation. After standing overnight, insoluble material is centrifuged from the virus solution.

Each 100 ml. of sap used as starting material contained from 2000 to 3000 mg. of protein. The final preparations from this quantity of sap contained from 1 to 10 mg. of protein and one tenth to one fifth the amount of virus found in the sap. Electrophoretic patterns of the preparations revealed one major and two minor components. Under the electron microscope the preparations were seen to consist predominantly of two types of spherical particles.

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