Technical Papers

Solution of Tobacco Mosaic Virus in the Aqueous Phase of a Chloroform-Water Emulsion and Application of this Phenomenon in Virus Assay

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Several workers (*1–3*) have found that proteins, including a bacterial nucleoprotein (*4*), after shaking in a chloroform-water emulsion, accumulate at the interface between chloroform and water. Experiments in this laboratory have shown that there is a relatively large accumulation of plant constituents at such an interface, but that tobacco mosaic virus (TMV), in contrast, remains dispersed in the aqueous phase. (Chlorophyll and certain other constituents dissolve in the excess chloroform.) The virus retains its infectivity after the treatment. Stream double refraction and electron microscopic tests[2] have shown that the typical rod-shaped structure is also retained. The dispersion of the virus in the aqueous phase after shaking provides a means of separating it from host proteins and has therefore been utilized as the basis of a method for virus assay.

Briefly, the assay procedure developed is as follows: Frozen interveinal leaf disks from Turkish tobacco plants (total weight *ca.* 0.35 g) are homogenized, after thawing, in an electrically driven homogenizer. The homogenate is transferred to a 10 ml glass-stoppered volumetric flask, and the homogenizer is rinsed with a 0.1 *M* phosphate buffer, pH 6, to give a final tissue weight to buffer volume ratio of 1:10. One ml of Squibbs USP chloroform and 0.5 ml *N*-amyl alcohol (an antifoam agent) are added. The sample is shaken vigorously for 15 min on a Boerner vibrating shaker, transferred without rinsing to a 12 ml conical centrifuge tube, and held at 1° C for 15 min. During this period the chloroform settles to the bottom, leaving an aqueous phase at the top. The sample is then centrifuged 5 min at about $1500 \times g$ on an International No. 2 centrifuge. Three definite layers are now present. A packed solid layer separates the lower chloroform and upper aqueous phases. The supernatant aqueous layer is poured along the clean portion of the tube wall to another centrifuge tube, and the centrifuging is continued 30 min longer. A 2 ml aliquot of the resulting supernatant is transferred to tubes for a small spinning-top air-driven ultracentrifuge. The solution is centrifuged 1 hr at about $79{,}000 \times g$, the supernatant is poured off, the tube is drained on paper towels, and the upper half of the inside wall is wiped with filter paper. One ml 0.1 *M* phosphate buffer (pH 7) is added to cover the resulting pellet, and the sample is stored overnight, corked, at 1° C. To resuspend the virus, samples processed as above are fastened on the platform of the Boerner shaker and are agitated 1 hr. The virus suspension is transferred to a Servall centrifuge tube, and the ultracentrifuge tube is rinsed with three more 1 ml aliquots of the pH 7 buffer. After twirling to ensure homogeneity, the sample is centrifuged on the Servall centrifuge at 10,000 rpm ($10{,}000 \times g$) for 15 min. Most of the supernatant is poured off, and its ultraviolet absorption (optical density) at 260 mμ is determined in a Beckman spectrophotometer. The optical density of this fraction (hereafter referred to as the assay fraction), minus the optical density of a comparable healthy preparation, is directly proportional to the concentration of TMV present.

Experiments were conducted to determine the best pH for the assay. When the method was carried out on homogenates from uninfected leaf disks in phosphate buffer at pH 6.5 to 7.0, consistently higher readings were obtained than when a buffer at pH 6.0 was used. The use of distilled water (resultant pH *ca.* 5.6) also gave low readings on samples of uninfected tissue, but reduced the amount of virus recovered when infected tissues were used.

To determine whether any virus was rendered insoluble in the chloroform emulsion step, a number of samples were prepared from a homogenate of infected leaf tissue. Samples were shaken for 2, 5, 10, 15, 45, and 75 min. The assay was completed, and the optical density (at 260 mμ) of the assay fraction determined. Homogenates from healthy tissue were also processed, and their readings were subtracted from the corresponding infected samples. If any virus was lost by shaking from 0 to 2 min, this should be detectable from the decreased readings obtained with those samples shaken longer. No decrease was detected; the optical density of samples shaken longer than 2 min was not significantly less than that for samples shaken 2 min.

Evidence was secured which indicated that most of the ultraviolet absorption in the assay fraction from infected plants is attributable to TMV, and not to host constituents. The ultraviolet absorption spectrum of the assay fraction from infected plants, taken at 5 mμ intervals in the range of 230–300 mμ is characteristic of that shown for purified TMV (*5*). Similar homogenates from uninfected leaf disks of the same weight (carried through the assay procedure) show very little absorption in the same range. For 20 samples of uninfected leaf disks that were assayed, the average optical density of the assay fraction (at 260 mμ) was only 0.011, with a maximum deviation of 0.011. As little as 0.03 mg of TMV/ml will absorb ten times this amount (*6*).

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[2] The preparation of the electron micrographs by A. Herbert Gold is gratefully acknowledged.

The optical density of a TMV suspension is proportional to its concentration (*6*). To determine whether the optical density of the assay fraction, minus that of the healthy control, is proportional to the concentration of virus in the homogenate, dilutions of homogenates from infected tissue were prepared by adding various volumes of homogenates from uninfected tissue, and the assay was completed (Fig. 1).

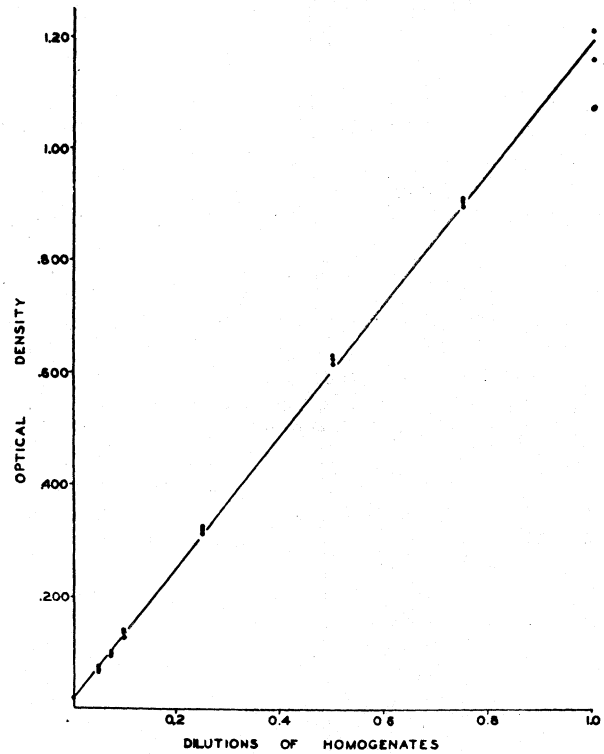

FIG. 1. Linear relationship between known dilutions of TMV in homogenates and the optical density obtained (circles) by use of the chloroform emulsion method.

It was found that a straight line could be drawn which falls very close to the mean of each dilution, including the mean of the control. The average deviation of all samples from this straight line is 4.5%, with a maximum mean deviation of 8.1% for any dilution. It can be seen that in the twentyfold dilution range tested (Fig. 1), the optical density (at 260 mμ) of the assay fraction of each dilution, minus that of the healthy check, is inversely proportional to the dilution of the homogenate, or directly proportional to the virus concentration in the homogenate.

To estimate percentage recovery, 0.5 ml aliquots of various dilutions of purified virus having a known optical density were added to incompletely homogenized healthy tissue. The homogenizing was completed, and the samples were assayed. For three experiments, the average recovery was 89%, with a maximum mean deviation of 9% for any dilution. Within a single experiment, the average recovery for any virus dilution did not differ from that of any other dilution by more than 4%. Takahashi has shown that not all the virus present in the tissue is extracted by homogenizing, and that additional virus may be recovered from the residue of the first slow-speed centrifugation (*7*). Therefore, the percentage of virus recovered by this assay method is based on the free virus, and not on the total amount in the infected tissue.

The results obtained show that the chloroform emulsion technique provides a rapid and relatively accurate assay method for TMV. Even with young leaf tissue, where it is difficult to separate virus from chlorophyll, the chloroform method is very effective. It is possible that the technique may also be useful in the purification or assay of animal viruses or other plant viruses.

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The Disruption of Mitochondria of Saccharomyces by Ultraviolet Irradiation[1]

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Coagulation of cellular protein has been the principal cytoplasmic change attributed to ultraviolet irradiation (*1, 2*). We report here a characteristic disruption of mitochondria which becomes microscopically detectable prior to the onset of cytoplasmic precipitation. The effect is first observed with ultraviolet doses sufficient to reduce irradiated yeast suspensions to approximately 1% survival, and becomes more pronounced as dosage increases.

For this study we have employed a procedure which consistently leads to optimal production of mitochondria in baker's yeast. Actively growing cells were harvested, washed once with distilled water, and transferred to a solution of 3% monopotassium phosphate and 3% dextrose, the final dry weight of yeast being about 1%. Two hundred and fifty ml of this suspension was shaken in a 500 ml flask, 8–12 hr at 30° C, until the sugar was completely exhausted. The yeast cells from such a preparation, with their large mitochondria, are shown in Fig. 1. In general, an inverse relation exists between the size of the mitochondria and the metabolic activity of the cell (*8*). Large mitochondria may be considered to consist of an enzyme protein core essentially isolated from the cytoplasm by a lipoid cortex (*9*). As cellular activity declines, mitochondria increase in size.

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