VIRUS RECONSTITUTION

II. COMBINATION OF PROTEIN AND NUCLEIC ACID FROM DIFFERENT STRAINS*

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The *in vitro* formation of typical TMV particles from small molecular fractions of virus protein and virus nucleic acid has been described[1], as well as the finding that some infectivity similar in nature to that of the original virus was restored in this process. These studies have now been extended to various strains of TMV. Of particular significance, both from a theoretical and a potentially practical standpoint, appeared the incorporation into one virus particle of protein and nucleic acid originating from different strains of the virus. This has been achieved with various combinations of nucleic acid from 4 different strains and of protein from 3 strains. The biological and immunological characteristics of such mixed virus preparations have supplied what appears to be incontrovertible evidence that the infectivity of the reconstituted virus is actually a property of the newly formed virus particles. The biological and chemical nature of the progeny of a number of preparations of virus reconstituted from one or two strains has been studied. Some of the conclusions have been described in a preliminary note[2].

METHODS AND MATERIALS

Virus preparations and fractions

The different strains of TMV were the same as used in earlier studies from this laboratory[3,4]. All virus preparations were isolated by differential centrifugation. Nucleic acid was prepared from these strains by a slight modification[5] of the detergent method previously used[1]. About 90% of the experiments yielded preparations of biologically active nucleic acid, stable for periods up to several months, if stored at —60°.

For the preparation of native protein, the virus was degraded at 3° and at pH 10.0 to 10.5[1]. Recently 2-amino-2-methylpropanol-1 and ethanolamine have been suggested as advantageous buffers for that purpose**. After dialysis for 16 hours of a 1% solution of virus (20–50 ml) against 1000 ml of an 0.1% solution of the amine adjusted with HCl to pH 10.5, degradation was almost complete, as indicated by the small amount of material sedimented upon ultracentrifugation (1 hour at 40,000 with refrigeration). The clear supernate was brought to 0.28 saturation with ammonium sulfate and centrifuged. The precipitated protein was redissolved in water, freed from small amounts of material precipitating at low ammonium sulfate concentrations, and the bulk of the protein reprecipitated between about 0.15 and 0.25 salt saturation. The nature and the amount of material in each fraction was ascertained spectrophotometrically. The final protein precipitate generally showed a sharp maximum at 280 mμ and an *R*-value (max/min) of 2.2 to 2.4, and of 2.4 to 2.5 after dialysis. A contamination with 0.1% nucleic acid decreased this ratio

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** Unpublished results of P. E. AND M. NEWMARK.

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by 0.1. After thorough dialysis in the cold, the protein solutions were adjusted to pH 8.0, and subjected to ultracentrifugation (2 hours, 40,000 r.p.m., refrigerated). The marked tendency of the protein solutions to spoilage could be counteracted by storing them in the frozen state. Lyophilization caused some denaturation and decreased their suitability for reconstitution.

The preparation of protein from the masked strain was possible by the same method. Protein from the ribgrass strain (HR) could be prepared only with considerable difficulty and in poorer yield. A lower pH was required for splitting (9.8–10), because of the great tendency of this protein to become denatured by alkali. From the YA strain no native protein could be isolated, probably for the same reason.

Antisera

The rabbit antisera and γ-globulin fractions were kindly prepared and placed at our disposal by Dr. R. C. BACKUS and Mrs. G. PEREZ-MENDEZ. The sera were prepared in customary manner by biweekly intramuscular injections of about 1 mg of TMV or HR with mineral oil and aquaphor as adjuvants. After 3 weeks the rabbits were bled and then injected intermittently and bled weekly.

The γ-globulin fraction was separated as the trailing component in the analytical electrophoresis cell.

The efficacy of the antisera was tested by means of precipitin and neutralization tests. As an example of the latter, 1 ml of an 0.01 % solution (0.075 *M* sodium chloride) of TMV or HR virus was treated for 16 hours at 3° with varying amounts of the homologous or heterologous antiserum. Of the homologous sera 0.01 to 0.025 ml were required to reduce the infectivity of the virus (100 γ) by a factor of 5 or 10, as indicated by the number of lesions produced, after suitable dilution, by the usual assay procedure. There was, however, considerable cross reaction in the case of both the unfractionated serum and the γ-globulin fraction, particularly between anti-TMV sera and the HR virus. To decrease this hetero-specificity, the antisera were treated with varying amounts of the heterologous virus (0.16–4.0 mg/ml), and ultracentrifuged after several hours. Anti-HR sera and γ-globulins were thus obtained which had very little if any effect on TMV while reducing the infectivity of HR by about 95 %.

From anti-TMV sera no similarly selective antibody could be isolated. Repeated pretreatment with great amounts of HR virus removed all antibody activity from the solutions. Serum preparations cross-absorbed with less HR reduced the infectivity of TMV by about 97 %, and that of HR to a somewhat variable extent, averaging 44 %. Fortunately the latter antibody preparations were quite adequate to permit clear-cut serological identification of the two virus strains (Table I).

TABLE I

NEUTRALIZATION AND CROSSREACTION OF VIRUS STRAINS AND ANTISERA

Anti-serum		*Percentage neutralization of infectivity* of*	
Type	*ml/mg virus*	*HR*	*TMV*
Anti-HR-Serum**	0.1	90	0
Anti-HR-γ-globulin**	4	95	15
Anti-HR-γ-globulin	0.4	87	0
Anti-TMV-γ-globulin I**	4	62	97
Anti-TMV-γ-globulin II**	8	26	98

* Average of 2–8 experiments, each tested on about 8 half leaves at levels giving about 20 lesions per half leaf.

** Cross-absorbed with heterologous virus.

Analytical methods

For amino acid analysis, virus preparations (about 6 mg in 0.2–0.4 ml) were mixed with 2 ml of twice-redistilled constant boiling HCl, sealed *in vacuo*, and heated to 108° for 16 hours. After repeated evaporation of the acid in a desiccator, the hydrolysates were taken up in a 50-fold amount of water (50 γ per mg virus). Aliquots were chromatographed one-dimensionally on paper for the detection and analysis of histidine, methionine, tyrosine, and arginine by a recently described technique[6]. Another aliquot (1 mg) was dinitrophenylated for complete amino acid analysis, in principle according to LEVY[7]. The correction factors for the recovery of the amino acids as DNP-derivatives have been reinvestigated, and several were found to differ from those

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obtained two years ago under seemingly similar conditions[8]. Since only comparative data were required, hydrolyses for varying time periods were not carried out, and the analyses were not corrected for destruction of acid-sensitive amino acids during hydrolysis. Di-DNP-Cystine was almost absent, and not accounted for by a corresponding amount of DNP-cysteic acid. For histidine, methionine, tyrosine and arginine the results of the DNP-analyses were not readily reproducible, and the colorimetric analyses after chromatographic separation[6] were regarded as more reliable.

Tryptophane was determined, in conjunction with tyrosine on unhydrolyzed protein preparations by the spectrophotometric method as applied by BEAVAN AND HOLIDAY[9]. The tyrosine values were generally in good accord with those obtained by colorimetry[6]. The results of the amino acid analyses for TMV and HR are listed on Table II together with those available from the literature.

TABLE II

COMPARISON OF AMINO-ACID COMPOSITION OF TMV AND HR WITH VALUES IN THE LITERATURE*

	TMV					*HR*			
	Present methods	*Microbiol.*		*Average***	*Ion-exchange column****	*Present methods*	*Microbiol.*		*Average***
Glycine	2.3	1.8	2.5	2.1	2.7	1.6	1.3	1.7	1.5
Valine	9.6	9.2	10.9	10.1	9.1	5.9	6.3	7.3	6.8
Alanine	6.5	5.1	7.4	6.3	7.9	8.5	6.4	9.2	7.8
Leucine + Isoleucine	14.2	15.9	13.9	14.9	15.1	12.2	15.2	13.1	14.2
Proline	5.0	5.8	5.5	5.7	6.3	5.0	5.8	5.5	5.7
Serine	9.0	7.3	9.1	8.2	8.7	8.1	5.7	7.2	6.5
Threonine	8.9	9.9	11.9	10.9	10.5	7.2	8.2	9.8	9.0
Lysine	1.9	1.5	1.4	1.5		2.4	1.5	1.4	1.5
Arginine	9.5	9.8	9.7	9.8		8.9	9.8	9.7	9.8
Histidine	0.0	0.0	0.0	0.0	0.0	0.7	0.7	0.7	0.7
Phenylalanine	7.2	8.4	8.2	8.3	7.6	5.3	5.4	5.3	5.4
Tyrosine	4.1	3.8	3.7	3.8	4.2	6.3	6.7	6.6	6.7
Tryptophan	2.8§	2.1	1.9	2.0		2.2	1.4	1.4	1.4
Methionine	0.0	0.0	0.0	0.0	0.0	2.0	2.2	2.2	2.2
Glutamic acid	12.4	11.3	11.0	11.2	13.5	16.4	15.5	15.1	15.3
Aspartic acid	13.8	13.5	11.9	12.8	14.5	15.0	12.6	11.2	11.9
	107.2	105.4	108.1			106.9	104.7	107.4	

* All values expressed as g of amino acid per 100 g virus, not corrected for destruction during hydrolysis. It must be noted, however, that the values of FRASER AND NEWMARK are percentages of the material recovered from the ion-exchange column. Cysteine (about 0.6%) was not determined, nor listed.

** The two sets of analyses for TMV published by KNIGHT[3] and by BLACK AND KNIGHT[11] are listed, as well as the average. In the case of HR, the second column represents the expected values, had HR been again analyzed in 1953 and shown changes parallel to those observed wth TMV

*** FRASER AND NEWMARK[10].

§ The possibility of different protein fractions differing in their tryptophan content is still under investigation (2.6–3.2%). See footnote on p. 547.

RESULTS

Reconstitution of virus from common TMV protein and nucleic acid

The technique generally used for reconstitution was as follows: To 1–10 mg of protein (0.5–1.0% solution of pH 8) was added one tenth the amount of nucleic acid, and 3 *M* pH 6 acetate (10 γ per ml reaction mixture). Phosphate buffer (pH 6.8 *M*, 50 γ per ml) has also often been used and has at times given higher yields of active virus. Below pH 5.0 and above pH 8.5 little or no active virus was formed. The solutions

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were held at room temperature at least for the first few hours. Aliquots of the reaction mixture, which rapidly gets opalescent, were diluted for assay after various time periods, *e.g.*, 15 minutes, 1 hour, 20 hours. Upon assay, maximal activity was sometimes found after the short reaction periods, but more generally maximal activity was obtained after 20 hours. Activity has also occasionally been observed to decrease or disappear from reaction mixtures. Many of these experiments, however, were performed before the intrinsic infectivity of the nucleic acid, and its instability in the assay medium[5], were recognized, and these experiments are being repeated with due regard to the properties of the two types of infectious agents. When ribonuclease is added at various time intervals to inactivate any free nucleic acid, the infectivity is a true measure of the extent of reconstitution. It appears that at room temperature reaction is quite rapid in phosphate and proceeds to the same point in 24 hours in acetate (Fig. 1).

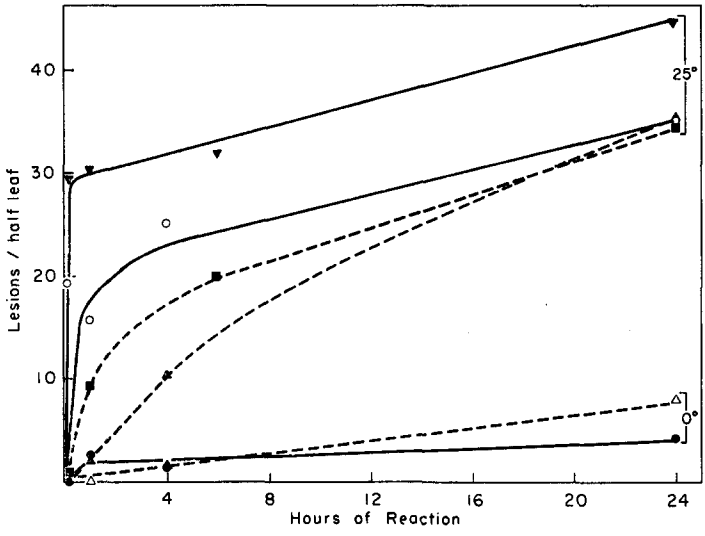

Fig. 1. Rate of reconstitution of active virus in reaction mixtures containing TMV protein (1 %), nucleic acid (0.1 %) and buffer. All samples are treated with ribonuclease (10 λ 0.1 % per ml, 16 hours) prior to assay, to inactivate any uncombined nucleic acid. Solid lines are results obtained with pH 7 phosphate (0.05 *M*), broken lines are for pH 6 acetate (0.03 *M*). Experiment I (▼, ■) yielded higher activity and was assayed at a protein concentration of 5 γ/ml. The final activity reached indicated a yield of about 5 %. Experiment II (○, ●, ▲, △) was assayed at 25 γ/ml.

Considerable effort has been put into establishing the extent of contamination, if any, of the protein and nucleic acid fractions with undegraded virus. The protein preparations usually gave no lesions when tested at 1–5 mg/ml, or at 100- to 500-fold levels of those at which reconstituted preparations gave 20–50 lesions per half leaf. That contaminating TMV would be pathogenic under these conditions was shown when TMV (0.1–0.5 γ/ml) added to the protein (1–5 mg/ml) gave 10–50% of the expected lesions. Assays of typical protein preparations by Dr. W. TAKAHASHI with a particularly sensitive assay technique suggested the presence of less than 0.0001 % virus.

A similar search for contaminating virus in nucleic acid preparations has led to

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the discovery that the nucleic acid *per se* is infectious[2,5*]. It was shown also that contaminating virus can be removed very effectively by ultracentrifugation of nucleic acid preparations. Upon repeated ultracentrifugation rod-like particles most of which are usually shorter than 300 mμ may at times be sedimented from such solutions. Their appearance seems to be favored by the presence of salts, and is probably due to reconstitution involving traces of contaminating protein. These findings have rendered definitive proof for the absence of the last traces of contaminating virus from the nucleic acid both less crucially important and more difficult. In view of the much higher yields in activity obtained in some recent reconstitution experiments, such hypothetical "last traces" become progressively more irrelevant. Furthermore, the experiments with strain mixtures to be described below have definitely shown that the activity in reconstitution experiments is inherent in newly formed particles, and cannot be attributed to undegraded virus particles.

Mixed strains

The viruses employed were common TMV, and the masked (M), yellow aucuba (YA) and Holmes ribgrass (HR) strains[3]. Active virus rods were successfully reconstituted from (1) TMV-nucleic acid and M-protein, (2) M-nucleic acid and TMV-protein, (3) YA-nucleic acid and TMV-protein, (4) YA-nucleic acid and M-protein, (5) HR-nucleic acid and TMV-protein, (6) TMV-nucleic acid and HR-protein and (7) HR-nucleic acid and M-protein. The infectivity of these mixed virus preparations was within the same range as that obtained for TMV-nucleic acid + TMV-protein. This was surprising in the case of the HR strain, because the original virus showed only 5% of the activity of TMV on a weight basis (to be referred to as specific infectivity, henceforth). The lesser infectivity of HR seems to be due to the protein component, however, since HR nucleic acid was found to be as infectious as TMV nucleic acid. It has in this case been possible to obtain a reconstituted preparation showing a four-fold higher specific infectivity than that of the original virus supplying the nucleic acid, the HR strain (mixed virus of type 5 above, to be referred to as M.V. HR/TMV).

Of greatest interest in these mixed virus experiments were the biological properties of the reconstituted virus, as compared to those of the two "parent" strains. When each of the reaction products was tested on *N. tabacum* and on *N. sylvestris*, it gave in every case the same symptoms as did the original strain supplying the nucleic acid. Thus in Turkish tobacco, M.V. TMV/M and TMV/HR gave a green mosaic disease, M.V. M/TMV produced virus without visible symptoms, M.V. YA/TMV and YA/M gave a yellow mosaic disease and M.V. HR/TMV and HR/M gave typical ringspot lesions**. These findings strongly suggest that the nucleic acid is the genetic determinant in TMV, and related strains, playing the same decisive role which DNA seems to play in the bacteriophages.

In striking contrast to the nature of the infection which is determined by the nucleic acid component, the serological characteristics of mixed virus preparations resemble those of the protein component. When anti-TMV serum or its γ-globulin

* The same conclusion was reached independently by A. GIERER AND G. SCHRAMM, *Nature*, 177 (1956) 702.

** The difference between HR- and TMV-like virus is also quite evident from the size of the local lesions on *N. glutinosa*. The differentiation of TMV and M on the one side, and HR and YA on the other is based on the type of response obtained in *N. sylvestris*.

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TABLE III

NEUTRALIZATION OF INFECTIVITY OF TMV, HR, AND THE TWO MIXED VIRUS PREPARATIONS DERIVED FROM THESE*

Assay level (γ/ml)	TMV 0.17	HR 1.7	HR/TMV** 0.44	TMV/HR** 22	TMV and TMV Prot. 0.11	TMV and HR Prot. 0.11	HR and TMV Prot. 1.7	HR and HR Prot. 1.7	HR/TMV 0.45	TMV/HR 9.7
Untreated	22	21	19	29	30	28	37	56	22	14
+ Anti-TMV γ-globulin	0.9	8.0	3.6	11	0.2	0.8	17	33	1	9
+ Anti-HR γ-globulin	23	1.1	16	2.9	6	3	2	4	11	3

* All figures in the table represent average number of lesions per half leaf on *N. glutinosa* plants. Most assays were performed 2 or 3 times on 8 or 10 half leaves per sample.

Two experiments selected out of about 30 are listed, all of which showed the same phenomenon though some were less complete, others showed more cross reaction. Of TMV and HR 5 γ were used, of the mixed virus preparations 20–30 γ; of the γ-globulin fractions, pretreated with the heterologous virus (see EXPERIMENTAL) 10 or 20 λ were used, and the final volume of the reaction mixtures was 0.3 ml. When protein was added to the reaction mixture, 25 γ was used. Combinations showing specific neutralization have been printed in italics.

** HR/TMV represents mixed virus prepared from HR-nucleic acid and TMV-protein; TMV/HR represents the virus prepared from TMV-nucleic acid and HR-protein.

(see METHODS AND MATERIALS) was added to the two preparations, it neutralized M.V. HR/TMV to a similar extent as it did TMV, but had little effect on M.V. TMV/HR. Anti-HR serum, on the other hand, neutralized M.V. TMV/HR much more effectively than it did M.V. HR/TMV (Table III). At the same time, and in the same assays, the nature of the lesions clearly showed that the latter was of HR character, while M.V. TMV/HR was of TMV character. Control experiments in which the neutralisability of the two viruses by anti-sera was tested in the presence of excess homologous or heterologous protein confirmed the validity of the experimental procedure. Thus there appears to be no doubt that the activity appearing in reaction mixtures containing HR-nucleic acid and TMV-protein is due to a particle containing a genetically determining HR-nucleic acid core, and an immunologically determining TMV-protein coat. No alternate explanations seem able to explain the observed facts. Thus reconstitution of infectious virus particles from two chemical components appears to be definitely established.

Nature of the progeny of mixed virus

The finding that TMV-nucleic acid will combine with HR-protein, and vice versa, is particularly surprising in view of the great differences between the proteins of these two strains. It appears from KNIGHT's analyses[3], as confirmed by us, that only 2 to 4 amino acids occur in the same amounts in HR and TMV. Histidine and methionine are completely absent from all strains that have been investigated by KNIGHT, with the exception of HR. An important functional property of the virus proteins probably resides in their specific tendency to aggregation in a superhelical array around, if they are present, nucleic acid strands. In view of the exchangeability of different virus proteins, as observed in the present experiments, one must conclude that this activity is dependent only upon a few suitably situated key sites surrounded by non-specific areas.

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In view of the marked difference in amino acid composition of TMV and HR, the mixed virus preparations obtained from these two were of particular interest and value in establishing the nature of the progeny of reconstituted virus. Paper chromatographic comparison of the hydrolysates of TMV, HR, and the progeny of the two mixed virus preparations has clearly demonstrated the presence of about 0.7% histidine and about 2% methionine in HR and the progeny of M.V. HR/TMV, and the absence of these amino acids from TMV and the progeny of M.V. TMV/HR. Complete amino acid analyses were then carried out for these 4 types of preparations (see Table IV). The first impression, based on the presence or absence of histidine and methionine was generally confirmed by these analyses. The protein of each progeny closely resembled that of the virus supplying the nucleic acid to the mixed virus from which it was derived. Only very minor differences were noted (less than 10% of the content in any one amino acid). However, in the case of glycine, that small difference was observed in 8 separate hydrolysates of 5 different progeny preparations from M.V. HR/TMV as compared to HR, and a small difference in the lysine contents between these has been observed almost as consistently. Yet these differences are too small to be regarded as more than a suggestion that the protein component may slightly influence the genetic message transferred by the nucleic acid.

TABLE IV

COMPARISON OF AMINO ACID COMPOSITION OF HR AND TMV WITH PROGENY OF EXPERIMENTAL PREPARATIONS*

	HR	*Progeny of HR/TMV*	*TMV*	*Progeny of TMV/HR*	*Mutant strain from TMV-nucleic acid*
Glycine	1.6	*1.8*	2.3	2.3	2.5
Alanine	8.5	8.5	6.5	6.9	5.5
Valine	5.9	6.3	9.6	9.0	9.6
Leucine + Isoleucine	12.2	12.2	14.2	14.3	13.0
Proline	5.0	5.1	5.0	5.1	4.3
Serine	8.1	8.1	9.0	8.8	7.8
Threonine	7.2	7.5	8.9	8.9	8.8
Lysine	2.4	2.3	1.9	1.8	2.1
Arginine	8.9	8.5	9.5	9.7	*7.6*
Histidine	0.70	0.70	0.0	0.0	0.0
Phenylalanine	5.3	5.4	7.2	7.1	6.8
Tyrosine	6.3	6.2	4.1	4.3	*5.4*
Tryptophan	2.2	2.2	2.8	2.6	2.7
Methionine	2.0	2.2	0.0	0.0	0.6
Glutamic acid	16.4	17.3	12.4	12.1	12.4
Aspartic acid	15.0	14.8	13.8	14.2	15.5

* All values expressed as g of amino acid per 100 g virus.
Values are not corrected for destruction during hydrolysis.
The first 2 columns represent averages of 8 hydrolysates each; the next three columns averages of 3 hydrolysates each.
Seemingly significant differences have been printed in italics.

It must further be noted that the observed differences are definitely less than one amino acid residue per subunit, and their acceptance requires the assumption that not all subunits can be identical. This assumption appears also required to explain the tryptophan content of TMV which is close to 2.5 residues per 18,000 molecular weight subunit. That value was obtained both by a chemical method[8], and by

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spectrophotometry both in 0.1 *N* sodium hydroxide and at neutral pH[8,9*], and appears definitely more probable than the lower value obtained by microbiological methods.

Another difference between HR and the progeny of M.V. HR/TMV is shown by the specific infectivity, which has been higher for many of the latter preparations than the highest obtained for any HR sample (20–45% *vs* 12%).

The significance of these differences between the original virus strains and the M.V. preparations will be dependent on whether they recur in successive progeny preparations. This seems to be the case for the specific infectivities, but as yet no sufficient number of amino acid analyses have been completed with successive single-lesion progeny preparations to warrant any definite conclusions in this regard. At the present time, one can only conclude from all this work that the nucleic acid of each strain has the ability to provoke the synthesis, within the host cell, of new virus protein very similar to, if not identical with, its own homologous protein; and that it retains this ability even when packaged, *in vitro*, in the protein of another strain.

Heritable modifications

In observing the plant-pathogenic nature, and the protein composition of the progeny from single lesions of many preparations of mixed virus or of isolated nucleic acids, a marked variation was noted in at least one instance. This apparent mutant was characterized by differences in both disease symptomatology and amino acid composition of progeny virus (Table IV). Its appearance may be regarded as indicative of the labilisation of the genetic material through chemical exposure and manipulation, since no similar variations have been observed from single lesion propagation of the original virus strains. In contrast to other instances where genetically more labile variants were observed, this mutant strain has continued to produce a striking necrotic disease in Turkish tobacco through several passages and isolations, including the separation of its nucleic acid and protein.

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SUMMARY

1. A method of preparation of native protein from TMV and other strains has been described.
2. The reconstitution of virus particles from protein and nucleic acid of different strains has yielded very active preparations, one of which showed higher infectivity than one of its parent strains.

* The spectrum of TMV protein (1 mg/ml) differs markedly in shape from that of an *ad hoc* mixture of the amino acids contributing to its absorption (30 γ tryptophan, 40 γ tyrosine, 65 γ phenylalanine, 7 γ cysteine, and 1.0 mg triglycine per ml). However, the maximum, though at 282 and 278 mμ respectively, is the same (O.D. = 1.25). When solutions of protein and amino acids were prepared in 67% acetic acid, the O.D.'s were the same and the spectra very much more similar in shape (maxima at 279 and 278 mμ, respectively).

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3. The nature of the disease provoked by mixed virus preparations resembled in each case that characteristic of the virus supplying the nucleic acid.

4. The chemical nature of the progeny of mixed virus preparations also closely resembled that of the virus supplying the nucleic acid, although the significance of minor differences in amino acid composition has not yet been established.

5. In contrast to these properties the serological characteristics of mixed virus preparations were those of the virus supplying the protein.

6. The dual nature of the activity of reconstituted virus particles has thus been clearly demonstrated.

7. Variants or mutants of different biological and chemical properties have occurred randomly in the course of this work, and are regarded as indications of a labilisation of the genetic material through chemical manipulation.

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ÉTUDE DE L'ACTION DU CHLORURE DE SODIUM SUR L'OXYHÉMOGLOBINE

I. MESURES DE DIFFUSION DE LA LUMIÈRE

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L'hémoglobine et ses dérivés soumis à diverses actions physiques ou chimiques (dilution[21] – pH[2,10,17] – urée[3,6,19] amides[19] – sels[11,13]) tendent à se dissocier en unités plus petites. La stabilité des molécules ne serait du reste pas la même suivant le matériel traité: elle varierait d'une espèce d'hémoglobine à l'autre, d'un dérivé à l'autre. L'action particulière du chlorure de sodium sur l'hémoglobine avec scission en deux moitiés a déjà été signalée[2,14].

Nous nous sommes proposé ici d'étudier l'effet du chlorure de sodium sur des solutions d'oxyhémoglobine humaine, au voisinage de son pH isoélectrique, en comparant la masse moléculaire de cette protéine dans des solutions contenant ou non du sel.

Nous avons mesuré les masses moléculaires par diffusion de la lumière.

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