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INFECTIVITY OF VIRAL NUCLEIC ACID*

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Methods have been described for the isolation of nucleic acid and protein from tobacco mosaic virus (TMV), yielding preparations which combined under suitable conditions to form particles resembling the original virus in physico-chemical and pathogenic respects[1]. Evidence has also been presented that "mixed" virus could be produced from protein and nucleic acid derived from different strains of TMV, and it has been shown that the progeny of such virus always resembles that strain which has supplied the nucleic acid, both in regard to symptomatology and chemical composition[2]. Thus the ribonucleic acid appeared to be the prime genetic determinant of a plant virus, just as the deoxyribonucleic acid is believed to be in bacterial viruses. Furthermore,

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observations have been described that indicate that the virus nucleic acid alone can initiate infection, even though it represents an inefficient pathogen compared to intact or reconstituted virus[2]. Similar conclusions have recently been reached independently by GIERER AND SCHRAMM[3]. It is the purpose of the present paper to describe in greater detail some of the characteristics of the infective activity of nucleic acid preparations.

METHOD OF PREPARATION

Twenty ml of a 1.0% solution of TMV, containing versene (0.0001 *M*), is warmed to 50°, adjusted to pH 8.5 at that temperature, mixed with 5 ml 5% sodium dodecylsulfate (SDS) (Duponol C, twice recrystallized from ethanol, and washed with low-boiling petroleum ether), and held at 50 ± 1° for 5 minutes. Under these conditions, the virus particles are degraded and the solution loses its characteristic opalescence. Some strains of TMV (*e.g.*, the Holmes ribgrass strain) require only 1 minute of heating for clarification, even if the initial pH is only 8.3; with other strains a longer heating period or readjustment of the pH is required to obtain almost clear solutions. Fresh preparations of common TMV also sometimes require a higher initial pH (*e.g.*, 8.8) for splitting. Complete clearing is not a necessary requirement, however, for release of active nucleic acid. The solution is rapidly cooled to 26°, and then brought to 0.33 saturation by the addition of 12.5 ml of saturated ammonium sulfate. After a few minutes the protein precipitate is separated by centrifugation, and the supernatant which may show beginning turbidity is held in a refrigerator overnight to permit the macromolecular nucleic acid to precipitate. The precipitate is then centrifuged off, redissolved in cold de-ionized water (2–4 ml), and precipitated by two volumes of cold ethanol and one to three drops of 3 *M* pH 5 acetate to facilitate flocculation. The alcohol precipitation is repeated. Any water-insoluble material is removed by centrifugation and discarded. The final aqueous solution (2 ml) is freed from aggregated material, and any contaminating virus by ultracentrifugation. This is performed for 2 hours with refrigeration at 40,000 r.p.m. in a Spinco 40-rotor with nylon adaptors and tubes of 2 ml capacity. The freely flowing supernatant is poured off quickly without draining, thus separating it from any pellet and a small amount (about 0.1 ml) of more viscous fluid remaining in the tube. This ultracentrifugation is sometimes repeated.

At times the reaction mixture was ultracentrifuged immediately after heating with the detergent, and prior to addition of ammonium sulfate. The resultant pellet then contained about 0.2% of the original virus, as determined by quantitative electron microscopy and by assay. This material, defined as "damaged" TMV, was used as a control in later experiments. After this added step isolation of the nucleic acid proceeded as usual. The final ultracentrifugation in these cases sedimented fewer virus rods. The concentration and the approximate purity of the nucleic acid in the final solution was established by spectrophotometry after a dilution of about one hundredfold in distilled water. The ratio (*R*) of optical densities at maximum/minimum (about 258/228 mμ) was usually 3.0 and sometimes up to 3.1*. The solutions were stored at —60°.

* This ratio is not a very sensitive expression of freedom from protein, since addition of 3 to 4% protein decreases it only by 0.1. An *ad hoc* mixture of the component nucleotides showed a ratio of 3.2.

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ASSAY OF VIRUS AND NUCLEIC ACID

Common TMV was diluted in 0.1 *M*, pH 6.8 phosphate buffer, and a determination made of the concentration of virus required to produce under the standard assay conditions about 25 (10 to 50) lesions per half leaf of *N. glutinosa*, or Holmes necrotic-type tobacco. About 0.25 γ/ml (0.05 to 0.5, depending on the season) and 0.025 γ/ml of TMV were required for the two test plants, respectively. The virus solutions were formerly applied by rubbing gauze pads soaked in the solution across the leaves, previously sprinkled with carborundum. In more recent experiments glass spatulas were employed instead of gauze, and definite volumes of solution were applied to each half leaf (usually 25 or 50 λ). Each solution was applied to 6-10 equivalent staggered half leaves on 6 to 10 plants, including one or two standard TMV solutions in each experimental set of 4-10 unknown solutions.

In the assay of nucleic acid it was found advisable to dilute the solutions to 110% of the desired assay level with ice water and to add one tenth of a volume of *M* phosphate buffer (pH 6.8) in an ice bath to each solution immediately prior to application. This was necessary since the infectivity of the nucleic acid was found sensitive to the phosphate ionic medium*, but fewer lesions were produced in more dilute phosphate or other solvents. Inactivation in phosphate and other media was particularly rapid if the same gauze pad was used for several plants, apparently owing to the action of absorbed plant materials on the nucleic acid. TMV showed no consistent loss of activity when applied with a gauze pad, but the activity of nucleic acid solutions dropped often from several hundred to less than 10 lesions when applied in this way to 3 or 4 consecutive half leaves.

A considerable number of experiments failed to uncover an assay medium which would favor both infectivity and stability. Other 0.1 *M* buffers at pH 6.8 (histidine, tris, veronal), and lower or higher pH's (acetate, phosphate, borate, bicarbonate), led to fewer lesions but no greater stability. The addition of serum albumin, versene, or inactive nucleic acid (2 mg/ml), did not stabilize the nucleic acid activity.

The problem of contending with *rapid* inactivation was simplified when it was found that the effect was due to plant materials. It was then prevented by the use of a fresh gauze pad for each half leaf, or, more simply, by application with clean glass spatulas. On the other hand, the *slower* inactivation occurring in pH 6.8 phosphate (0.1 *M*) in the absence of plant products was employed as a useful index of the extent to which the activity of a given solution was due to nucleic acid or to contaminating or added virus particles. To this end the solutions were applied immediately upon dilution with phosphate and again after exposure to 36° for 1 hour. Nucleic acid infectivity usually dropped to less than 10%, while control TMV showed no consistent change of activity under these conditions.

Within the limits of about 10-50 lesions per half leaf, proportionality between virus concentration and lesion number was close enough to permit approximate calculation of the relative infectivity of unknown compared to standard TMV solutions assayed simultaneously. All assays were repeated several times, at concentration levels selected to approximate those of the various standard TMV levels, and usually quite concordant results were obtained.

* This instability of the nucleic acid in the assay medium was responsible for the previously reported erroneous observation of differences in the infectivity in different hosts, and at different assay levels as compared to TMV[2].

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RESULTS

The essential difference in the nature of the infectious material of nucleic acid as compared to that of virus solutions is demonstrated by a number of experimental procedures. These were (1) sedimentability in the ultracentrifuge, (2) virus particle counts in solution and pellet, (3) effects of ribonuclease and other enzymes, (4) stability in 0.1 *M* phosphate and other ionic media, (5) effects of anti-TMV sera or γ-globulins, and (6) chemical studies on the purity of the nucleic acid.

1. The efficiency of sedimentation of dilute solutions of TMV under our conditions of ultracentrifugation (as described in the experimental section) was much greater than expected. As illustrated on Table I, solutions containing 5 to 50 γ/ml of virus could be freed from at least 99% of the virus under these conditions. When the concentration was only about 1 γ/ml, results were more erratic, owing to variable contamination of the material poured off with the solution near the bottom of the centrifuge tube. However, if nucleic acid (at about 0.5 to 5 mg/ml) was added to the virus solution, it greatly favored the sedimentation and attachment to the bottom of the tube of even traces of virus. Recovery of activity and of particles in the bottom of the tube was then always excellent, and no significant amounts were detected in the supernatant. These model experiments thus definitely showed that at least 99% of any virus present would be sedimented under the routine conditions used in the preparation of the nucleic acid. In contrast, the residual activity of our nucleic acid preparations was unaffected by 2 or more additional centrifugations (see next section).

TABLE I

SEDIMENTABILITY OF TMV FROM DILUTE SOLUTIONS*

TMV γ/ml	*Nucleic acid*** mg/ml	*Percentage of infectivity in*		
		Solution	*Drain*	*Pellet*
50	—	0.2	18	82
5	—	0.04	43	57
5	2.5	0	4	96
1	—	2	21	77
1	0.5	0.05	2	98
1	0.4	0.02	2	98
1	—	70	23	7
1	0.5	0.3	66	34

* A few experiments selected from a considerable number to illustrate the range of sedimentation efficiency observed. Centrifuged for 2 hours with refrigeration at 40,000 r.p.m. in a 40-rotor with nylon adaptors to fit 2 ml tubes. "Solution" is the bulk of the sample, poured off rapidly after arrest of the centrifuge, "Drain" the last 0.1–0.2 ml, "Pellet" the material that adheres to the bottom of the tube, resuspended in water.

** Non-infectious nucleic acid was used for these experiments. At concentrations of less than 0.2 mg/ml, the nucleic acid did not always exert a distinct favorable effect on the quantitative aspects of virus sedimentation.

2. The average number of full-length (< 300 mμ) particles found in solutions containing 0.1 γ TMV per ml is $1.1 \cdot 10^9$ ($1.8 \cdot 10^9$ if fragments are summated and included). When TMV at a known concentration was sedimented as described above, and the number of virus rods was determined by electron microscopy, the counts agreed within about 20% with the known amount of virus in the original solution. In contrast, the sediment obtained upon recentrifuging the nucleic acid contained very much fewer rods (< 1%) than required to account for the infectivity of the preparation, and upon additional centrifugations of the supernatant fluid, few, if any, rods were generally found sedimented. At the same time, the infectivity of the solution remained constant through several cycles of centrifugation, corresponding,

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for different preparations, to 1–60 γ of TMV per mg of nucleic acid. Also, the specific infectivity assays of the nucleic acid from the very top of the centrifuge tube was not different from that of the bulk of the material, nor from that of the solution near the very bottom of the tube. Yet the gradient in concentration observed in such centrifuge tubes indicated that mixing had been prevented (Table II).

TABLE II

EFFECT OF ULTRACENTRIFUGATION ON ACTIVITY OF NUCLEIC ACID

Expt. No.	*Fraction**	*Concentration** mg/ml*	*Lesions/h.l.****
I	Nucleic acid, once ultracentrifuged, supernate	5.6	25
	2 × ultracentrifuged, supernate	3.0	18
	3 × ultracentrifuged, supernate	2.6	23
II	Nucleic acid, once ultracentrifuged, supernate	1.25	52
	Same, 2 × centrifuged, top 25 %	1.0	30
	Same, 2 × centrifuged, bottom 20 %	1.7	18
III	Nucleic acid, once ultracentrifuged	8.2	154
	Same, 2 × centrifuged, top 25 %	3.2	74
	Same, 2 × centrifuged, middle 60 %	4.9	102
	Same, 2 × centrifuged, bottom 15 %	8.3	73
IV	Nucleic acid, once centrifuged	4.0	30
	Same, 2 × centrifuged, top 13 %	1.0	24
	Same, 2 × centrifuged, bottom 25 %	3.2	24
	Same, in 0.5 *M* ammonium acetate, top 10 %	0.8	34
	Same, in 0.5 *M* ammonium acetate, bottom 25 %	3.0	21

* Technique of centrifugation as described in Table I. Percentages are relative amounts of nucleic acid in fraction analyzed.

** Concentration of ultracentrifuge fraction, indicative of the gradient produced by the centrifugal field.

*** Lesions per half leaf at 20–25 γ/ml in Holmes necrotic-type tobacco. Standard TMV, assayed parallel, produced 25 lesions at concentrations of 0.02–0.05 γ/ml.

When the nucleic acid was ultracentrifuged in the presence of 0.1 *M* ammonium acetate or 0.05 *M* phosphate of pH 7, many more rods were found in the sediment (observation of C. A. KNIGHT AND W. TAKAHASHI). It appears probable that these rods many of which are often quite short, represent the re-assembly of small amounts of contaminating protein with the nucleic acid under the influence of the added buffers.

3. Crystalline ribonuclease (RNase) in extraordinarily low concentrations (one hundred millionth of the nucleic acid concentration: 10^{-8} mg/ml, 10^{-12} *M*) abolished the infectivity of the RNA in 24 hours at room temperature. A thousandfold higher concentration of enzyme had no effect on the activity of TMV. The effects of other enzymes at higher levels were somewhat erratic. Crystalline pancreatic deoxyribonuclease (DNase) usually inactivated at about 10^2 to 10^3 times higher concentrations. However, this effect seemed not to require the presence of Mg^{++} and like the effect of ribonuclease it was not abolished by heating the enzyme to 90° for 15 minutes at pH 4.7. As expected, DNase activity, as tested with DNA as substrate, was destroyed by this heat treatment. It thus appears very probable that the in-

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activation of virus RNA by DNase is actually due to a contamination with about 1% of RNase. The same interpretation is suggested for the inactivating action occasionally produced by trypsin at a hundred to thousand fold concentration of the inactivating concentration of RNase. This effect of trypsin was also heat-stable (Table III).

TABLE III

EFFECT OF ENZYMES ON RNA-ACTIVITY*

	γ/100 ml	% Inactivation
RNase	0.05	98 (95 to 100)
	0.005	0 (40 to —300**)
RNase, heated	0.05	95 (93 to 98)
DNase + $MgCl_2$	5	18 (18, 90***)
DNase no $MgCl_2$	5	23 (18 to 31)
DNase, heated + $MgCl_2$	5	25
DNase, heated no $MgCl_2$	5	24 (11 to 48)
Trypsin	50	95
	5	45
Trypsin, heated	50	97
	5	90

* 0.1 ml containing 25 γ of nucleic acid and 0.005 M pH 7.0 phosphate were treated with 5 λ of appropriate dilutions of the enzymes for 2 hours at 36°.

** Infectivity higher than in the control tube has been obtained repeatedly with the samples treated with less than inactivating concentration of ribonuclease.

*** In unaccountable manner $MgCl_2$ (0.002 M) alone caused loss of infectivity in about half of the experiments, including this one. In other experiments there was no loss of activity in the control tube containing $MgCl_2$, at 0.002, 0.02, and 0.2 M concentration. Citrate acted in similarly unpredictable manner.

The clarity of these as well as other experiments was at times obscured by variations in the stability of the infectivity of the nucleic acid under the experimental conditions. A considerable number of experiments had to be rejected because the nucleic acid in the control tube, containing only phosphate buffer, lost most or all of its activity during the enzyme digestion period. This effect was minimized by working at low buffer concentrations (0.005 M pH 6.8 phosphate) and using short digestion periods (2 hours at 36°), but single experimental or control samples occasionally and unaccountably gave unpredictable results.

4. The loss of infectivity of the nucleic acid in 0.1 M phosphate has been mentioned. In a series of experiments nucleic acid (0.001%) was exposed for 1 hour at 36° to various buffers and other ionic media, and subsequently assayed in the customary medium, 0.1 M pH 6.8 phosphate. It then became evident that the infectivity was very sensitive to any salt at 0.1 M concentration, but that in 0.01 M buffers it was comparatively stable as over the wide range of pH 4.6 to 9.2 (Fig. 1). At higher nucleic acid concentrations (0.5%) the rate of inactivation in 0.1 M sodium chloride seemed to be decreased but not that in phosphate. Inactivation by phosphate also showed a smaller temperature coefficient than that by chloride. In contrast to the inactivating action of 0.1 M salts, very low salt concentrations (0.001 M), particularly phosphate, protected much of the infectivity during incubation. High salt concen-

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trations (M to 2 M), paradoxically, had an even more marked protective and at times even an activating effect, possibly because they caused aggregation and precipitation of the nucleic acid (Table IV). When the pH-stability of the infectivity was determined in 0.001 M buffers (2 hours at 36°), over 50% was retained in phosphate of pH 5 to 8, with pH 6 being the optimum. Other buffer salts consistently caused more inactivation, but an appreciable fraction of the infectivity remained over the entire range of pH 3 to 9 (Fig. 2).

TABLE IV

EFFECT OF VARIOUS MOLARITIES OF SODIUM CHLORIDE ON NUCLEIC ACID INFECTIVITY*

Experiment	2 M	M	0.2 M	0.02 M	0.002 M
I	180		19	47	
II	131		5	53	55
III	163	131	9		53
IV**	58		2		49

* Held at 36° for 2 hours at the indicated molarity, then diluted at least five-fold with water and assayed after addition of one-tenth volume of M phosphate (pH 7). Infectivity expressed in percentage of that of frozen-stored nucleic acid.

** Experiment performed with a preparation of nucleic acid obtained by Dr. R. HART by the procedure of GIERER AND SCHRAMM[3].

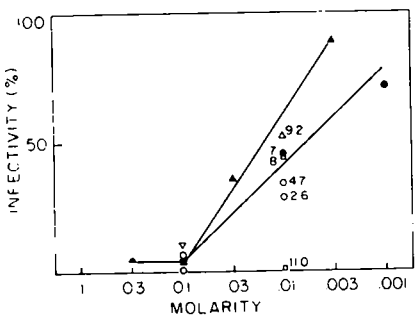

Fig. 1. Infectivity of TMV nucleic acid after incubation for one hour at 36° in various ionic media, as compared to the original infectivity of the same nucleic acid preparation. Most points are the averages of 2 to 4 experiments. ▲ = sodium chloride, ● = phosphate (pH 7.0), ○ = acetic acid or acetates, △ = borate (pH 9.2), ▽ = ammonium sulfate, and □ = carbonate or bicarbonate. The figures on the graph represent the approximate pH of the buffer.

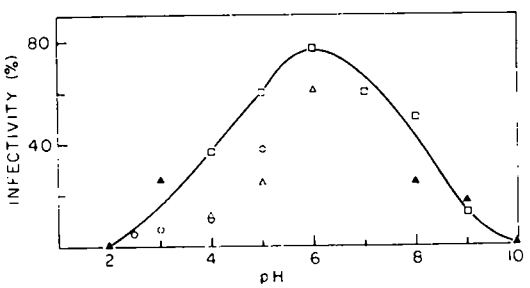

Fig. 2. Infectivity of TMV nucleic acid after incubation for two hours at 36° in various 0.001 M buffers as compared with unincubated control. Most points are the averages of 2 to 5 experiments. □ = phosphate, △ = acetate, ○ = citrate, and ▲ = HCl, and carbonate buffers. The curve represents the results obtained with phosphate buffers. More inactivation occurred generally in other buffer media.

A study of the effect of various cations is only in its beginning stages. Of the divalent metals tested, many appear to cause inactivation at low concentrations (Table V).

5. Anti-TMV serum was often found to cause inactivation of the infectivity of the nucleic acid (though not, or only slowly, of its ability to reconstitute active virus particles in the presence of protein) but normal serum had similar effects. Therefore, the γ-globulin fraction of both types of sera were employed in further experiments.

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TABLE V

EFFECT OF VARIOUS CATIONS ON NUCLEIC ACID INFECTIVITY

*Cation**	*Percentage of infectivity of control***			
Ca^{++} (0.01 *M*)	20	22	(0.001 *M*)	3
Mg^{++} (0.01 *M*)	74	172		
Mn^{++} (0.01 *M*)	40	73		
Ba^{++} (0.01 *M*)	2	3	(0.001 *M*)	10
Cu^{++} (0.001 *M*)		5	(0.001 *M*)	3
Fe^{++} (0.001 *M*)			(0.001 *M*)	0
Hg^{++} (0.001 *M*)			(0.001 *M*)	0

* Incubated at 36° for 2 hours in presence of 0.001 *M* phosphate (pH 5 for the heavy metals, pH 6 for the others). The first 4 were added as chlorides, copper and iron as sulfate, mercury as the acetate. The final pH of all solutions was between 5.6 and 6.3, except Cu and Hg (pH 5.1, 4.8).

** In the first experiment, the control was also incubated (in 0.001 *M* phosphate). In the further experiments the control was held undiluted and frozen.

It was then found possible to show that the activity of the nucleic acid was not appreciably affected by low levels of anti-TMV-γ-globulin, while the infectivity of TMV was largely neutralized by the same amount of antiserum fraction. TMV that had been exposed to the SDS treatment ("damaged TMV") was inhibited by the antisera to a similar extent (Table VI). This infectious material was also resistant to RNase, and to 0.1 *M* phosphate (see Table VII). These experiments again demonstrate the different nature of the two infectious agents, virus and nucleic acid.

TABLE VI

PERCENTAGE OF INFECTIVITY RETAINED AFTER TREATMENT WITH ANTI-TMV-γ-GLOBULIN*

Nucleic acid	95 (8 experiments, range 66–142 %)
TMV	23 (7 experiments, range 2–30 %)
"Damaged TMV"	35 (3 experiments, range 33–36 %)

* Amounts giving similar lesion numbers (25 to 100 γ nucleic acid and 0.1 γ TMV) were treated in 0.1 ml with about 5 λ of a 1:100 dilution of anti-TMV-γ-globulin for 1 hour at 36°, and then diluted for assay.

TABLE VII

STABILITY OF DETERGENT-TREATED VIRUS

Infectious agent and conditions			*Percentage of activity remaining*
Damaged virus*,	0.1 *M* phosphate,	36°, 2 h	98
Damaged virus*,	RNase (0.01 γ/ml) treated, in 0.005 *M* phosphate)	36°, 2 h	89
TMV	0.1 *M* phosphate,	36°, 2 h	98
TMV	RNase treated, as above	36°, 2 h	80
TMV-RNA	0.1 *M* phosphate,	36°, 2 h	5
TMV-RNA	RNase treated, as above	36°, 2 h	2

* Ultracentrifugal sediment obtained after the usual sodium dodecylsulfate treatment (5 min at 50°). Contains 0.5 % of the original number of 300 mμ-rods, and 0.1 % of the original infectivity. RNase was found to be active in this reaction mixture since it produced a hyperchromic effect on the small amounts of nucleic acid present.

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6. Further studies have been carried out concerning the purity of the nucleic acid as isolated from TMV. Phosphorus analyses indicated 8.1–8.5% P for preparations which were lyophilized and equilibrated with laboratory air (about 5% moisture). The UV spectrum, with particular reference to the depth of the minimum at 228 mμ and the resulting high R value (3.0–3.1) suggested a degree of freedom from extraneous absorbing material higher than any other nucleic acid preparation that has been described and was available to the authors. The extinction coefficient at the maximum (258 mμ) for a 1.0% solution in water was 250. The height of the absorption peak differed in various ionic media. It increased upon degradation of the nucleic acid by ribonuclease and alkali by, maximally, 25 and 40%, respectively.

The micro-biuret test proposed by HOLDEN AND PIRIE[4] for the detection of protein in nucleic acid indicated no detectable protein in 5.5 mg, indicative of an upper limit of 0.5% protein contamination. Qualitative and quantitative amino-acid analysis by two-dimensional chromatography and by the DNP-method[5], after hydrolysis of 12 mg nucleic acid, showed the expected great amount of glycine and another lesser nucleic-acid degradation product resembling alanine chromatographically, and giving a similar DNP-derivative. However, only serine, and aspartic plus glutamic acid, could be identified as probable hydrolytic products of the protein, and these occurred in amounts suggesting 0.4% protein contamination.

DISCUSSION

By a variety of methods it has been shown that the infectiousness of nucleic acid preparations from TMV is a property of the nucleic acid itself. The same conclusion was reached on the basis of similar evidence by GIERER AND SCHRAMM[3]. It has been shown elsewhere that with the infectivity is associated the ability to transfer all information needed for the production of complete new virus by the host cell, including the amino acid composition and structure of the virus protein[2,6,7]. A question of obvious interest is the minimal size of the nucleic acid molecule or complex of molecules required for this transfer of information. The bulk of the material in our preparations has an S_{20} of about 6–10, similar to preparations described by COHEN AND STANLEY[8] and others. This sedimentation coefficient could represent a single polynucleotide chain of molecular weight of about 200–300,000. However, it seemed possible that the infectivity in our preparations actually resided in a minor component of high molecular weight, which might correspond to the entire nucleic acid core of one virus particle. Such core material of a molecular weight of 2 to 3 million has been described by HOPKINS AND SINSHEIMER[9], and was identified with the infectious agent by GIERER AND SCHRAMM[3]. These same hypothetical undegraded cores might also be required for reconstitution. However, much recent evidence has rendered this hypothesis more and more untenable. When some high-molecular weight nucleic acid prepared according to SINSHEIMER[9] was put at our disposal by Dr. H. K. SCHACHMAN and Mr. GLENN RICHARDS of this laboratory, it proved to contain little if any nucleic acid that was infectious or able to combine with protein to reconstitute infectious rods. On the other hand, when infectious nucleic acid was ultracentrifuged, no difference was detected in the specific infectivity of material from the very top and from the bottom of the tube (Table II). This was so for material prepared both by our detergent method, and by that described by GIERER AND SCHRAMM[3] (kindly given

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to us by Drs. W. TAKAHASHI AND C. A. KNIGHT). That the latter preparation was of appreciably higher average molecular weight than our preparations was supported by the finding that the concentration in the top and middle (0.5 and 1.2 ml) of the tube was less than 1% of that in the bottom fraction (0.3 ml) quite in contrast to the much smaller concentration gradient observed for our nucleic acid under identical conditions (Table II). A detailed physico-chemical study of the sedimentation behavior and other molecular parameters of virus nucleic acid is being carried out by Dr. H. K. SCHACHMAN, Dr. V. SCHUMAKER and Mr. G. RICHARDS of this laboratory. However, the preliminary observations which have been described, and others, seem to favor the concept that the activity is associated with the bulk of the material and shares with it, therefore, the molecular weight of about 250,000 and the tendency to secondary aggregation to heavier material which may, up to a certain point, retain its infectiousness. Independent biological evidence[6] favors the same concept.

The infectivity of the most active preparations has been about 1–5% of that of TMV. Since the virus contains 6% nucleic acid, this infectivity is only of the order of 0.1% of the theoretical. However, in view of the sensitivity of the isolated nucleic acid to enzymes, ionic media, etc., no great efficiency of infection is to be expected. The finding of considerably higher infectivities for the same preparations upon reconstitution to rods[7] supports the concept that the relatively low infectivity of the nucleic is not an indication of "impurity" but rather a function of the assay conditions.

SUMMARY

A method of preparation of infectious nucleic acid from TMV is described.

The material is of similar infectivity at 10 γ/ml as is TMV at 0.02 to 0.5 γ/ml.

No significant number of virus particles can be found in the ultracentrifuge sediment of such preparations, while TMV added to nucleic acid is quantitatively sedimented under the same conditions.

The instability in 0.1 *M* salts, the remarkable sensitivity to ribonuclease, and the resistance to anti-TMV-γ-globulin clearly differentiate the nucleic acid infectivity from that of intact or detergent-damaged virus.

From the behavior in a centrifugal field and other evidence it is concluded that the activity is associated with the bulk of the nucleic acid in the preparations (Mol. wt. = 200,000 to 300,000) and not with a small fraction of high molecular weight.

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