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Infectivity of Ribonucleic Acid from Tobacco Mosaic Virus

In their experiments with bacteriophages, Hershey and Chase[1] have shown that only the nucleic acid component plays a part in the intracellular multiplication. There are also indications that in simple viruses containing ribonucleic acid the nucleic acid plays a dominant part in the infection. Thus, experiments with tobacco mosaic virus have shown that the protein can be changed chemically without affecting the activity and the genetic properties[2]; recently, it was even found[3] that part of the protein can be removed from tobacco mosaic virus without destroying the activity.

We have now obtained evidence that after complete removal of the protein, the ribonucleic acid itself is still infectious.

The protein was extracted from tobacco mosaic virus with phenol by a procedure elaborated by Schuster, Schramm and Zillig (to be published). After a short treatment at low temperatures, a preparation of ribonucleic acid is obtained which has a high molecular weight during the first few hours but depolymerizes in the course of time. Its physical properties will be described elsewhere.

A solution of 10 per cent tobacco mosaic virus in 0·02 *M* phosphate buffer of *p*H 7·3 is shaken for 8 min. at 5° C. with an equal amount of water-saturated phenol. The aqueous phase which contains the ribonucleic acid is separated by centrifugation, and the process of extraction with phenol is repeated at least twice for 2 min. The phenol is then extracted by ether from the aqueous phase. The whole procedure is carried out at 5° C. and takes about 50 min.; it is followed immediately by testing the infectivity.

For that purpose, five to ten plants of *Nicotiana glutinosa* with five leaves each were inoculated with a diluted solution of the ribonucleic acid, and an equal number of plants with a standard solution of tobacco mosaic virus. The number of local lesions produced by ribonucleic acid and tobacco mosaic virus are compared in Table 1. It is found that 10 μgm. of ribonucleic acid produces about the same number of lesions as does 0·2 μgm. tobacco mosaic virus. The infectivity of the ribonucleic acid preparation is thus about 2 per cent of that of the native virus.

Table 1. Comparison of the Infectivity of Ribonucleic Acid and Tobacco Mosaic Virus in 0·1 *M* Phosphate Buffer

*p*H	Ribonucleic acid μgm./ml.	lesions	Tobacco mosaic virus μgm./ml.	lesions
6·1	10	153	0·09	95
			0·8	445
7·3	10	815	0·27	1,048
7·3	1	524	0·05	795
7·5	10	998	0·27	685

The following experiments, the results of which are collected in Table 2, have been carried out to show that the infection is due to the nucleic acid rather than to contamination of the ribonucleic acid with native virus.

(*a*) In the ribonucleic acid solution protein was not detectable by chemical methods (Schuster, Schramm and Zillig, unpublished work); thus the amount must be less than 0·4 per cent of the ribonucleic acid content. By serological methods (complement fixation) it was shown that the ribonucleic acid contains less than 0·02 per cent of native tobacco mosaic virus protein.

(*b*) Treatment of both the ribonucleic acid and tobacco mosaic virus with normal rabbit serum (concentration 3×10^{-3}, applied for 10 min. at 4° C.) somewhat reduces the infectivity. There is no significant further reduction if the ribonucleic acid is treated with the same amount of tobacco mosaic virus antiserum, whereas with antiserum the infectivity of the virus itself is almost completely destroyed.

(*c*) Incubation of the stock solutions of ribonucleic acid (0·3 per cent) and tobacco mosaic virus (0·06 per cent) with 2 μgm. per ml. of ribonuclease at 4° C. for 10 min. reduces the activity of the ribonucleic acid to 0, whereas that of the virus remains almost unaffected.

(*d*) The sedimentation constant of the ribonucleic acid is 12–18 *S*, compared with 180 *S* for tobacco mosaic virus. We have centrifuged the stock solution of ribonucleic acid for 30 min. at 50,000 rev. per min. and found the supernatant liquid to be only a little less active than the original solution. If the solution of the virus is treated in the same manner, the activity of the supernatant liquid is very low.

(*e*) The ribonucleic acid is known to be unstable; and, as would be expected, its infectivity is much reduced after 48 hr. at 20° C., whereas that of the virus is much less affected.

These experiments show that protein, if present at all, is only there in very small amounts and does not resemble closely the native protein of tobacco mosaic virus. We are thus led to conclude that the infectivity is due to the nucleic acid itself.

Table 2. Comparison of Ribonucleic Acid (10 μgm./ml.) and Tobacco Mosaic Virus (0·27 μgm./ml.) in 0·1 *M* Phosphate Buffer of *p*H 7·3

(Infectivity expressed as lesions per 30 leaves)

	Ribonucleic acid	Tobacco mosaic virus
Normal	488	629
With normal serum	180	117
With antiserum	145	0
With ribonuclease	0	473
After ultracentrifugation	367	31
After 48 hr. at 20° C.	2	130

The infectivity of the ribonucleic acid preparation is about 0·1 per cent of that of the same amount of ribonucleic acid contained in native tobacco mosaic virus. Whether this relatively low value is due to a large inactive fraction of the ribonucleic acid preparation, or to low efficiency of the mechanism of infection, has still to be determined.

Studies on the combination of the ribonucleic acid with proteins are being carried out and may elucidate the connexion between our findings and the reactivation experiments of Fraenkel-Conrat and Williams[4], of Lippincott and Commoner[5], and of Hart[6].

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A detailed account of this work will be published in the *Zeitschrift für Naturforschung*.

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