aortic mechanisms. Assuming the mechanism to be reflex in nature, the site of origin of the impulses causing digitalis vomiting is still undetermined. The possibility remains that afferent impulses may be set up in a structure or structures not yet eliminated. It is perhaps more probable that impulses may arise from any one of several structures all of which would have to be denervated in the same animal to abolish the emetic response to digitalis.

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The Electron Micrography of Plant Virus-Antibody Mixtures.

L. M. BLACK,* W. C. PRICE,† AND RALPH W. G. WYCKOFF.‡

From the National Institute of Health, Bethesda, Md., and the Department of Animal and Plant Pathology, the Rockefeller Institute for Medical Research, Princeton, N.J.

Electron microscopy is a promising new way of studying the interaction between an antigen and its specific antibody. Such a study is of practical importance because the minute amounts of required material make possible the development of unusually sensitive tests for antigen and antibody. From a more fundamental standpoint the ability of the microscope to record particles comparable in size to antibody molecules may provide a way of checking directly the several hypotheses that have been developed to explain the nature and specificity of the immune reaction.

Very few papers dealing with the electron microscopy of antigen-antibody mixtures have thus far been published. In two[1,2] the antigens are bacteria; another[3] describes the apparent swelling of tobacco mosaic virus particles when mixed with antibody. The published pictures of sensitized typhoid and paratyphoid bacilli give clear evidence of the adsorption of antibody onto both the cell walls and the flagella. In the tobacco mosaic-serum photographs isolated fibers show the same kind of thickening as these bacterial flagella. It was suggested[3] this broadening of the tobacco mosaic particles, from a normal 150A to *ca.* 600A, was due to each being coated with a single layer of antibody molecules arranged with their long axes at right angles to the axis of the virus particle.

The visibility of viruses[4] and other objects of macromolecular dimensions[5] is improved by the oblique evaporation upon the preparation of a very thin metallic film.[6] We are using this "shadow" technic to see what new information it will give concerning the reaction between certain plant viruses and sera prepared against them. Preliminary photographs are reproduced in this note.

Antigens used were tomato bushy stunt and southern bean mosaic viruses purified by means of a preliminary clarification with $(NH_4)_2SO_4$ followed by fractionation in a high speed centrifuge. Antisera for the bushy stunt virus and for the bean virus[7] were prepared by intraperitoneal injection of rabbits with puri-

* Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

† Department of Biology, University of Pittsburgh, Pittsburgh, Pa.

‡ National Institute of Health, Bethesda, Maryland.

1 Mudd, S., Heinmets, F., and Anderson, T. F., *J. Exp. Med.*, 1943, **78**, 327.

2 Mudd, S., and Anderson, T. F., *J. Immunology*, 1941, **42**, 251.

3 Anderson, T. F., and Stanley, W. M., *J. Biol. Chem.*, 1941, **139**, 339.

4 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265; *Science*, 1945, **101**, 594; Price, W. C., Williams, R. C., and Wyckoff, R. W. G., *Science*, 1945, **102**, 277.

5 Williams, R. C., and Wyckoff, R. W. G., *Nature*, 1945, **156**, 68.

6 Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1944, **15**, 712; 1946, **17**, No. 1.

7 Price, W. C., and Black, L. M., *Phytopathology*, in press.

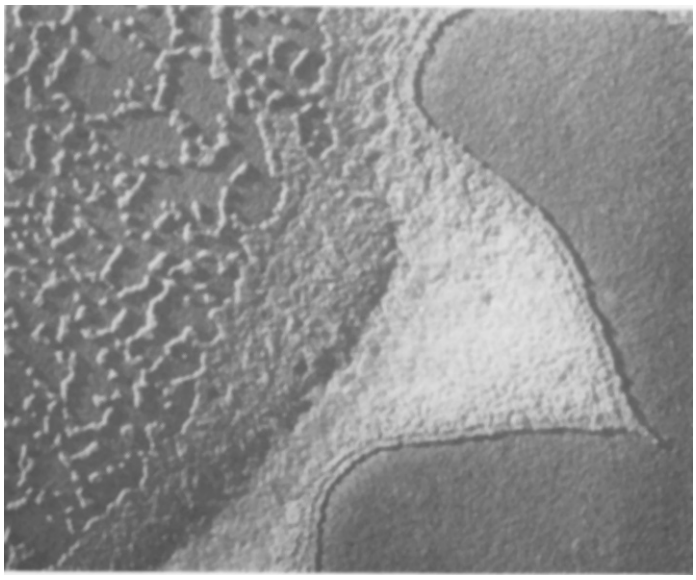

FIG. 1.
An electron micrograph of a field in a preparation of purified southern bean mosaic virus. At the top of the picture the bean virus particles, singly and in groups, are widely separated from one another on the substrate. In the middle they are more closely packed into a nearly continuous layer. At the bottom these layers of particles are stacked on top of one another to give the beginnings of a crystalline array. Magnification 16,800 ×.

FIG. 2.
An electron micrograph of a field in a preparation consisting of a mixture of purified southern bean mosaic virus with its antiserum. The individual particles in the floc do not have the same degree of regularity in arrangement that is evident in Fig. 1. Magnification 16,800 ×.

fied virus suspensions. The anti-bushy stunt serum was originally made for another set of experiments for which it was important that the serum be freed from traces of tobacco mosaic antiserum that might be present. To insure this it has been absorbed with purified tobacco mosaic virus, the excess of which provides the rod-forms seen in Fig. 3.

Preparations for electron microscopy were made in the following way. Purified virus containing *ca.* one milligram per cc was mixed with an equal volume of undiluted serum and the mixture immediately diluted with from 10 to 100 volumes of distilled water. This diluted serum-virus mixture was examined immediately and after varying intervals up to 24 hours. Time seemed to have little effect on the character of the electron photograph obtained. The concentrated serum-virus mixture soon became turbid with the eventual precipitation of a part of its contents but the more dilute mixtures remained clear through-

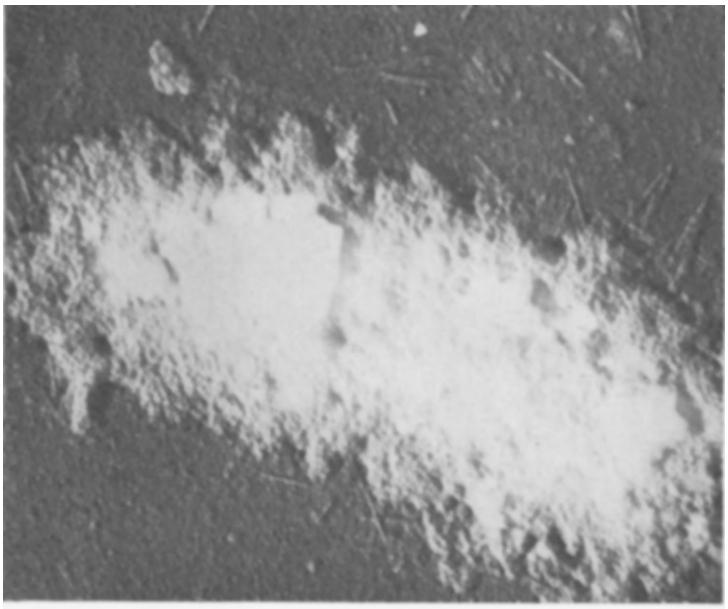

FIG. 3.
An electron micrograph of a similar preparation consisting of a mixture of purified bushy stunt virus with antiserum. Magnification 20,500 ×.

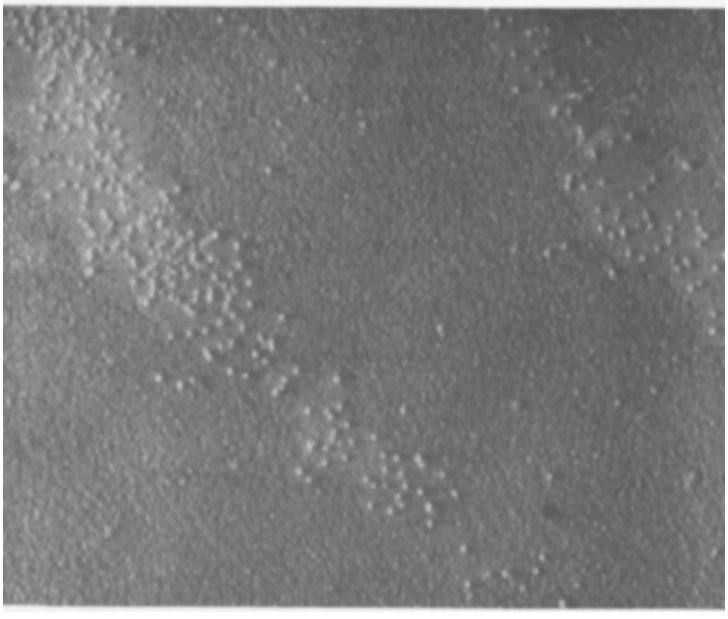

FIG. 4.
An electron micrograph of a field of a preparation consisting of a mixture of bushy stunt virus with normal rabbit serum. Magnification 20,500 ×.

out the period of observation. Micro-drops of the diluted mixtures were placed on the usual collodion-covered screens, left several minutes and then withdrawn as completely as possible. After being allowed to dry in the air the screens containing the less dilute mixtures were washed several times by the addition and subsequent withdrawal of droplets of distilled water. Final preparations were shadowed as usual in the vacuum chamber of a metal-evaporating unit.§ Gold in an aver-

§ We wish to express our gratitude to Perry C. Smith and R. G. Picard of the Radio Corporation of America, Camden, N.J., for the use of evaporating equipment.

age thickness of *ca.* 8A was laid down, the angle of evaporation being such that an object on the preparation would cast a shadow five times its height.

Two sorts of preparations were made and examined as controls for the foregoing virus-antibody mixtures. One consisted of mixtures containing normal in place of immune serum, the other was of serum without admixed virus. These were shadowed in the usual fashion before examination.

Both viruses have elementary bodies that are essentially spherical; in pure suspension and at sufficient concentrations they show a pronounced tendency to associate in regular crystal-like arrays.[8] When mixed with specific antiserum, however, these particles appear aggregated without the same regularity into microclumps. The character of this micro-agglutination will be clear from a comparison of Fig. 1 and 2. Fig. 1 is a typical field from a purified preparation of the southern bean mosaic virus; Fig. 2 shows a mixture of this virus with its antibody. Similar microflocs are produced when the bushy stunt virus and its antiserum are mixed (Fig. 3). Micro-flocculation has also been observed with the tobacco mosaic virus protein and with other antigens when mixed with their anti-substances. Similar agglutinations were not seen when normal rabbit serum replaced specific serum in the mixtures. For example, in Fig. 4, which is taken of a mixture of bushy stunt virus and normal serum, virus particles are separate from one another in their enmeshing serum. The amount of virus in single microflocs on preparations such as those giving Fig. 2 and 3 often is no more than *ca.* 10^{-12} g.

In these photographs the background structure due to the collodion is often less sharply defined than usual. This apparent smoothing-over of detail in the substrate is probably due to an irreversible deposition of serum proteins over the entire surface of the preparation.

It is not so simple as might at first be imagined to measure accurately the apparent increase in particle size of the virus in these mixtures. The agglutinated particles of Fig. 2 and 3 are close to one another but their outlines are often diffuse and the irregularity of their vertical stacking prevents many determinations of particle-separation. Best results are obtained by seeking places where several particles are lined up in rows. Preliminary measurements on such rows indicate that the apparent separation of both agglutinated bean mosaic and bushy stunt virus particles is about twice that found in pure suspensions. More accurate studies are being made of mixtures containing different proportions of antigen in order to obtain more precise data.

Summary. Shadowed electron micrographs of mixtures of purified southern bean mosaic and bushy stunt viruses with their specific antisera show their spherical elementary bodies aggregated into microflocs in which the particle-separations seem to be about twice their normal values. More detailed electron micrographic study of these systems is underway.

[8] Price, W. C., Williams, R. C., and Wyckoff, R. W. G., *op. cit.; Arch. Biochem.*, in press.