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[1] H. P. KLEIN AND Z. K. BOOHER, *Proc. Soc. Exptl. Biol. Med.*, 89 (1955) 43.
[2] H. P. KLEIN AND Z. K. BOOHER, *Bacteriol. Proc.*, (1955) 136.
[3] H. P. KLEIN, *J. Bacteriol.*, 69 (1955) 620.

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Morphological changes accompanying thermal denaturation of tobacco mosaic virus

The inactivation of tobacco mosaic virus (TMV) at high temperature was described in the last century by MAYER[1], who noted that the sap of mosaic-diseased plants became non-infectious after exposure to temperatures of 80° or more. Later, with purified preparations of the virus, the thermal inactivation was found to be accompanied by denaturation and precipitation of the nucleoprotein[2]. However, when the virus is heated in salt solution at 100°, the nucleoprotein complex dissociates and the protein precipitates, leaving the ribonucleic acid (RNA) in suspension[3]. The mechanism of this dissociation is somewhat difficult to understand in view of the recent observation that the RNA lies within a tube of protein in the rod-shaped virus particles[4]. In the present note, the effects of heating TMV are described as seen in electron micrographs and from these observations a mechanism is proposed for the thermal release of virus RNA in salt solution.

The method of treatment was as follows: A suspension containing the purified TMV at a concentration of about 0.5 mg/ml was drawn into the lower part of a thin-walled glass capillary tube bent into the shape of a U. The tube was dipped into a hot-water bath for a timed interval, and then removed and emptied into a volume of distilled water sufficient to dilute the tube contents about 30-fold. Immediately after this dilution the material was sprayed upon electron-microscope grids and shadowed with uranium.

In Fig. 1 are shown the results of heating TMV in pyrex-distilled water. The same morphological changes occur over the entire temperature range from 80° to 98° (though the rate at which they occur is strongly dependent on temperature). The first visible change is a swelling at one or both ends of the rod. As heating continues the swelling takes the form of a terminal ball which increases in diameter as the attached rod becomes shorter. Finally the rod is completely

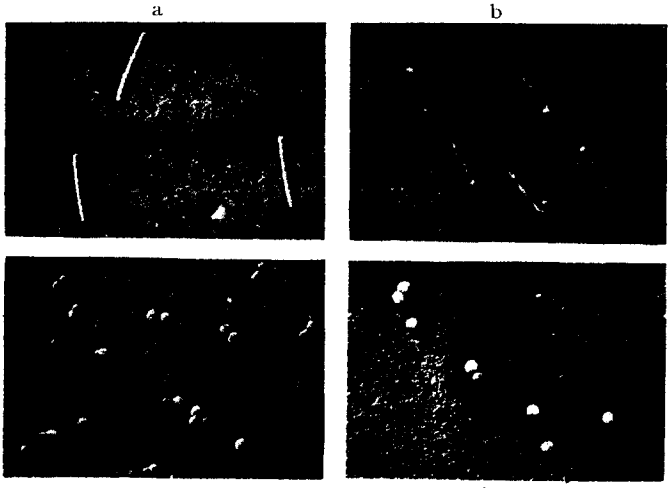

Fig. 1. The effect of heating tobacco mosaic virus in distilled water. Although different temperatures were used for the samples shown here, the entire conversion of virus rods to balls occurs at any of the temperatures used. These representative fields were chosen merely to illustrate stages in the conversion. The larger balls shown in d have approximately the same volume as the original virus rods. × 50,000. (a) untreated; (b) 15 seconds at 85°; (c) 10 seconds at 90°; (d) 10 seconds at 98°.

transformed and in its place is a ball* having the approximate volume of the original virus rod.

From a comparison of the results of a number of heat treatments in pyrex-distilled water, the time rates of such morphological changes at 80°, 85°, 87.5°, 90° have been found to be in the approximate ratios 1:30:120:300. These data are arranged in an Arrhenius plot in Fig. 2. The line of best fit, as determined by the method of least squares, has a slope of 0.58/degree, indicating an activation energy of 150,000 calories/mole for the reaction (conversion of rods to balls) at 85°. This value agrees with that found in a macroscopic study[6] of the denaturation of TMV.

When the virus was heated at 100° in a 0.1 *M* solution of ammonium acetate, the bulk of the material was apparently precipitated, since the spray droplets of such preparations were seen to contain only occasional rods and balls in addition to a fibrous material resembling RNA. Because of the rapidity of precipitation under these conditions, it would be difficult to study in detail the morphological changes of the individual particles. The presence of a few unprecipitated balls may indicate, however, that the thermal precipitation in salt solution is preceded or accompanied by morphological changes similar to those which take place in distilled water.

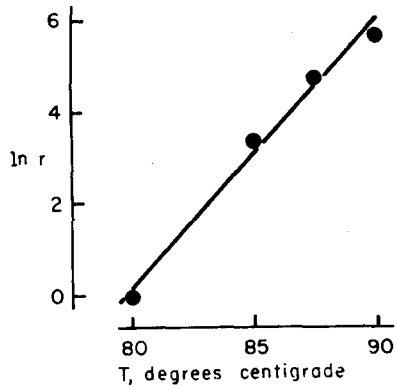

Fig. 2. Natural logarithm of the relative reaction rate, *r*, plotted against temperature, *T*, for the thermal denaturation of tobacco mosaic virus in distilled water. The points were estimated by comparison of a number of micrographs similar to those shown in Fig. 1. The straight line was determine by the method of least squares and has a slope of 0.58/degree.

From the above observations, a mechanism may be postulated for the thermal release of NRA in salt solution. The chemical evidence to date[7] indicates that the RNA is bound to the virus protein by a salt linkage. Although the protein as a whole is predominantly acidic, a salt linkage is easily conceivable if the protein tube has a sufficient number of basic groups so arranged along its interior as to engage the RNA phosphate groups. The disruption occurring in thermal denaturation would destroy the original arrangement of charged groups and could lead to an electrostatic repulsion between the ball of disrupted protein and the RNA. As more and more of the protein tube collapses and "rounds up" under the action of interfacial tension, the RNA would tend to be expressed from the un-collapsed end of the tube (which is presumably "open"). The requirement that the medium contain salt[3] can be explained by postulating that salt ions in the medium would permit the RNA fibers to "slip" more easily past the positively charged groups in the intact portions of the protein tube. In distilled water the RNA would remain locked in place while the entire tube collapses, and thus would be trapped in the protein precipitate.

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[1] A. MAYER, *Landwirtsch. Vers. Sta.*, 32 (1886) 451.
[2] W. M. STANLEY, *Science*, 81 (1935) 644.
[3] F. C. BAWDEN AND N. W. PIRIE, *Proc. Roy. Soc. (London), B* 123 (1937) 274; W. M. STANLEY AND H. S. LORING, *Cold Spring Harbor Symposia Quant. Biol.*, 6 (1938) 341.
[4] R. G. HART, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 261.
[5] R. C. WILLIAMS, *Advances in Virus Research*, 2 (1954).
[6] M. A. LAUFFER AND W. C. PRICE, *J. Biol. Chem.*, 133 (1940) 1.
[7] C. A. KNIGHT, *Advances in Virus Research*, 2 (1954).

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* The circular outline of such particles as projected in the micrographs would suggest a spherical shape, but their shadows indicate that the true shape when dried is more like an oblate ellipsoid of revolution with its minor axis perpendicular to the grid surface and about half as long as the major axes. The latter shape was assumed in calculating the volume. However, it is quite possible that the balls are approximately spherical in water, since there is a well-known tendency for small, non-rigid objects in spray droplets to become flattened as the droplets dry[5].

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