The Action of Metal Ions on Tobacco Mosaic Virus Ribonucleic Acid*

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Added transition metal ions stabilize the secondary structure of tobacco mosaic virus ribonucleic acid (TMV-RNA) as evidenced by a reduction in the absorbancy change of heated nucleic acid solutions. In spite of this stabilization of secondary structure, heating in the presence of metal ions results in the loss of biological activity due to the hydrolysis of phosphodiester bonds. Thus, all heavy metal ions of the IIB and first-transition elements studied caused a rapid loss of biological activity of TMV-RNA at *p*H 6.5 and 65°. Calcium and magnesium ions, while not affecting secondary structure at 65° and *p*H 8.5, caused a rapid loss of biological activity. At *p*H 5.8 and room temperature lead ions catalyzed the hydrolysis of RNA to 2′(3′)-mononucleotides; on long standing, nucleosides were formed.

The presence of metals in ribonucleic acids has been reported by several groups of investigators (Wacker and Vallee, 1959; Loring and Waritz, 1957; Wacker *et al.*, 1963). These "intrinsic" metals are bound very firmly to ribonucleic acid and cannot be removed completely by any known nondestructive means (Wacker and Vallee, 1959; Wacker *et al.*, 1963). The metal ions may play an important role in stabilizing regions of critical secondary structure by imposing a tertiary structure (Fuwa *et al.*, 1960).

Several groups of investigators have noted that the addition of heavy metal ions inactivated preparations of infectious nucleic acid from tobacco mosaic virus (Bawden and Pirie, 1959; Fraenkel-Conrat, 1957–58). The present report deals with an investigation of the effects of such added metal ions, i.e, extrinsic metals, on the primary, secondary, and tertiary structure of TMV-RNA.

EXPERIMENTAL

Materials.—The virus used in these experiments was the common strain of TMV grown in Turkish tobacco var. Maryland, and isolated according to the procedure of Knight (1962). TMV-RNA was prepared by phenol extraction of aqueous solutions of TMV (Gierer and Schramm, 1956), containing 0.001 M EDTA (Haschemeyer *et al.*, 1959). The samples of ^{32}P-labeled TMV-RNA were the same as those used in previous studies (Gordon and Huff, 1962).

Commercial yeast RNA (Schwarz Bioresearch, Inc., Orangeburg, N. Y.) was purified by decolorization with charcoal followed by two precipitations with ethanol. The ribonucleotides were commercial preparations. The chlorides of the metals were used, except when otherwise stated, and these salts were all spectrographically pure (Johnson Matthey, Ltd., London, U.K.). All other chemicals were of reagent grade. Deionized water was used containing less than 1 ppm of conducting salts (expressed as sodium chloride). Phenol was redistilled immediately before use.

Melting curves of TMV-RNA were obtained by measuring the increase in absorbance at 260 mμ as a function of temperature. RNA (25 μg/ml) was dissolved in 0.025 M ammonium acetate buffer, *p*H 6.8, which was 0.025 M in sodium chloride. The solution was placed in glass-stoppered 1-cm cuvets and the absorbance was measured in a Beckman DU spectrophotometer equipped with thermospacers. Temperature was controlled within 0.1° in a thermostated water bath. Temperature was measured directly in a cuvet containing 3 ml of buffer. Cuvets were weighed before and after each experiment to allow correction for evaporation. Each metal, as the chloride, was dissolved in metal-free water (Fuwa *et al.*, 1960) and added to the RNA solution. The duration of a given experiment was about 45 minutes.

Effect of Metals on Heat Inactivation of TMV-RNA.—TMV-RNA, at a concentration of 25–50 μg/ml, was heated with the desired concentration of each metal salt in 0.025 M sodium chloride–0.025 M ammonium acetate buffer, *p*H 6.5 (Fuwa *et al.*, 1960), in a water bath maintained at 65.0 ● 0.1°. Aliquots for assay were removed at various times and cooled to 0°. A control without added metal salts was treated identically. When the effects of EDTA were to be determined, the solutions were made 0.01 M with respect to EDTA. Each series of experiments was repeated at least three times.

Assays of Infectivity.—The infectivity of TMV-RNA was determined by the standard half-leaf assay using *Nicotiana tabacum* var. Xanthi, n.c. (Fraenkel-Conrat, 1959). The method has an error of about ±10%. The metal-ion concentration in the cooled control was adjusted to equal that of the experimental solution of TMV-RNA, and then both solutions were assayed immediately. This addition of metal ions to controls did not affect the assay except in the case of the chromic ion (E. V. Merriam, C. Smith, and M. P. Gordon, unpublished results).

The Effect of Nickelous Ions on the Infectivity of TMV-RNA at Room Temperature.—A solution of TMV-RNA containing 460 μg RNA/ml (1.23 μmoles P/ml) and 1.23 μmoles/ml of $NiCl_2$ in the above buffer was allowed to stand at room temperature for 15 days. A control solution which did not contain $NiCl_2$ was run in parallel. Toluene was added to avoid bacterial growth. Aliquots were removed periodically and stored at −70° until assayed.

Degradation of RNA by High Concentrations of Metals.—Two 2.1-mg samples of TMV-RNA in 0.5 ml of water

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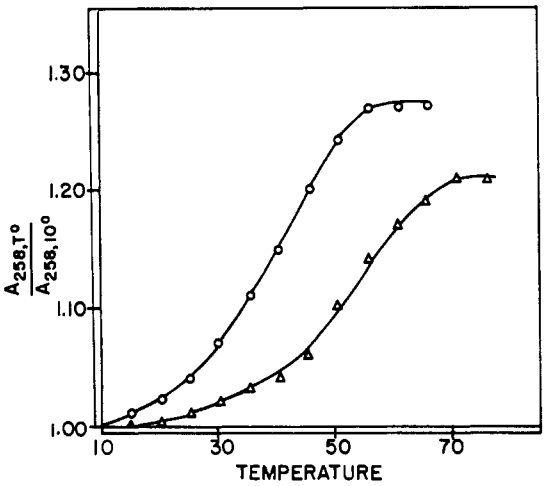

FIG. 1.—The effect of nickel on the phase-transition curve of TMV-RNA. The increase in A_{258} as a function of temperature is expressed as the ratio of the absorbancy at any given temperature to the absorbancy at 10°, $A_{258,t°}/A_{258,10°}$. The absorbancy of TMV-RNA after complete hydrolysis expressed as this ratio is 1.34. RNA content = 25 μg/ml. Buffer = 0.025 M NH_4 (acetate) + 0.025 M NaCl, *p*H 6.8. (○) control, (△) 10^{-3} M Ni^{2+}.

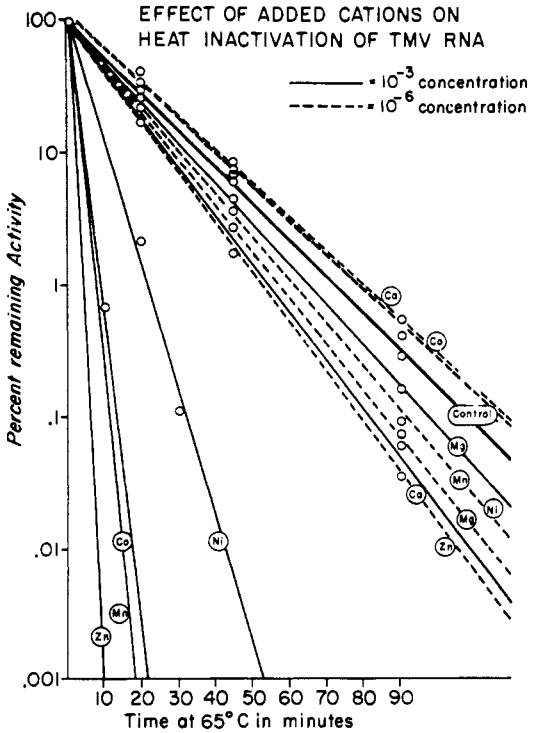

FIG. 2.—Decrease of infectious titer of TMV-RNA as a function of time when heated with various metals salts in 0.025 M sodium chloride–0.025 M ammonium acetate, *p*H 6.5, at 65°.

were treated separately with 0.5 ml of 1.5 M lead acetate solution (*p*H 5.8). The mixtures, which contained heavy precipitates, were shaken at room temperature for 24 and 72 hours. Lead ion was precipitated by hydrogen sulfide treatment and removed by centrifugation. The supernatant was evaporated to dryness, and the residue was examined by electrophoresis and chromatography.

The hydrolysis of RNA by Ni^{2+} was studied as follows: Purified (500 mg) yeast RNA was treated with a final concentration of 10^{-2} M nickelous chloride in a total volume of 5 ml. The precipitate which formed was dissolved by stirring, and the solution was then incubated for 1 week at room temperature. The Ni^{2+} was then removed by passage through a Dowex 50-X12 column (potassium form), and the oligonucleotides were precipitated with alcohol. The supernatant was concentrated and the precipitated potassium chloride was separated by filtration. Upon chromatographic examination the residue contained materials that migrated in solvent A to positions characteristic of mononucleotides. The spots were eluted and identified as the 2′(3′)-mononucleotides of adenine, guanine, cytosine, and uracil by electrophoresis at *p*H 3.5 and 9.2. RNA incubated in absence of Ni^{2+} showed no degradation to mononucleotides.

Chromatographic and Electrophoretic Systems.—The chromatographic solvents used in this investigation were: (A) 70% aqueous isopropanol with 0.35 ml of 28% aqueous ammonia added to the bottom of the tank for each liter of tank volume (Markham and Smith, 1952); (B) isoamyl alcohol layered over 5% aqueous disodium phosphate (Carter, 1950); (C) saturated ammonium sulfate–water–isopropanol (79:19:2, v/v, adapted from Markham and Smith, 1951). Electrophoretic separations were carried out at 30 v/cm in either ammonium formate, 0.05 M, *p*H 3.5, or sodium borate, 0.05 M, *p*H 9.2, on Whatman 3 MM paper.

Sedimentation Studies.—Sedimentation patterns were obtained using a Spinco Model E ultracentrifuge equipped with ultraviolet-absorption optics. TMV-RNA was at a concentration of 0.028 mg/ml in 0.025 M ammonium acetate–0.025 M sodium chloride. All runs were at 42,040 rpm and 19°. The films were scanned in a Spinco Analytrol and the sedimentation coefficients were determined from these scans. The values are reported as $s_{20,b}$; no correction for buffer viscosity was made. For each series of experiments controls (without metal ions) were maintained at the same temperature as the metal-treated RNA samples.

Detection of Terminal Phosphomonoester Groups.—In preliminary experiments use was made of *Escherichia coli* alkaline phosphomonoesterase obtained from the Worthington Biochemical Corp., Freehold, N. J. In later experiments a highly purified enzyme obtained from Rev. Donald J. Plocke, S. J., Biophysics Research Laboratory, Harvard Medical School, Peter Bent Brigham Hospital, Boston, Mass., was employed. RNA labeled with ^{32}P was used in these studies. The inorganic phosphate liberated by the enzyme from monoesterified phosphate groups was separated by electrophoresis and determined as the amount of the radioactivity traveling at the same rate as inorganic phosphate (Gordon and Huff, 1962; Gordon *et al.*, 1960). The action of the enzyme on adenosine 2′(3′)-phosphate was not affected by the concentration of Ni^{2+} used in these experiments.

RESULTS

A solution of TMV-RNA measured at 258 mμ shows marked hypochromicity compared to that of its component bases. On heating, RNA becomes hyperchromic, asymtotically approaching a limiting value of absorbancy which is less than that obtained on complete hydrolysis to mononucleotides. For native TMV-RNA upon heating there is a reproducible maximal increase of absorbancy of 1.26-fold. This ratio of the maximal absorbancy to the absorbancy at 10° will be referred to as $A_{258,t°}/A_{258,10°}$.

$A_{258,t°}/A_{258,10°}$ is markedly affected by metals of the first transition series. Figure 1 shows the melting

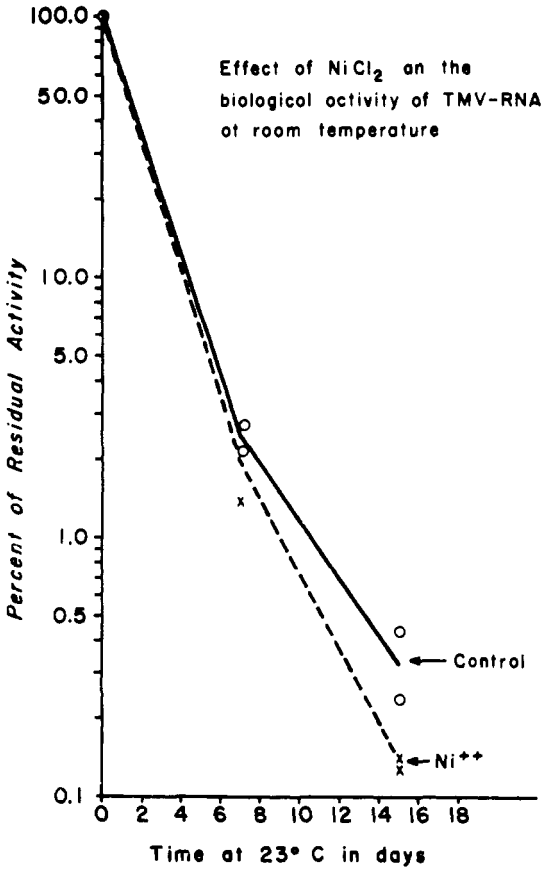

FIG. 3.—Effect of $NiCl_2$ on the stability of TMV-RNA at room temperature. A solution of TMV-RNA (1.23 μmoles P/ml) and $NiCl_2$ (1.23 μmoles/ml) in sodium chloride–ammonium acetate buffer was allowed to stand at room temperature. Samples were removed for assay at the indicated times.

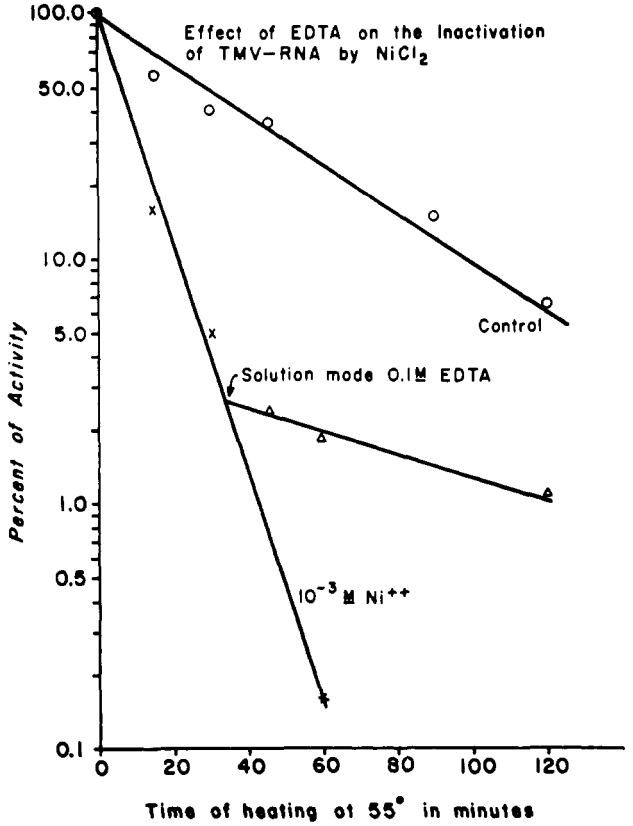

FIG. 4.—Attempted reversal of the inactivation of TMV-RNA. A solution of TMV-RNA containing 10^{-3} M $NiCl_2$ was made 0.1 M with respect to EDTA at 34 minutes (designated by the arrow). TMV-RNA content = 75 μg/ml in all samples.

profile of TMV-RNA, in 0.025 M NaCl–0.025 M ammonium acetate, *p*H 6.8, and TMV-RNA in the same buffer with added Ni^{2+} 10^{-3} M. $A_{258,t°}/A_{258,10°}$ is markedly reduced in the presence of added metal. Similar reductions in $A_{258,t°}/A_{258,10°}$ are also obtained upon the addition of other metals of the first-transition series (Table I). Magnesium and other alkaline earths, however, do not decrease $A_{258,t°}/A_{258,10°}$ (Table I) al-

TABLE I

EFFECT OF ADDED METALS ON THE MELTING CURVE OF TMV-RNA

Sample[a]	$A_{258,t°}/A_{258,10°}$
Control	1.26
Cr^{3+} 1×10^{-4} M	1.08
Zn^{2+} 1×10^{-3} M	1.19
Mg^{2+} 1×10^{-3} M	1.27

[a] All in 0.025 M NaCl–0.025 M NH_4 acetate, *p*H 6.8.

though T_m, the mid-point of the temperature profile, is displaced to higher temperatures.

When TMV-RNA (4×10^{-5} M with respect to phosphorus) in ammonium acetate–sodium chloride buffer, *p*H 6.5, was heated in the presence of 10^{-3} M Zn^{2+}, Mn^{2+}, Co^{2+}, or Ni^{2+}, a very rapid first-order loss of biological activity ensued (Fig. 2). At a concentration of 10^{-6} M, however, these metals had little or no effect on the rate of inactivation of the infectious RNA.

In view of several reports on the action of Ni^{2+} on RNA from different sources (Vizoso and Burness, 1960; Cheo *et al.*, 1959), the effect of Ni^{2+} on TMV-RNA was extensively studied. In the present experiments no stabilizing effect of $NiCl_2$ on the infectivity of TMV-RNA was noted; indeed, the samples containing $NiCl_2$ lost infectivity at a slightly greater rate than did controls (Fig. 3). Attempts were made to reverse the Ni^{2+} induced inactivation of TMV-RNA by addition of EDTA. The experiments were performed at 55° in order to decrease the rate of inactivation so that a greater number of experimental points could be obtained. Two solutions of TMV-RNA were heated in in the presence of 10^{-3} M in $NiCl_2$. After 34 minutes of heating, one solution was made 10^{-1} M with EDTA. The results (Fig. 4) show that the inactivation of TMV-RNA by Ni^{2+} ions is not reversed by EDTA. The expected decrease in the rate of inactivation due to the chelation of Ni^{2+} was obtained upon the addition of EDTA. The slightly lower rate of inactivation as compared to the control in the presence of EDTA is perhaps due to the higher ionic strength which is known to stabilize the infectivity of TMV-RNA (Gordon *et al.*, 1963).

Examination of the sedimentation behavior of TMV-RNA after heating with 10^{-3} M $NiCl_2$ or $CoCl_2$ in ammonium acetate–sodium chloride buffer suggested that a breakdown of the nucleic acid molecule might be taking place. The sedimentation coefficients obtained for TMV-RNA in these experiments ranged from 26.9 to 30.1 S (Table II) in agreement with the value of about 30 S obtained by Boedtker (1960). When Ni^{2+} or Co^{2+} was added at a concentration of 10^{-3} M

and the sedimentation was performed immediately, the RNA was found to sediment slightly more rapidly than in the control. While heating of the control RNA reduced the velocity of sedimentation slightly, striking decreases in sedimentation coefficients were observed on heating RNA solutions which contained either 10^{-3} M Ni^{2+} or Co^{2+} ions (Table II). The period of heating in the case of each metal was sufficient to destroy all but 0.1% of the initial biological activity. Uniform boundaries were obtained during sedimentation of the unheated control (Fig. 5, top row). The addition of Ni^{2+} did not change the shape of the boundaries (Fig. 5, second row). Heating the control to 65° introduced some lack of uniformity (Fig. 5, third row) but the presence of Ni^{2+} during heating introduced a much greater lack of uniformity (Fig. 5, fourth row). This is seen most strikingly in the 36-minute tracing, where a comparison of the heated control with that heated in the presence of nickel shows the much wider boundary in the latter. This evidence of heterogeneity, together with the decreased weight-average sedimentation coefficient, suggested that phosphodiester bonds had been broken under these conditions. Treatment of yeast RNA with $NiCl_2$ at room temperature for 7 days, indeed, led to the formation of detectable amounts of mononucleotides.

That catalysis of hydrolysis is not limited to metals of the first transition series is indicated by the effect of lead salts on TMV-RNA. Treatment with 0.75 M lead acetate at *p*H 5.8 for 24 hours at room temperature hydrolyzed the RNA almost completely to mononucleotides. The mononucleotides were separated by electrophoresis at *p*H 3.5, eluted, and resubmitted to electrophoresis in sodium borate buffer at *p*H 9.2, along with standards of the corresponding 2′-, 3′-, and 5′-nucleoside monophosphates. The mobilities of the isolated materials in both systems corresponded to those of the corresponding 2′- or 3′-nucleoside monophosphate standards. Paper chromatography of the isolated purine mononucleotides in solvents B and C showed separation into the individual 2′- and 3′-monophosphates. The spectra of the isolated materials corresponded to those of the standards at several *p*H values.

An attempt was made to obtain information concerning the mechanism by which TMV-RNA is broken down on heating in the presence of $NiCl_2$ by correlating the loss of infectivity with the appearance of phosphomonoesterase labile groups in ^{32}P-labeled TMV-RNA. The samples and conditions were identical to those previously described (Gordon and Huff, 1962). The loss of biological activity of samples heated in the presence of 10^{-3} M Ni^{2+} was much greater than that of controls; nevertheless, the number of detectable phosphomonoester groups in Ni^{2+} treated samples was not significantly different from that of control samples (Table III).

TABLE II

THE EFFECT OF METALS ON SEDIMENTATION COEFFICIENTS OF TMV-RNA

	$s_{20,b}$ (S)	
	Ni^{2+} (10^{-3} M)	Co^{2+} (10^{-3} M)
At room temperature		
Control	30.1	26.9
Control + metal	33.3	31.9
At 65°		
Control	28.6[a]	25.1[b]
Control + metal	20.9[a]	19.7[b]

[a] Solution heated for 30 minutes at 65°. [b] Solution heated for 15 minutes at 65°.

TABLE III

PHOSPHOMONOESTERASE DETECTABLE END GROUPS OF TMV-RNA UNDER VARIOUS CONDITIONS OF HEATING

Experiment Number[a]	RNA Preparation[a]	Time of Heating at 65° (min)[b]	Average Length of Polynucleotide Chain[c]	Ratio of Average Length of Control Chain to Average Length Heated Chain	Number of Detected Chain Breaks	Number of Chain Breaks Calculated from Actual Infectivity	Actual Infectivity (% Control)
3	B	0	14,500				
		80	7,700	1.9	0.9	4.3	1.4[d]
		80 + Ni	7,000	2.1	1.1	8.5	0.02[d]
4	C	0	5,000				
		80	3,200	1.6	0.6	3.9	2.0[e]
		80 + Ni	4,000	1.3	0.3	6.9	0.1[e]
5	D	0	6,500				
		80	2,500	2.6	1.6	3.7	2.6[e]
		80 + Ni	1,700	3.8	2.8	7.6	0.05[e]

[a] Experiment numbers and RNA preparations correspond to those previously published (Gordon and Huff, 1962). In experiments 3 and 4 the Worthington enzyme was used. In experiment 5 Father Plocke's enzyme was used. [b] The samples designated + Ni^{2+} were heated in the ammonium acetate–sodium chloride buffer made 1×10^{-3} M with respect to $NiCl_2$. [c] Average length of the polynucleotide chain = cpm in the nucleic acid sample/cpm in the inorganic phosphate released by the phosphomonoesterase. [d] In this experiment the infectivities of controls and heated sample declined 10-fold during the incubation with the phosphomonoesterase. [e] The infectivities of these samples were not affected by treatment with the phosphomonoesterase.

DISCUSSION

Metals of the first-transition series are firmly bound to the nucleic acid of tobacco mosaic virus and are considered to be intrinsic components (Wacker *et al.*, 1963). Exhaustive dialysis against orthophenanthroline does not lead to the removal of iron; instead, a mixed complex, RNA-Fe-orthophenanthroline forms (Wacker and Vallee, 1959; J. Huff, unpublished data). Precipitation of TMV-RNA from hot EDTA does not lead to any detectable removal of heavy metals. The extremely firm binding of the metals of the first-transition series and of the IIB elements to TMV-RNA observed in earlier experiments, precludes simple electrostatic binding to the phosphate groups and sug-

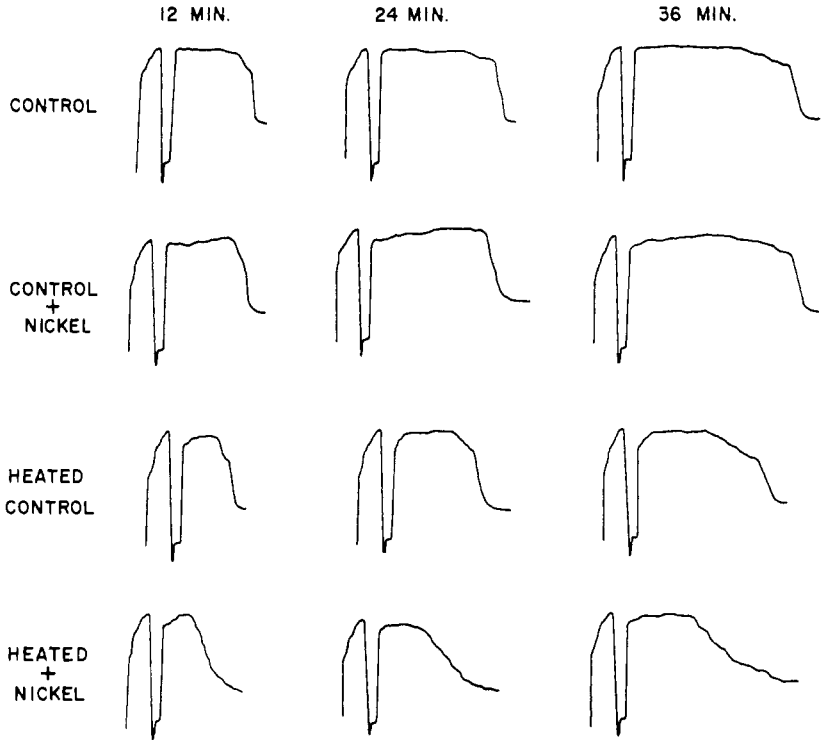

FIG. 5.—Sedimentation patterns of TMV-RNA after treatment with 10^{-3} M Ni^{2+} for 30 minutes at room temperature and at 65°, together with the corresponding controls. The RNA concentration was 0.028 mg/ml of 0.025 M ammonium acetate–NaCl buffer, *p*H 6.8. The vertical axis is per cent transmission and sedimentation is from left to right. The cell was filled completely so no meniscus trace is seen. Photographs were taken at the designated times after reaching a speed of 42,040 rpm. Figures are densitometer tracings of ultraviolet-absorption patterns.

gests that the metals are present either as chelate complexes of the nitrogenous bases or as "sandwich" complexes of the ferrocene type (Wacker and Vallee, 1959).

Previous studies on beef liver RNA demonstrated that the addition of metals of the first-transition series stabilized the secondary structure of the molecule (Fuwa *et al.*, 1960). The present study indicates a similar effect of added metals on the stabilization of the secondary structure of TMV-RNA. Transition metal ions when added to TMV-RNA bring about large reductions in the ΔA_{258} of the phase transition curve obtained when the solution is heated. This effect is not obtained with either alkali metals or alkaline earths, indicating a different type of binding for the transition metals in accord with the proposed binding of these metals by either chelation or through the formation of "sandwich" complexes.

In view of the molecular stabilization evidenced by these physicochemical experiments, studies were carried out to determine whether metals added to TMV-RNA would bring about a similar stabilization of infectivity. The addition of Ni^{2+} ions has been reported to result in the stabilization of the biological activity of infectious RNA, probably as a result of the inactivation of contaminating nucleases (Cheo *et al.*, 1959; Vizoso and Burness, 1960). Mercuric salts, silver acetate, and indium chloride have, in fact, been found to protect TMV-RNA from degradation by various ribonucleases (Singer and Fraenkel-Conrat, 1962). The loss of biological activity upon the addition of various metals, particularly Fe^{2+} (Bawden and Pirie, 1959) and Cu^{2+} (Fraenkel-Conrat, 1957–58) has also been observed. In the present experiments it has been found that all added heavy metals cause a rapid loss of biological activity of TMV-RNA at 65° (Fig. 2). The inactivation of TMV-RNA by Ni^{2+} could not be reversed by EDTA. The first-order loss of biological activity, indicative of a "one hit" process, and the sedimentation studies (Table II, Figs. 2 and 5) suggest that cleavage of internucleotide bonds is the basis of the loss of biological activity. Model experiments in which yeast RNA was incubated with $NiCl_2$ showed that mono- and oligonucleotides were formed under these conditions. The mononucleotides were mixtures of the 2′ and 3′ isomers. If it is assumed that the inactivation of TMV-RNA in the presence of $NiCl_2$ occurs solely by means of chain breakage, the number of phosphomonoester groups formed during brief periods of heating is about one order of magnitude smaller than would be calculated from the observed loss in infectivity (assuming a Poisson distribution of chain breaks each of which would lead to a terminal monoesterified phosphate group, Table III). These results, together with the findings of nucleoside 2′- and 3′-monophosphates, suggest that the cleavage occurs with the initial formation of phosphomonoesterase stable cyclic 2′,3′-phosphate end groups. Matsushita and Ibuki (1960) have also observed a nonenzymic hydrolysis of ribonucleic acids catalyzed by metal ions. They also found some 2′- and 3′-nucleoside monophosphates produced as a result of hydrolysis by metal ions at 100° and at different *p*H values.

The effectiveness of various metallic ions in destroying the biological activity of TMV-RNA varied widely. Cu^{2+}, Cr^{3+}, Fe^{3+}, and Pb^{2+} at concentrations of 10^{-5} M, 2×10^{-7} M, 10^{-6} M, and 10^{-5} M, respectively, were as effective as 10^{-3} M Ni^{2+} at 65°. Very dilute solutions of lead acetate rapidly inactivate TMV-RNA at 65°. Britten has observed that concentrated solutions of this salt cause extensive breakdown of RNA at room temperature (Britten, 1962). In 24 hours TMV-RNA is

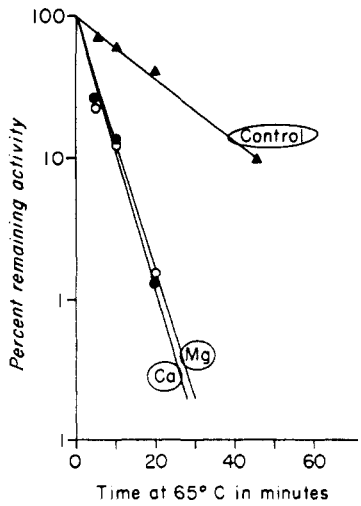

FIG. 6.—Decrease of the infectious titer of TMV-RNA as a function of time when heated with calcium or magnesium chloride in 0.025 M sodium chloride–0.025 M ammonium acetate, *p*H 8.0, at 65°.

completely cleaved to mononucleotides by 0.75 M lead acetate, and on further standing nucleosides are formed.

Experiments reported here demonstrate the cleavage of internucleotide links in TMV-RNA by metal ions. In view of the current concepts that the integrity of the entire virus RNA molecule is needed for biological activity, the rapid loss of infectivity by internucleotide bond breakage is readily explained. It should be noted that Ca^{2+} and Mg^{2+} ions were also found to catalyze the hydrolysis of RNA at alkaline *p*H values. In the present studies 10^{-3} M Ca^{2+} and Mg^{2+} caused a rapid loss of the biological activity of TMV-RNA at *p*H 8.0 and 65° (Fig. 6). These observations, which demonstrate a destruction of RNA structure, are of particular importance in view of the known dependence of ribosomal structure as well as their ability to carry out protein synthesis on the apparent concentration of magnesium (Watson, 1963).

Initially these data presented the paradox that added metal ions, while stabilizing the secondary structure of TMV-RNA when it is exposed to high temperature, cause a rapid loss of biological activity. Further studies have resolved this paradox. The rapid loss of infectivity on heating TMV-RNA in the presence of metal ions is clearly due to metal ion–catalyzed hydrolysis of phosphodiester bonds. This effect appears to be more nonspecific than the stabilization of secondary structure as measured by hypochromicity, since hydrolysis is catalyzed not only by the IIB and transition elements but also by lead, calcium, and magnesium ions. As pointed out previously, stabilization of the secondary structure may be the result of the formation of internucleotide metal bonds. These metal bonds between nitrogenous bases appear to stabilize the secondary structure in a manner analogous to the stabilization of the hydrogen-bonded secondary structure of proteins by disulfide bridges (Fuwa *et al.*, 1960). Such stabilization of secondary structures as measured by A_{max} would still be evident even after a single hydrolytic break in the polynucleotide chain had completely destroyed biological activity. The fact that melting curves obtained on heating in the presence of metals of the first-transition series are reversible on cooling (Fuwa *et al.*, 1960) is in agreement with this prediction. Thus it is apparent that two separate effects of added metal ions can be distinguished. First the stabilization of the helical secondary structure specifically by metals of the first-transition series, and second the loss of infectivity brought about by metal-ion catalysis of phosphodiester bonds by a wider range of metal ions including the alkaline earth elements.

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