has commenced to multiply about 7 hr after inoculation. There is also reason to suspect that the entity which begins to multiply is different from the intact virus particle which initiated the infection, and it is presumed that the changes in sensitivity are associated with the release of nucleic acid from the protein subunits of the intact particle, followed by reproduction of the nucleic acid. Support for this view is found in Siegel's observation of infections commenced with nucleic acids. The curve marked NA depicts the behavior for both strains. The nucleic acids exhibit identical behavior which is in marked contrast to the inactivation behavior of the intact viruses from which the nucleic acids were obtained. The resistance to inactivation begins to increase almost immediately after inoculation, compared to the lag for each of the intact strains, and furthermore, Siegel was able to show that the inactivation curves for the nucleic acids both changed to a multi-target character in about 3 hours after infection, compared to 7 hours or more for intact virus.

Evidently, we can view the protein surrounding the nucleic acid of TMV as an impediment to the reproduction of the nucleic acid, the degree of impediment being a specific property of the TMV strain. We can imagine also that the different levels of resistance encountered with the intact strains are in some way associated with the release of nucleic acid from the protein subunits.

Thus, various lines of experimental evidence derived from many laboratories scattered throughout the world have converged to illuminate a remarkably detailed picture of the construction of an entity so small as to permit only its outlines to be visualized by the most exacting electron microscopy. Furthermore, some comprehension is being gained to relate this structure to its biological function.

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*BIOPHYSICAL STUDIES OF INFECTIOUS RIBONUCLEIC ACID FROM TOBACCO MOSAIC VIRUS**

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Following the brilliant discovery by Fraenkel-Conrat[1] and by Gierer and Schramm[2, 3] of the infectivity of the ribonucleic acid (RNA) of tobacco mosaic virus (TMV), considerable attention has been devoted to this material. However, a number of important questions persist. It is known that this material is unstable

both with respect to its physical properties[4] and to its infectivity. The origin of this instability is as yet obscure. The nature of the products of the instability has not been carefully investigated. Conflicting reports[5-8] have been issued with respect to the possibility that fragments smaller than the intact RNA of the virus can retain biological activity. The low specific infectivity of the viral RNA continues to leave open the question as to whether the infectivity may reside in a minor, special component of the preparation or whether the bulk of the preparation is, at least potentially, infective.[9]

Factors Influencing the Stabiliiy of the RNA.—The addition of 8 *M* urea[10] or of 10^{-3} *M* versene[11] to a fresh phenol preparation of RNA has no significant effect upon its infectivity. In addition, the presence of these agents was found to have no influence upon the rate of loss of infectivity at 25°C. However, the addition of certain metal ions[11] has been found to have significant effects upon the stability of the RNA at 25°C.

Of the cations studied (in concentrations of 5 ppm) the alkali metals (Na^+, K^+, Rb^+, Cs^+) and the alkaline earths (Mg^{++}, Ca^{++}, Sr^{++}, Ba^{++}) were ineffective.

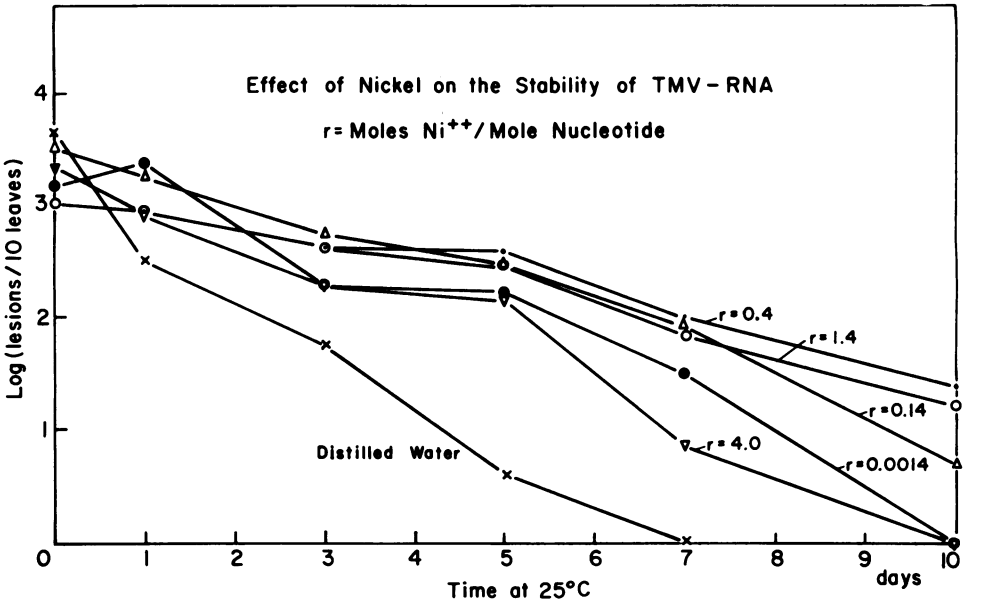


FIG. 1.—Influence of the concentration of added Ni^{++} ion on the stability of the infectivity of TMV RNA at 25°C.

Cupric and plumbous ions were toxic, destroying all infectivity. Chromous, ferrous, and manganous ions produced a slight stabilization of infectivity. Zinc, cobalt, and aluminum provided significant protection, while nickelous ion proved to be extraordinarily effective in stabilization of the infectivity (Fig. 1).

A broad optimum of protection was found about a concentration which provided one mole of nickel per mole of nucleotide. At this optimum concentration, the rate of loss of infectivity was reduced by a factor of four. However, a significant stabilization of infectivity was obtained at a concentration as low as 1 nickel ion

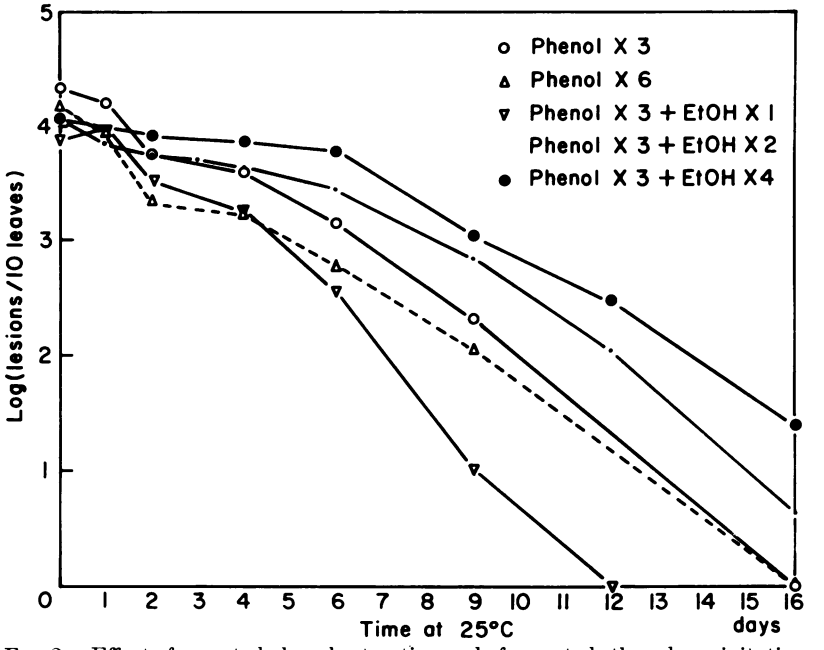


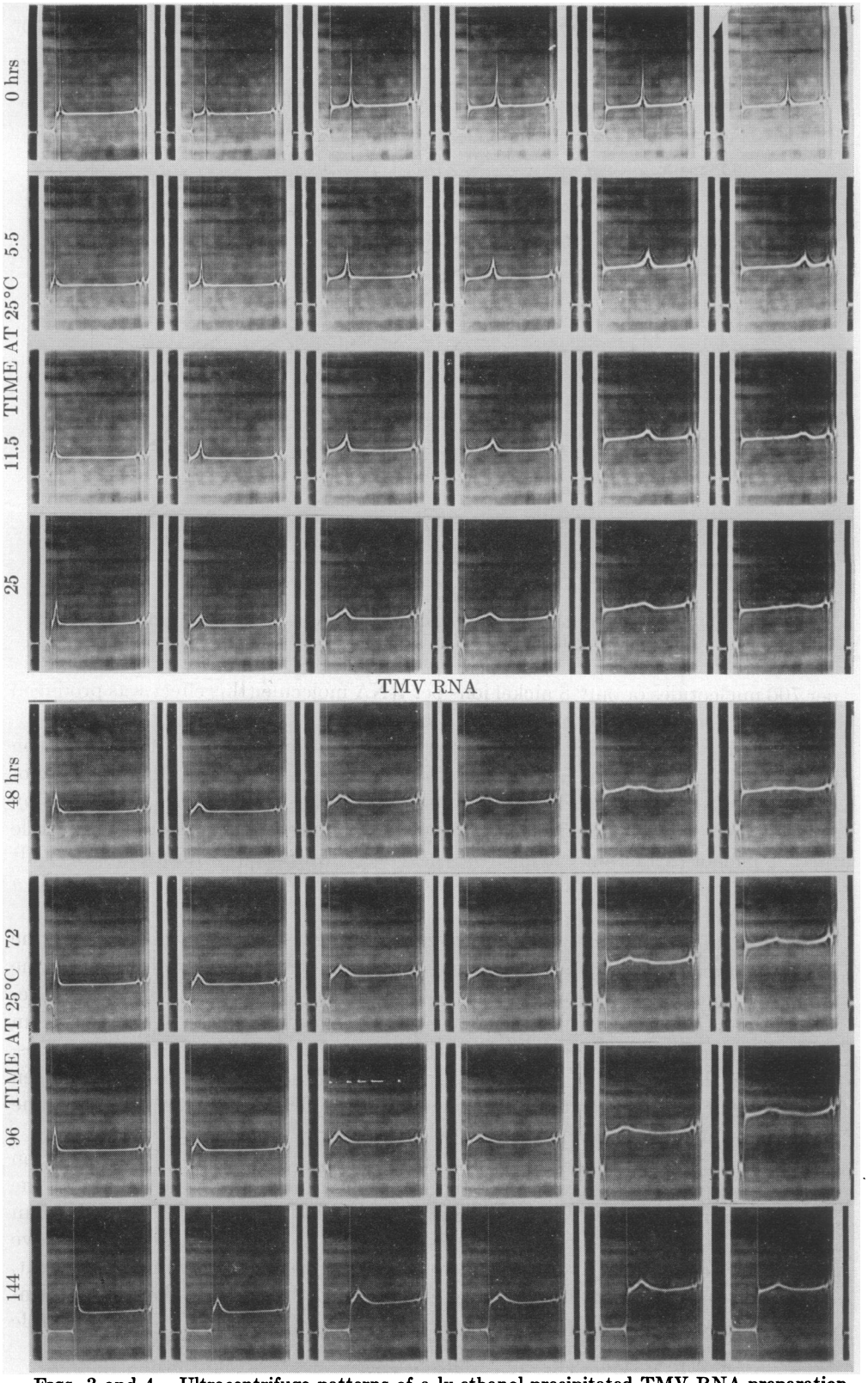
FIG. 2.—Effect of repeated phenol extraction and of repeated ethanol precipitation on the stability of the infectivity of TMV RNA at 25°C.

per 700 nucleotides or only 8 nickel ions per RNA molecule (this effect was produced with a Ni^{++} concentration of 5×10^{-9} g/ml).

The effectiveness of nickel at such low concentrations suggested that its action was to inhibit an extrinsic labilizing factor, rather than to stabilize the structure of the RNA molecule. Therefore, attempts were made to stabilize the infectivity by further purification of the RNA. As shown in Figure 2, it was found that while further phenol extraction was ineffective, repeated alcohol precipitation did result in a stabilization of the infectivity, presumably by removal or inactivation of a labilizing factor.

Ultracentrifugal Studies of the Degradation Products.—To learn more about the nature of the instability, a series of ultracentrifuge patterns was obtained as the biological infectivity disappeared upon standing at 25°C (Figs. 3 and 4). Accompanying the loss of infectivity, there is a physical disintegration of the RNA from its initial nearly monodisperse, rapidly sedimenting form, to a range of smaller sizes. During this degradation, a remarkably good correlation has been observed between the area remaining under the leading component (11S) at various times and the residual infectivity at those times (Fig. 5).

This correlation indicates rather conclusively that at no stage of the degradation do the smaller molecules carry any significant fraction of the infectivity. If the small fragments were active, the infectivity should fall off much more slowly than does the area of the leading component. However, this correlation does not prove the identity of the infective material with the material of the leading component, but merely indicates that the infective material and the RNA of the leading component are subject to disintegration, under these conditions, at very comparable rates.



FIGS. 3 and 4.—Ultracentrifuge patterns of a lx-ethanol-precipitated TMV RNA preparation after varying duration of storage in 0.02 *M* phosphate buffer, pH 7.5, at 25°C. The centrifuge patterns were obtained at 56,100 rpm, at 5°C, at a RNA concentration of 1 mg/ml.

Analysis of the centrifuge patterns obtained at various stages of degradation indicates that the breakdown of the RNA is not a completely random process, e.g., that the changes in the pattern do not appear to be those that would be expected from any process or enzyme acting completely at random along the length of the

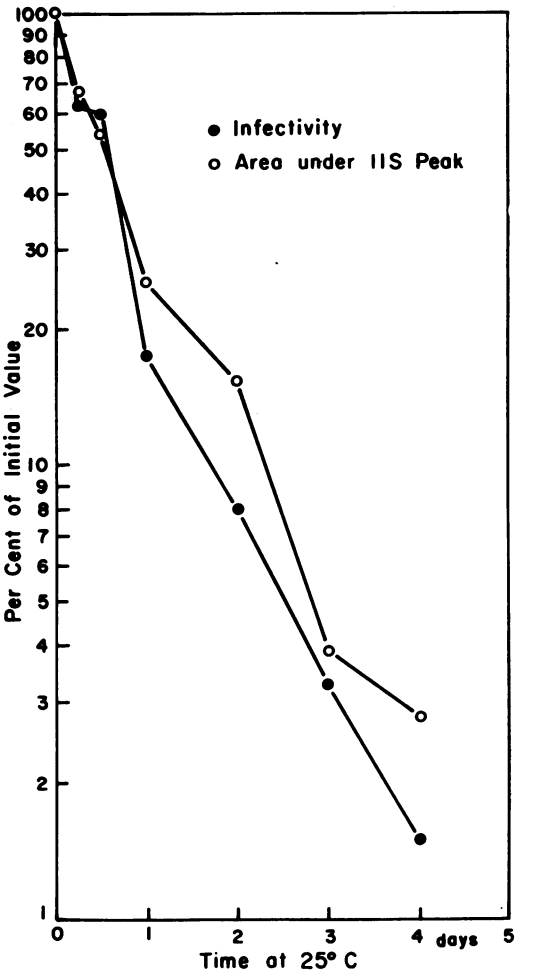


FIG. 5.—Correlation between the area of the leading component of the centrifuge pattern of TMV RNA remaining after varying periods at 25°C. with the infectivity remaining after similar periods.

RNA chain.[12, 13] The material initially present in the leading component appears, in early stages, in discrete, slightly slower components, and at later stages as more-or-less discrete, still slower components. This process is better observed in studies made upon the pattern of degradation of a 4-times alcohol precipitated preparation, in which the action of external labilizing factors has been minimized. Figure 6 illustrates the centrifuge pattern of such a preparation after 6 days at 25°C. Five distinct centrifugal components can be observed, of which the leading component corresponds to the residue of the original nearly monodisperse preparation. The existence of these discrete components provides clear evidence for the existence of subunits of the RNA, or more correctly, of preferred points of cleavage.

Fresco and Doty,[14] using synthetic polyribonucleotides, and Gierer,[12] using ribo-

nuclease digests of TMV RNA, have shown that at concentrations about 1 mg/ml the sedimentation constant for polyribonucleates varies as $M^{0.45}$. If, as a first approximation, the corrections for concentration dependence are omitted, the relative masses of the RNA components observed in the degradation pattern can be estimated from a log-log plot of the sedimentation rate versus the degree of polymerization; the slowest component is taken to be of unit degree of polymerization, and it is assumed that the observed sedimentation rates should lie on a line

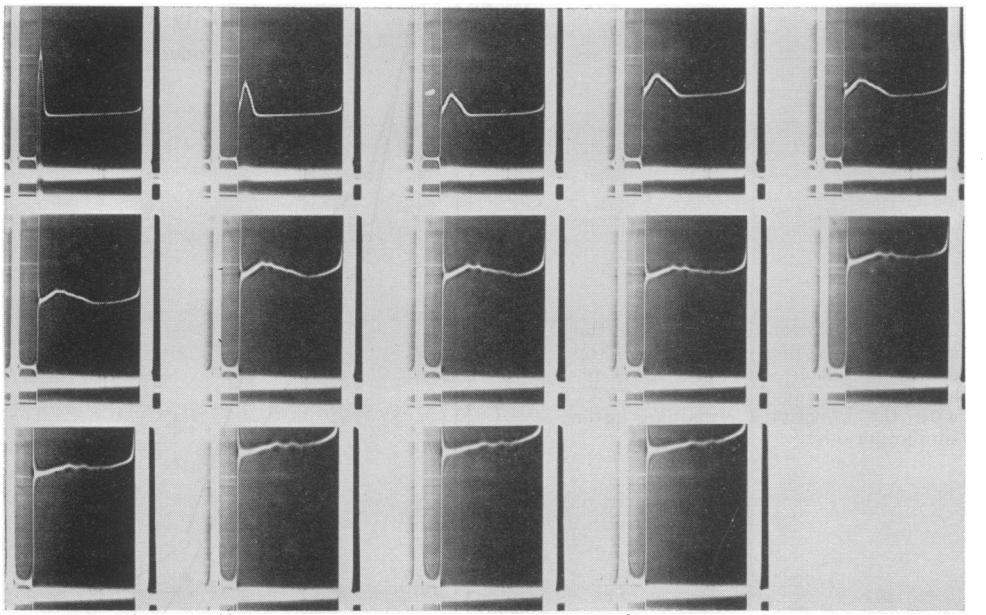


FIG. 6.—Ultracentrifuge pattern of a 4x-ethanol-precipitated TMV RNA preparation after six days at 25°C in 0.02 *M* phosphate buffer, pH 7.5. The centrifuge pattern was obtained at 56,100 rpm, at 5°C, at a RNA concentration of 1 mg/ml in a cell of 30 mm depth.

of slope 0.45. As illustrated in Figure 7, this approximate procedure results in a set of regular and integral degrees of polymerization for the observed components. This result implies, conversely, a regular pattern of breakdown of the RNA, at first into two-thirds and one-third pieces, then into one-sixth components and then again to smaller fragments, perhaps one-eighteenth. The validity of the approximation employed here, however, will have to be established by future work.[15]

Partition Cell Ultracentrifuge Studies.—A series of experiments has been conducted with the moving partition ultracentrifuge cell[22] in order to establish the identity,

TABLE 1

PARTITION CELL EXPERIMENTS WITH TMV RNA AT C = 1 mg/ml

Fresh Preparations

Manner of Preparation	UV Recovery, %	$Q_{inf.}$	S(5°) Boundary	S(5°) UV	S(5°) Infectivity
Phenol	91	0.41	12.6	11.2	12.0
Phenol	101	0.20	12.7	11.5	12.3
Phenol	100	0.37	12.2	11.1	16.5
Phenol	100	0.31	11.9	11.3	12.0
SDS	92	0.14	14.7	12.9	13.9
Av			12.8	11.6	13.3

or nonidentity, of the sedimentation rate of the bulk of the RNA preparation with the sedimentation rate of the infective element. In these experiments, three sedimentation constants can be determined: the S of the boundary or leading component by photography, the S for ultraviolet absorption by measurement of the proportion of the absorbing material remaining above the partition after a given centrifugal period, and the S for infectivity by measurement of the proportion of infectivity remaining above the partition after centrifugation.

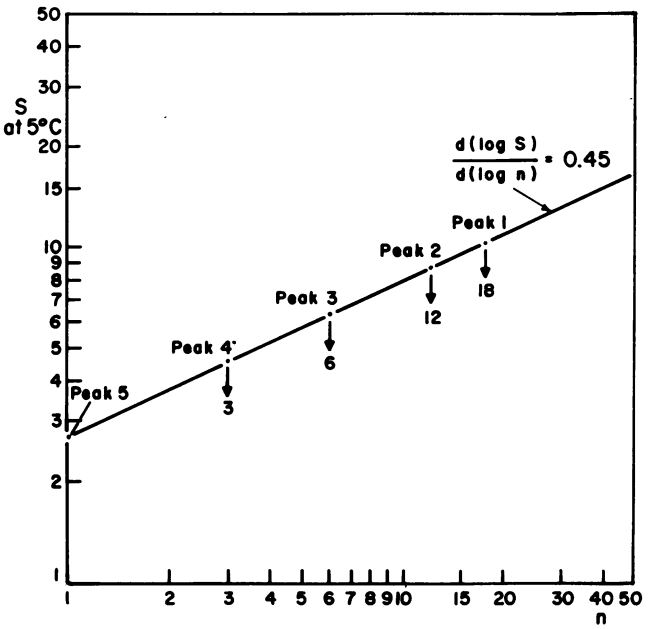


FIG. 7.—The observed sedimentation rates of the five centrifugal components of Figure 6 versus degree of polymerization, relative to the slowest component. It is assumed that the observed rates should lie upon a line of slope 0.45.

In Table 1 are summarized the results of five such experiments upon fresh RNA preparations, centrifuged at 1 mg/ml. The mean S for infectivity is 13.3 which is certainly not significantly different from the mean S of the schlieren component, 12.8. Table 2 summarizes the results of seven such experiments carried out, again at concentrations of 1 mg/ml, with aged preparations with varying degrees of

TABLE 2

PARTITION CELL EXPERIMENTS WITH TMV RNA AT C = 1 mg/ml

Aged Preparations

Manner of Preparation	Survival, %	UV Recovery, %	$Q_{inf.}$	S(5°) "Boundary"	S(5°) UV	S(5°) Infectivity
Phenol	10	...	0.06	11.7	9.6	14.5
Phenol	54	...	0.26	12.4	...	11.5
Phenol	61	110	0.04	12.2	8.1	16.7
SDS	59	100	0.26	...	7.2	12.1
SDS	43	97	0.17	12.0	8.4	12.4
SDS	4	113	0.05	11.5	7.5	15.5
SDS	40	94	0.05	12.9	6.8	15.6
Av				12.1	7.9	14.0

residual infectivity to as low as 4 per cent. In all cases the sedimentation rate for infectivity is essentially the same as is observed with the fresh preparations. Thus, the infectivity cannot be associated with any degradation products, but rather, in all cases, is associated with the residue of the original rapidly moving component.

However, these centrifugations were carried out using an RNA concentration of 1 mg/ml; at this concentration there is considerable molecular interaction and certainly not a free centrifugal movement. By the use of the more sensitive Xanthi strain of tobacco as test plants[23] it has been possible to carry out partition cell experiments at concentrations as low as 20 μg/ml, where the sedimentation rate of the RNA is the same as at infinite dilution. Under these conditions the sedimentation rate for the infectivity appears to exceed that of the ultraviolet boundary by a factor of 1.4 to 1.5 (Table 3).

TABLE 3

PARTITION CELL EXPERIMENTS WITH TMV RNA AT C $\leq$ 50 μgm/ml

Manner of Preparation	Survival, %	UV Recovery, %	Q_{inf}	S(5°) Boundary	S(5°) UV	S(5°) Infectivity
Fresh Preparations						
Phenol	...	..	0.038	19.4	...	28
Phenol	...	90	0.072	...	18.2	31
Phenol	...	90	0.25	22.1	20.8	35.7
Aged Preparation						
Phenol	40	..	0.11	17.7	...	26

Interpreted literally, this result would indicate that the infectious element in these TMV RNA preparations comprises a very minor component with a sedimentation rate, at infinite dilution, 40–50 per cent greater than that of the bulk of the RNA. Since, however, these experiments amount to a fractionation of the RNA in which only the component of lower specific infectivity is recovered, it would be wise to withhold any conclusions until it shall have become possible also to recover a fraction of increased specific infectivity.

Summary.—TMV RNA preparations are subject to both extrinsic and intrinsic modes of degradation. The extrinsic mode can be reduced by traces of certain metals, notably nickel, and can be markedly minimized by purification of the RNA The intrinsic mode reveals a set of discrete and apparently uniformly spaced points of preferred cleavage of the RNA, which must reflect some interesting discontinuities of structure. No element smaller than the intact RNA has been found to be infective. Partition ultracentrifuge studies at low RNA concentrations cast doubt upon the identity of the bulk RNA of these preparations and the infective component.

Note added in proof: More recent experiments have demonstrated that the apparent large sedimentation coefficient for the infectious element in dilute solutions of RNA is an artifact, resulting from inactivation of the RNA when in dilute solution, both during and after the sedimentation. When this inactivation is taken into account, by appropriate controls, the sedimentation rate of the infectious element of the RNA in dilute solution is very nearly the same as the sedimentation rate of the ultraviolet absorption boundary.

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*TWO CONFIGURATIONS OF TOBACCO MOSAIC VIRUS-RIBONUCLEIC ACID**

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Infectivity is now generally accepted as an intrinsic property of viral ribonucleic acid (RNA) although the detailed physical and chemical requirements for this biological activity are still largely unknown. Recent evidence favors the concept that the infective RNA from tobacco mosaic virus (TMV) represents a single strand[1] of approximately 2 million molecular weight.[2] The question of the natural physical state of such an enormous macromolecule is of considerable interest. X-ray scattering data[3, 4] have adduced evidence that in TMV itself, the RNA is located at a radius of about 40 A° from the center of the cylindrical axis of the rod-shaped virus and is embedded within a matrix of protein subunits which are stacked in a helical array. As suggested by Ginoza,[5] it also seems likely that the RNA represents a helix of the same pitch within the virus. Since the polynucleotide retains biological activity even outside the protein, it is of considerable importance to ascertain if any specific molecular configuration must be retained for this activity. While none of the physical properties of infectious TMV-RNA in solution indicate that it possesses a characteristic secondary structure, evidence will be given suggesting that such a structure may be induced under the proper conditions.

Preparation of RNA in Free and Metal-Complexed Form.—The isolation of RNA