

SOME PROPERTIES OF ROSE MOSAIC VIRUS FROM SOUTH AUSTRALIA

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Summary

Isolates of rose mosaic virus (RMV) from South Australia were purified by differential centrifugation of cucumber extracts clarified by emulsification with ether, followed by sucrose density-gradient centrifugation. The virus was shown to be serologically similar to and to have many physical properties in common with RMV from North America. However, the Australian isolates studied appear to have narrower host ranges.

It has been confirmed that RMV is serologically related to apple mosaic virus, *Prunus* necrotic ringspot virus, and Danish plum line-pattern virus, and a serological relationship between RMV and cherry rugose mosaic virus has been established. Although RMV has certain physical properties in common with cucumber mosaic virus and alfalfa mosaic virus, no serological relationships were demonstrated. The inclusion of RMV in the *Prunus* necrotic ringspot virus group is discussed and it is suggested that the name RMV be confined at present to virus isolates inducing line-pattern symptoms in rose plants.

I. INTRODUCTION

Virus diseases of rose have been observed in many parts of the world but little is known about the viruses themselves owing to difficulties encountered in their mechanical transmission from rose plants. Fulton (1952) managed to transmit rose mosaic virus (RMV) to herbaceous plants; this allowed him to study the properties of the virus and later (Fulton 1967a) to purify it and prepare an antiserum. Serological studies have shown that RMV is closely related to apple mosaic virus (AMV) and shares some antigens with *Prunus* necrotic ringspot virus (NRSV) and Danish plum line-pattern virus (DLPV) (Fulton 1968).

In preliminary work with RMV in South Australia we were confused by the simultaneous isolation of a plant-pathogenic *Pseudomonas* sp. (Basit, Francki, and Kerr 1970) which we found to be a common, apparently latent pathogen of both rose and cherry (Basit and Francki, unpublished data). In this paper we report studies on the isolation, purification, and some properties of four isolates of RMV from South Australia. The inclusion of RMV in the NRSV group is discussed.

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II. MATERIALS AND METHODS

(a) *Transmission of Virus to Herbaceous Hosts*

Virus was transmitted to peach seedlings [*Prunus persica* (L.) Batsch cv. Elberta] by grafting three pieces of bark from a young rose shoot to the base of each young peach seedling. These were then pruned back and kept in an insect-proof glasshouse. Young leaves were ground with water in a pestle and mortar 1-3 months later and the extract was mechanically inoculated to cotyledons of young cucumber seedlings (*Cucumis sativus* L. cv. Polaris). Virus isolates were maintained in cucumber seedlings by weekly to fortnightly transfers and in peach seedlings which were kept in pots for up to 3 years.

(b) *Infectivity Assays*

As no suitable local-lesion host could be found for RMV, infectivity is expressed as the percentage of inoculated cucumber seedlings developing symptoms (Diener and Weaver 1959). Although this method is not as accurate as the local-lesion assay, satisfactory dilution curves were obtained and the assay was considered adequate for the present work.

(c) *Sucrose Density-gradient Centrifugation*

Samples of virus (0.2-0.3 ml) were layered over linear sucrose density-gradients prepared in 5 ml Spinco 39 SW tubes using 10 and 40% sucrose solutions in 0.01M phosphate buffer, pH 7.5. The tubes were centrifuged at 37,000 r.p.m. for 2 hr and their contents scanned and fractionated with an ISCO model D density-gradient fractionator and flow densitometer assembly.

(d) *Spectrophotometry*

Virus preparations were examined in a Shimadzu QR spectrophotometer using cells of 1 cm path length.

(e) *Electron Microscopy*

Virus preparations were stained in 2% uranyl acetate or phosphotungstic acid, pH 6.8 (Davison and Francki 1969), and were examined in a Siemens Elmiskop I electron microscope.

(f) *Serological Techniques*

All serological tests were carried out by the double-diffusion precipitation technique (Randles and Francki 1965) using virus concentrated by a single sedimentation as antigen.

Antisera prepared against NRSV, RMV, DLPV, and AMV were donated by Dr. R. W. Fulton of the University of Wisconsin; against NRSV, DLPV, and AMV by Dr. R. Cropley of the East Malling Experimental Station, Kent; and against cherry rugose mosaic virus (CRMV), arabis mosaic virus, tomato ringspot virus, tobacco ringspot virus, and strawberry latent virus by Dr. R. G. Grogan of the University of California at Davis. Antisera to tobacco ringspot virus and cucumber mosaic virus (Q strain) were prepared in our laboratory (Randles and Francki 1965; Francki *et al.* 1966).

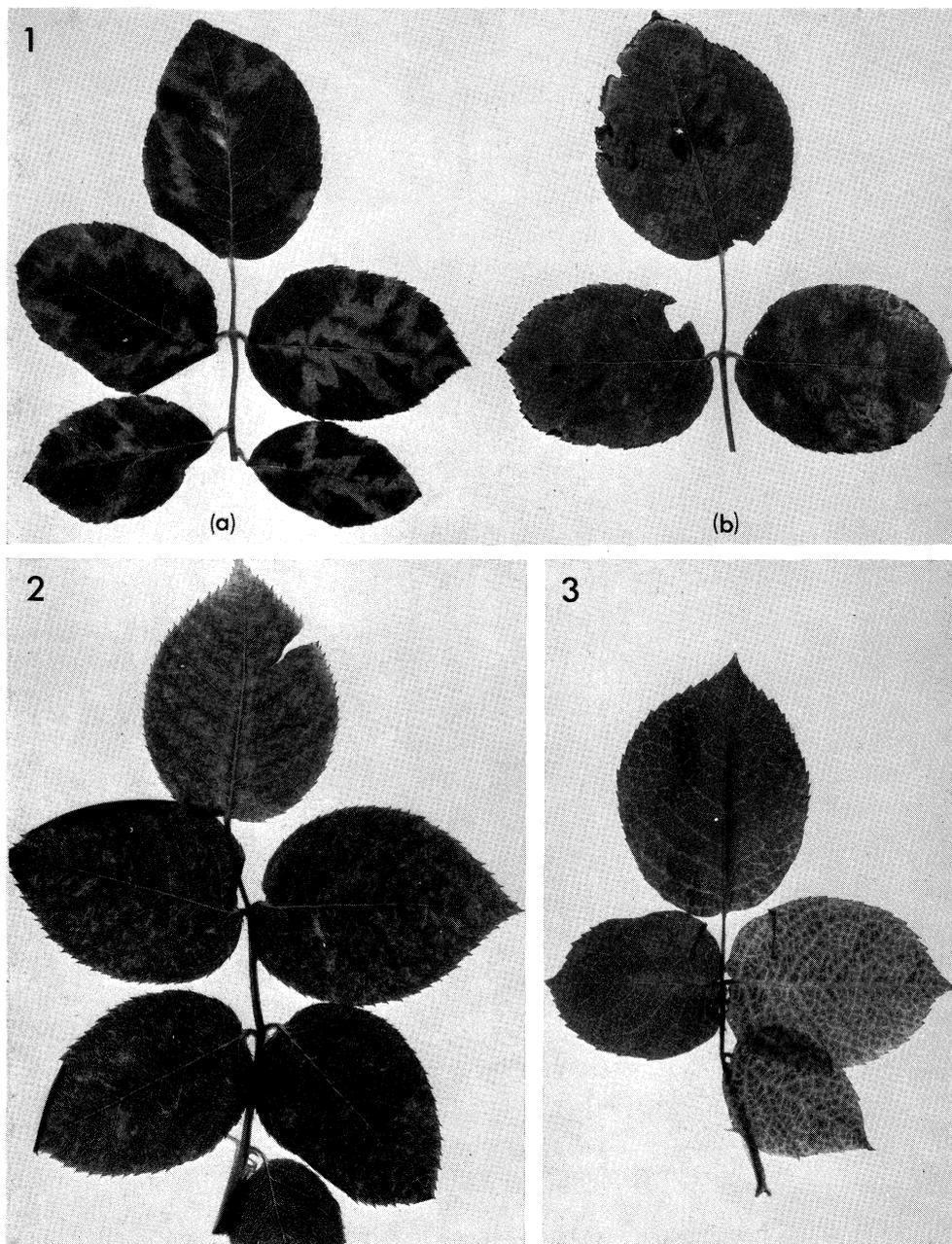
Antisera to the four isolates of RMV were prepared by injecting rabbits intravenously at weekly intervals with 2-ml samples of purified virus recovered from density-gradient columns without prior dialysis or concentration. After three or four injections the antisera had titres of 1/4 to 1/8 in gel-diffusion tests, and further booster injections failed to increase the titres above 1/8.

III. RESULTS

(a) *Disease Symptoms on Rose Plants and Virus Transmission to Herbaceous Plants*

Rose plants in South Australia show a variety of virus-like symptoms similar to those in New Zealand described by Fry and Hunter (1956) and Hunter (1965), the most common being line pattern, chlorotic ringspot, chlorotic mottle, and vein

banding (Figs. 1-3). The chlorotic ringspotting observed on some plants [Fig. 1(*b*)] can probably be considered as a variant of line-pattern symptoms. Very often all three of these symptoms occur on different leaves of the same plant.



Figs. 1-3.—Disease symptoms on rose plants. 1, line-pattern (*a*) and chlorotic ringspot (*b*) symptoms on leaves from the same rose plant (cv. Peace) infected with isolate C of RMV. 2, chlorotic mottle symptoms on leaf of rose (cv. Cuba). 3, vein-banding symptoms on leaf of rose (cv. Peace).

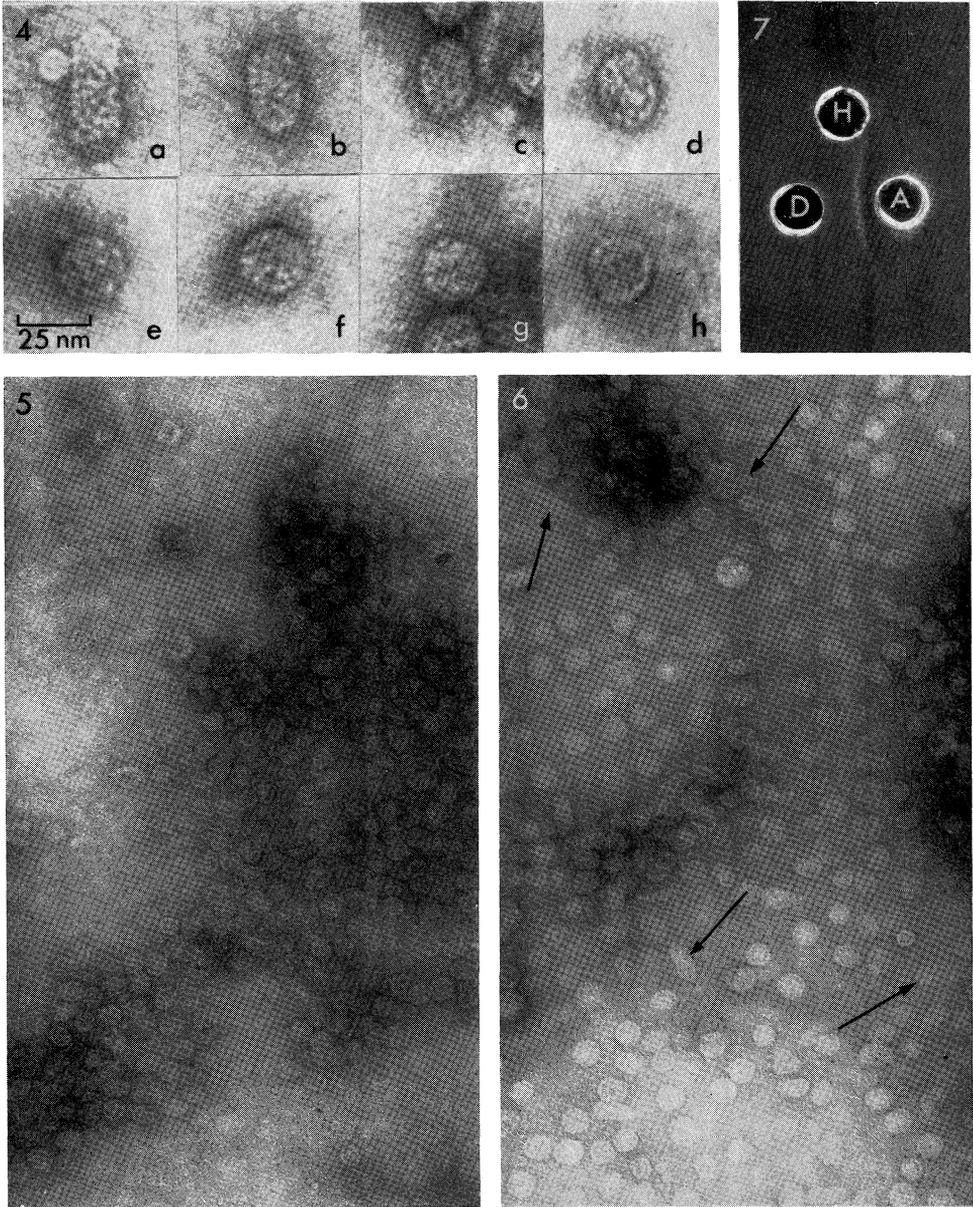


Fig. 4.—Electron micrographs of selected RMV particles from a partially purified virus preparation stained with uranyl acetate.

Fig. 5.—Electron micrograph of a preparation of T component of RMV stained with uranyl acetate. $\times 113,000$.

Fig. 6.—Electron micrograph of a preparation of B component of RMV stained with uranyl acetate. Note the bacilliform particles (arrows). $\times 113,000$.

Fig. 7.—Serological reaction in agar gel diffusion test between antiserum to RMV (A) and concentrated leaf extract from healthy cucumber seedlings (H) and those infected with RMV (D).

All attempts to transmit viruses directly from rose to herbaceous plants failed even after extensive attempts to counteract the possible adverse effects of tannin, oxidizing agents, and pH by using extracting buffers containing a variety of additives. The only method by which viruses could be transmitted from roses to herbaceous plants was via grafted peach seedlings. However, even this method was not completely satisfactory as only six virus isolates were obtained from 10 rose plants of eight different varieties showing line-pattern symptoms. No virus was isolated from 10 plants of different varieties which did not show any symptoms, nor from rose plants showing vein banding and chlorotic mottle.

Four of the virus isolates transmitted to herbaceous plants were selected for further study. These isolates will be referred to as RMV. One isolate from rose (cv. Peace) was found to be contaminated by a plant-pathogenic *Pseudomonas* sp. (Basit, Francki, and Kerr 1970) and was freed from it by passage through *Chenopodium quinoa* L. This isolate will be referred to as isolate C of RMV and was the most extensively studied; unqualified reference to RMV in this paper is to this isolate. The other isolates studied were from rose plants cv. Lady Alice (isolate A), cv. Chrysler Imperial (isolate B), and cv. First Love (isolate D).

The only two species of herbaceous plants successfully infected by mechanical inoculation with the isolates of RMV were cucumber and *Chenopodium quinoa* as reported by Basit, Francki, and Kerr (1970). We failed to infect several plant species which have previously been reported as hosts of RMV (Fulton 1952, 1967a). These were *Cyamopsis tetraganolobus* (L.) Taub., *Vigna sinensis* Endl., *Phaseolus vulgaris* L., *Nicotiana glutinosa* L., *Petunia hybrida* Vilm., and *Momordica balsamina* L.

(b) Virus Purification

RMV is unstable in plant extracts. In a typical experiment the infectivity of virus extracted in 0.01M phosphate buffer, pH 7.5, declined to less than 10% within 4 hr at 25°C. Extracts prepared at pH below 6 were very poorly infectious (Fig. 8). It was also found that the addition of 0.01% ascorbic acid to the extracting buffer helped to prevent virus inactivation. Other reducing agents such as cysteine hydrochloride, thioglycollic acid, sodium sulphite, and 2-mercaptoethanol were found not to be as effective and when added at concentrations greater than 0.1%, caused significant loss of virus.

Attempts were made to clarify virus extracts with calcium phosphate gel as used by Fulton (1967a, 1967b) but variable and unpredictable losses were encountered; hence several organic solvents were tested as clarifying agents. Butanol, Freon 113, and chloroform all caused serious virus loss and are considered unsatisfactory. Emulsification of extracts with ether and carbon tetrachloride caused only slight loss of virus (Fig. 9) and eventually ether was selected as the most satisfactory clarifying agent.

The procedure finally adopted for the purification of RMV was as follows. Cucumber seedlings were harvested 5–7 days after infection and stored at 4°C until required (up to 4 days). Tissue (100 g) was ground with 100 ml of 0.01M phosphate buffer, pH 7.5, and 20 ml of 0.01% ascorbic acid in a Waring blender for 3–5 min at 0–4°C and strained through a double layer of muslin. The resulting filtrate was centrifuged at 15,000 g for 20 min and the supernatant, pH about 7, was shaken

for 1 min with an equal volume of anhydrous ether. The emulsion was centrifuged at 15,000 *g* for 10 min, the lower buffer phase was recovered with a hypodermic syringe, and the virus was concentrated by centrifugation at 105,000 *g* for 90 min in a Spinco 30 rotor. The pellets were resuspended in one-tenth the original volume of 0.01M phosphate buffer, pH 7.5, and clarified by centrifugation at 15,000 *g* for 20 min. The virus was further purified by two or three further cycles of high- and low-speed centrifugation, the virus being sedimented at 225,000 *g* for 30 min in a Spinco 50 Ti rotor and clarified by centrifugation at 15,000 *g* for 10 min. The final pellets were straw-coloured and transparent. Those preparations of RMV which

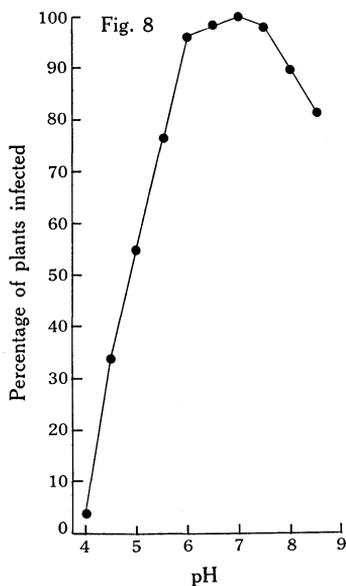


Fig. 8.—Effect of pH of leaf extracts from RMV-infected cucumber seedlings on virus infectivity.

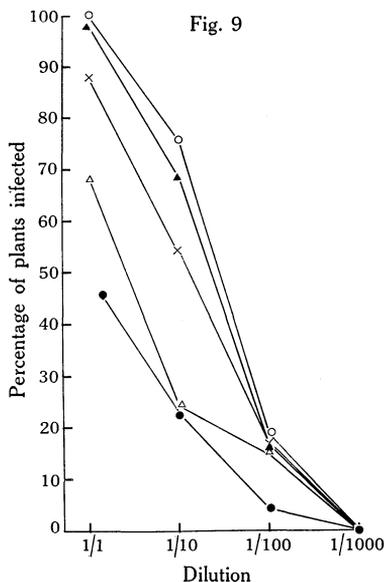


Fig. 9.—Effect of various clarifying agents on the infectivity of RMV in cucumber leaf extracts. ○ Control. ▲ Ether. × Carbon tetrachloride. △ Calcium phosphate gel. ● Chloroform.

were purified by three or four cycles of differential centrifugation will be called “partially purified”. “Purified” preparations are those which, in addition to differential centrifugation, were fractionated by sucrose density-gradient centrifugation, recovering peaks B, M, and T (Fig. 10). When peaks B and T are recovered separately these are referred to as the B and T components of RMV.

(c) Physical Properties of RMV

Partially purified RMV in 0.01M phosphate buffer, pH 7.5, was highly infectious after storage at 0–4°C for at least 7 days. These preparations had ultraviolet absorption spectra between 230 and 300 nm characteristic of a nucleoprotein, whereas preparations from equivalent amounts of healthy plant material showed relatively little absorption in ultraviolet light (Fig. 11). Similarly, analysis with the ISCO

apparatus of density-gradient columns containing preparations from healthy plants showed that they contained relatively little ultraviolet-absorbing materials (Fig. 10). However, preparations from virus-infected plants contained three peaks of ultraviolet-absorbing material (T, M, and B in Fig. 10). Infectious material was recovered only from the region of component B in density-gradient columns. Similar T, M, and B peaks were observed in all four isolates of RMV. The proportion of material in each peak varied slightly from experiment to experiment but there appear to be consistent differences in their proportions between different isolates, as already observed by Fulton (1967*a*).

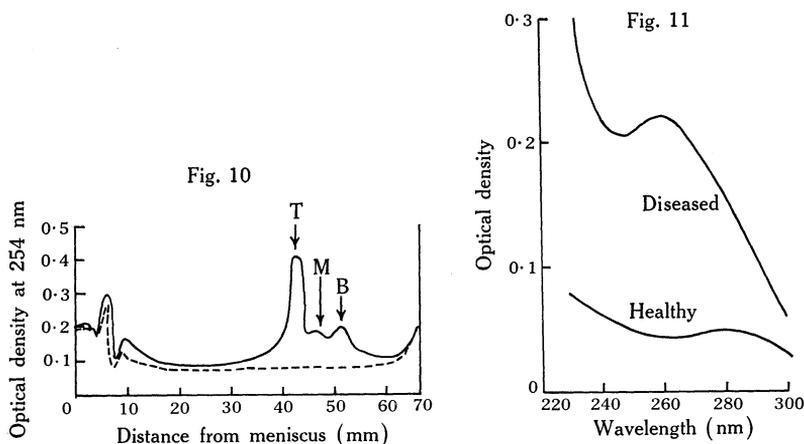


Fig. 10.—Density-gradient centrifugation profile of partially purified RMV (—) and a similarly processed extract from healthy cucumber seedlings (---).

Fig. 11.—Ultraviolet absorption spectra of partially purified RMV from a diseased plant and a similarly processed extract from healthy cucumber seedlings.

Purified RMV as well as isolated B and T components had ultraviolet spectra characteristic of nucleoproteins [Fig. 12(*a*)]. The virus was readily separated into protein and nucleic acid by freezing with 2*M* LiCl [Fig. 12(*b*)], and the nucleic acid fraction was found to be infectious.

Electron-microscopic examination of purified virus preparations stained with phosphotungstic acid or uranyl acetate showed small roughly spherical particles ranging from 23 to 29 nm in diameter, many of which showed signs of damage, especially when stained with phosphotungstic acid. Although the majority of particles were roughly spherical, some have a bacilliform outline (Fig. 4). Preparations of isolated T component contained spherical particles uniform in size (Fig. 5) with a mean diameter of 22.5 nm (based on measurements of 150 particles). However, preparations of B component contained both spherical and bacilliform particles (Fig. 6); spherical particles measured 28.7 nm in diameter (mean based on measurements of 150 particles) and bacilliform particles varied in length, some being as long as 82 nm.

(*d*) Serology

Preparations of all four RMV isolates produced single precipitin lines when tested against homologous antisera whereas similar preparations from healthy cucumber seedlings failed to produce any reactions (Fig. 7). When tested against

homologous and heterologous antigens placed in adjacent wells, antisera of all four isolates produce single confluent precipitin lines. These results indicate close antigenic relationships between all four RMV isolates although small antigenic differences may have gone undetected owing to the relatively low titres of the antisera used.

Concentrated preparations of all four RMV isolates reacted in gel-diffusion tests against antisera to NRSV, RMV, DLPV, AMV, and CRMV from North America and NRSV, DLPV, and AMV from Great Britain. Alfalfa mosaic virus did not react when tested against antiserum to RMV. RMV did not produce precipitin lines when tested against antisera to viruses of arabis mosaic, tomato ringspot, tobacco ringspot, strawberry latent, or cucumber mosaic.

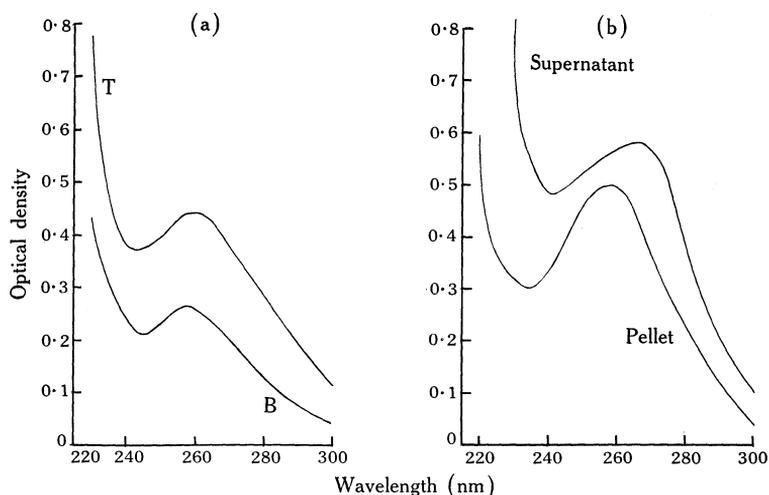


Fig. 12.—Ultraviolet absorption spectra of T and B components of RMV (a) and of RMV treated with 2M LiCl and separated into supernatant and pellet by centrifugation at 3000 *g* (b). The pellet was resuspended in 0.02M phosphate buffer, pH 7.2.

IV. DISCUSSION

RMV isolates from South Australia are serologically related to and have many physical properties in common with isolates from North America (Fulton 1952, 1967*a*, 1968). The most striking differences lie in the host ranges of isolates from the two regions. The Australian isolates studied have very much narrower host ranges.

At present, rose mosaic does not appear to be a well-defined disease. Fry and Hunter (1956) and Hunter (1965) recognized three types of symptoms on rose which they considered to be caused by different viruses but nevertheless grouped the viruses as RMV. In Australia all three symptoms (line pattern, chlorotic mottle, and vein banding) occur on roses and sometimes two, or all three, occur on the same plant. If these symptoms are indeed caused by different viruses, it seems likely that we have been isolating and studying only one of them—probably the virus causing line-pattern symptoms in rose. For the present it may be advisable to confine the name RMV to the virus causing line-pattern symptoms in rose. In the future it will be necessary to isolate viruses associated with each of the three types of symptoms, purify

them biologically as well as physically, and re-inoculate them to roses before we are sure how many viruses are involved and what their relationships are. This task is not straightforward owing to difficulties encountered in mechanical transmission of viruses from and to rose plants.

The RMV studied by Fulton (1952, 1967*a*, 1968) and by us appears to belong to the NRSV group (Gibbs 1969), a group containing a large number of virus strains and serotypes with varying degrees of relationship. AMV appears to be the closest relative of RMV (Fulton 1968) but NRSV and DLPV have also been shown to be serologically related (Fulton 1968); and CRMV has now been shown to have some antigens in common with RMV. DLPV was shown to be serologically unrelated to plum line-pattern virus isolates from other localities (Paulsen and Fulton 1969; Seneviratne and Posnette 1970) but these viruses can probably still be included in the NRSV group by virtue of similar physical characteristics. Prune dwarf virus, tobacco streak virus, and Tulare apple mosaic virus (Fulton 1967*b*; Gibbs 1969) may also be included in the group. The report that tomato ringspot virus is related to RMV (Halliwell and Milbrath 1962), however, appears to be ill-founded and its physical properties (Stace-Smith 1966) indicate that it belongs to the tobacco ringspot, rather than to the NRSV group.

The structure of RMV particles and those of the other members of the group is interesting because of the striking similarities of the spherical particles [Figs. 4(*e*)-4(*h*)] on the one hand to cowpea chlorotic mottle virus, brome mosaic virus (Bancroft, Hills, and Markham 1967), broad bean mottle virus (Finch and Klug 1967), and cucumber mosaic virus (Francki *et al.* 1966; Finch, Klug, and Van Regenmortel 1967) and on the other hand to alfalfa mosaic virus (Hull, Hills, and Markham 1969). The similarity of RMV to alfalfa mosaic virus [Figs. 4(*a*)-4(*d*)] may be due to artefacts, as it seems possible that RMV particles may degrade and reassemble during preparation for electron microscopy. Differences observed in the size of spherical particles of B and T components of RMV are remarkably similar to those observed by Seneviratne and Posnette (1970) with three virus isolates from plum causing decline, line-pattern, and ringspot symptoms.

V. ACKNOWLEDGMENTS

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