A Rapid Colorimetric Method for the Determination of Tobacco Mosaic Virus Concentration in Plant Saps

BANCROFT and CURTIS[1] reported that a copious white precipitate appeared when 2 *M* trichloroacetic acid (TCA) was added to preparations of chloroform clarified sap (SCHNEIDER[2]) from virus-infected plants; whereas the preparations from healthy plants remained clear. It is thus evident that the precipitate which appeared on addition of trichloroacetic acid to clarified sap from virus-infected (tobacco mosaic virus [TMV]) plants was of TMV-nucleoprotein.

COMMONER et al.[3] used a modified method of Folin reaction[4] for the estimation of μg quantities of TMV. In the present case the nucleoprotein, after precipitation with 2 *M* TCA, was dissolved in 1 *N* NaOH and the Folin-Phenol reaction used for the estimation of protein concentration.

Method. Frozen leaves from tobacco plants were homogenized after thawing. The homogenate was transferred to 10 ml glass stoppered volumetric flasks. The final tissue weight to buffer volume ratio was 1:10. 1 ml of chloroform + 0.5 ml of *N*-amyl alcohol were added. The sample was shaken vigorously for 15 min, and transferred without rinsing to a conical centrifuge tube and held at 1 °C for 15 min. The chloroform settled at the bottom, leaving an aqueous phase at the top. The sample was then centrifuged for 5 min at 2000 *g* in an electrical centrifuge. 3 definite layers were formed. A packed solid layer separated the lower chloroform and upper aqueous phases. The supernatant aqueous layer was poured off and again centrifuged and upper aqueous phase removed.

Different aliquots of the resultant supernatant were removed to acid-washed test-tubes and to each aliquot 2 *M* TCA was added at room temperature. The ratio of aliquot and TCA in each case was 2:1 v/v. The tubes were allowed to stand for 5 min and then centrifuged at 2000 *g* for 5 min. The supernatants were carefully decanted and the tubes were drained off thoroughly. In subsequent steps, instead of adding 0.3 *M* TCA as suggested by BANCROFT and CURTIS[1], the precipitate was dissolved in each case in 0.5 ml of 1 *N* NaOH. The nucleoprotein precipitate was soluble in 1 *N* NaOH. Different aliquots of this fraction thus obtained were taken for colour development. Readings were taken on a Junior Coleman Spectrophotometer. The optical density readings gave an indication of the virus content and corresponded with the virus content observed by biological assay.

The results of Table I and Figure 1 revealed that the optical density readings could be correlated with different amount of virus nucleoprotein present in the NaOH soluble sample and chloroform clarified sample. The nucleoprotein precipitate in each case was dissolved in 0.5 ml of 1 *N* NaOH and the colour developed as stated above.

In another experiment a large number of leaf samples infected with TMV were treated with different chemicals

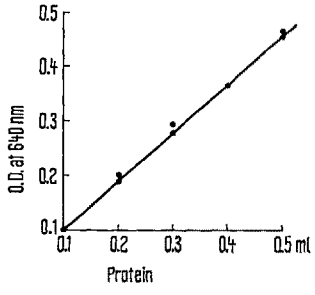

Fig. 1. Folin-Phenol reagent calibration curve for TMV nucleoprotein after precipitation of virus nucleoprotein with 2 *M* TCA and dissolving the precipitate in 1 *N* NaOH.

Table I. Estimation of virus nucleoprotein content in tobacco leaf sap by Folin-Phenol reagent

Volume of nucleoprotein sample	O.D. at 640 nm First replicate	Second replicate
(A) NaOH soluble fraction		
0.1 ml	0.10	0.10
0.2 ml	0.19	0.20
0.3 ml	0.28	0.29
0.4 ml	0.36	0.36
0.5 ml	0.45	0.46
(B) Chloroform clarified fraction		
1 ml	0.07	0.08
2 ml	0.14	0.13
4 ml	0.29	0.29
8 ml	0.58	0.59

[1] J. B. BANCROFT and R. W. CURTIS, Phytopathology *47*, 79 (1957).
[2] I. R. SCHNEIDER, Science *117*, 30 (1953).
[3] B. COMMONER, F. L. MERCER, P. MERRIL and J. A. SIMMER, Archs Biochem. *27*, 271 (1950).
[4] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FAN and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

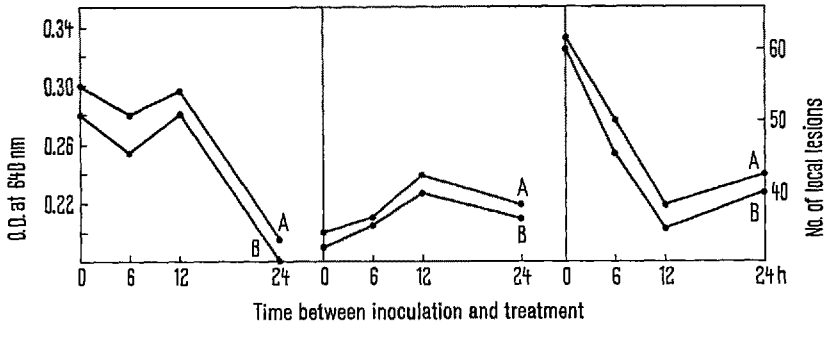

Fig. 2. Comparison of the virus content in 3 virus samples, at different times after treatment with amino acid analogues by active virus assay (A) and by estimation with Folinphenol reagent (B).

(amino acid analogues) for varying periods, and virus content estimated by active virus assay on *Nicotiana glutinosa* leaves and by Folin-Phenol reagent method, after 7 days of inoculation. The results are presented in Table II and Figure 2. The amount of virus nucleoprotein content in different virus samples was comparable to that obtained by active virus assay of different samples. The optical density readings were proportional to the number of local lesions in different samples. Therefore, the results were reproducible for different amounts of virus nucleoprotein and were also comparable to those obtained by active virus assay. It is thus a new simplified method for the estimation of TMV-content in plant saps and gives results comparable to those obtained by active virus assay. The method eliminates the use of costly apparatus like ultracentrifuge and UV-spectrophotometer, and also eliminates the use of trichloroacetic acid as blank, which sometimes gives significant absorption in the UV-range. The method may, therefore, be of great help to laboratories where facilities for costly instruments do not exist[5].

Table II. Comparison of the pattern of virus multiplication in different samples by active virus assay and by estimating virus nucleoprotein content by Folin-Phenol reagent

Sample No.	Virus estimation	0 h	6 h	12 h	24 h
1	Active virus[a]	50	45	50	30
	Virus nucleoprotein[b]	0.30	0.28	0.30	0.19
2	Active virus	32	35	40	35
	Virus nucleoprotein	0.20	0.21	0.24	0.22
3	Active virus	60	45	35	40
	Virus nucleoprotein	0.34	0.28	0.22	0.24

[a] No. of local lesions on 5 leaves of *N. glutinosa*. [b] Optical density at 640 nm.

Zusammenfassung. Es wird eine neue kolorimetrische Schnellmethode zur Bestimmung des Nucleoproteidgehaltes von Tabakmosaikvirus im Saft von Tabakblättern beschrieben. Der Saft wird durch die Chloroform-Wasser-Emulsionstechnik geklärt, das Virus-Nucleoproteid durch 2,0 *M* Trichloressigsäure gefällt und dann in 1,0 *N* NaOH gelöst. Die durch Zusatz von Folin-Phenolreagens entstandene Farbe wird kolorimetrisch bestimmt. Die mit der neuen Methode erhaltenen Resultate stimmen gut mit denjenigen von Aktivvirusbestimmungen überein.

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A System for Studying 'Target-Finding' by Schistosome Miracidia and Other Motile Organisms

This communication describes an experimental system which may be applicable to analyzing 'target-finding' by various motile organisms or cells. The system was devised to study influences on the capacity of the free-swimming miracidium of the human blood-fluke (*Schistosoma mansoni*) to locate and infect its usual intermediate host, an aquatic snail (*Biomphalaria glabrata*). Details of the findings will be published elsewhere[1].

The apparatus comprised a series of parallel channels, each 152.5 × 2.5 × 2.5 cm, levelled, partly filled with water, and arranged under appropriate conditions of light and temperature. Susceptible snails were confined as 'targets' in one end of each channel and counted miracidia were introduced in the opposite end. Preliminary experiments served to establish the number of miracidia and the interval of exposure required to produce infection in a high proportion (usually 90–100%) of the target animals. Other experiments disclosed that miracidial host-finding was not interfered with when pebbles, shells or other inanimate objects were interposed between the targets and the point of miracidial release. When, however, snails of the same species and size as the targets – and, of particular interest, when snails of several varieties refractory to infection – were interposed as 'decoys', the frequency of infection among targets was usually reduced to 0–10%. The findings in these and related experiments, while not wholly explicable at present, suggest that insusceptible snails may be involved covertly in the epidemiology of schistosomiasis.

The apparatus can be modified to accomodate various kinds of aquatic or terrestrial animals as decoys or targets; a miniature apparatus could accomodate cell or organ cultures as a target for motile micro-organisms or even gametes. Soluble or volatile substances, alone or incorporated into appropriate carriers, could be used to produce chemical gradients in mid-channel through which questing organisms must pass if they are to reach the target. Depending on circumstances it may be possible to observe the behavior of such organisms directly; failing this, some direct consequence of their behavior (infection of target snails in the case of miracidia) may serve as an indicator. The interference system can be thought of as a kind of biological 'black box' in that the specific mechanisms underlying the effects of interferents (decoy snails in this case) may not be understood.

The idea set forth is that of studying target-finding by interfering with it. The very simplicity of this notion suggests that it may not be novel; in this event, its previous description or application has escaped my notice[2].

Zusammenfassung. Die Infektionshäufigkeit durch Miracidien von *Schistosoma mansoni* in empfindlichen «Ziel»-Schnecken in gradlinigen Kanälen war stark vermindert, wenn empfindliche oder unempfindliche Schnecken als Köder zwischen «Ziel» und Eintrittsstelle der Miracidien eingesetzt wurden.

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[1] E. Chernin, J. Parasit., in press (1968).

[2] This investigation was supported in part by Research Grant No. AI-00513 and by a Research Career Award from the U.S. Public Health Service.