The virus, after a subsequent passage in mice, was inoculated onto the cornea of a young adult rabbit. This rabbit developed a kerrato-conjunctivitis typical of Herpes infection.§ After a period of 8 days this rabbit developed a fulminating encephalitis and died. Pathological study of the brain revealed findings typical of Herpetic encephalitis.

In another rabbit, No. 548, following the precipitation of active encephalomyelitis from the latent state by anaphylactic shock 120 days after inoculation of Herpes simplex virus into the quadriceps femoris muscle group, a virus agent was again recovered from the central nervous tissues. The technics of recovery of the virus and its identification by biological characteristics were the same as were used in Rabbit No. 370. The virus after 3 mouse passages was identical in all respects to the virus inoculated 4 months previously.

Summary. 1. An infectious agent was recovered from the central nervous system of a rabbit which had been inoculated 9 months previously with Herpes simplex virus and which had suffered 3 acute relapses of the disease. The virus recovered exhibited characteristics identical with those of the original virus. 2. A second instance of virus recovery 4 months after inoculation is reported.

§ Corneal scrapings were obtained from the infected eye three days after inoculation and showed typical intranuclear inclusion bodies in the epithelial cells.

15794

Artifacts in Gold Shadowed Electron Micrographs Due to Electrons of High Intensity.

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The electron microscope has been used widely for the examination of virus preparations, for the sizes of many viruses are below the range in which the light microscope can be used to good advantage.[1] However, because of the low contrast which is afforded by small viruses, difficulties in securing good electron micrographs have been encountered. Electron microscope studies on small viruses were aided immensely by the development of the metal shadow-casting technic by Williams and Wyckoff.[2,3] This technic has also proved of value in securing good definition of virus particles in the presence of a background of material of smaller particle size. By means of this technic Sigurgeirsson and Stanley[4] and Oster and Stanley[5] have secured good delineation of the particles of tobacco mosaic virus in the unpurified juice and contents of hair cells, respectively, of plants diseased with tobacco mosaic. Although there was no difficulty in these studies in showing that most of the rods present in fresh virus preparations possessed a length of 280 mμ, occasional pictures were obtained which showed segmentation of the virus rods similar to that described by Williams and Wyckoff.[6] Further study has shown that this segmentation into shorter pieces of variable

1 Stanley, W. M., *Chronica Botanica*, 1943, **7**, 291.

2 Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1944, **15**, 712.

3 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.

4 Sigurgeirsson, T., and Stanley, W. M., *Phytopathology*, 1947, **37**, 26.

5 Oster, G., and Stanley, W. M., *Brit. J. Exp. Path.*, 1946, **27**, 261.

6 Williams, R. C., and Wyckoff, R. W. G., *Science*, 1945, **101**, 594.

lengths is due to an artifact. This artifact and its controlled formation are described in the present paper.

Methods. Tobacco mosaic virus from infected Turkish tobacco plants, isolated and purified by differential centrifugation,[7] was prepared in a suitable dilution and placed on the copper screens covered with a thin film of collodion in the normal fashion of preparing specimens for use in the electron microscope. The screens were then subjected to the oblique deposition of a very thin evaporated metal film after the manner described by Williams and Wyckoff. The apparatus used in this laboratory consists of a glass chamber mounted on a polished brass plate in which there are 4 supports for the 1×3 glass slides which carry the mounts. The chamber is evacuated to less than 10^{-5} mm. of mercury. The gold *used* for the shadow casting was deposited from a hot tungsten filament 20 cm. above and at an angle of 15° to the glass slides. The amount of gold deposited was controlled by observing the color of the light transmitted through the slide and comparing it to a sheltered "standard" slide placed within the chamber. Since the slides were arranged with their long axis toward the gold source, a gradation of gold was obtainable on each slide so that it was possible to select the screen with the desired amount of deposited gold.

An RCA Console Model (type EMC-1) electron microscope yielding a magnification of 5800 diameters was used for the observation of the specimens. In order to obtain the initial electron micrographs the minimum intensity that would still allow accurate focusing of the image on the fluorescent screen was used. After a suitable field had been procured, a micrograph was taken with as little delay as possible. The exact field was maintained and the intensity was raised to the maximum. Following the exposure of the specimen to the high intensity electron beam for such time as was necessary to secure the desired effect, usually about 5 minutes, a micrograph was again taken. In this manner it became possible to compare images of identical virus particles following exposure to low and to high intensity electron beams.

Discussion of Results. Fig. 1 shows the virus particles as they appear in a micrograph taken at low intensity. The background is uniform and the particles appear well defined. Fig. 2 shows the same particles after they had been subjected to the high intensity beam for 3 minutes. The background has become very granular and the virus particles appear segmented. This segmentation appears to be similar to that described by Williams and Wyckoff.[6]

Fig. 3 and 4 show another set of micrographs treated in a similar manner except that Fig. 4 was taken after 10 minutes of intense irradiation. It can be seen that, although the background has become quite granular, the degree of segmentation appears to be considerably less than in Fig. 2. Continued irradiation did not produce the extreme segmentation noted in Fig. 1. This may have been due to a difference in the thickness of the gold deposited on the surface of the particles. It can also be seen from a comparison of Fig. 3 and 4 that there are many particles missing or obscured in Fig. 4. The letters correspond to the same particle or group in each figure. There appears to be some correlation between the direction of the deposition of the gold in relation to the orientation of the particle and the obscuring of the particle.

Fig. 5 shows an extreme effect which can result from prolonged exposure at the maximum intensity. Some of the particles appear segmented and others show only a single very dense circular area. It seems likely that on prolonged exposure to a high intensity electron beam the gold becomes fluid and forms globules on the surface of the virus particle and on the collodion membrane. Since the gold globules may coalesce to different sizes, the resulting appearance may then vary from the smallest ones, giving the effect of segmentation of the particle, to the larger globules which are shown in Fig. 5. The fine beading or segmentation shown in Fig. 2 could easily be mistaken as evidence for the existence of small segments of the tobacco

[7] Stanley, W. M., *J. Biol. Chem.*, 1940, **135**, 437.

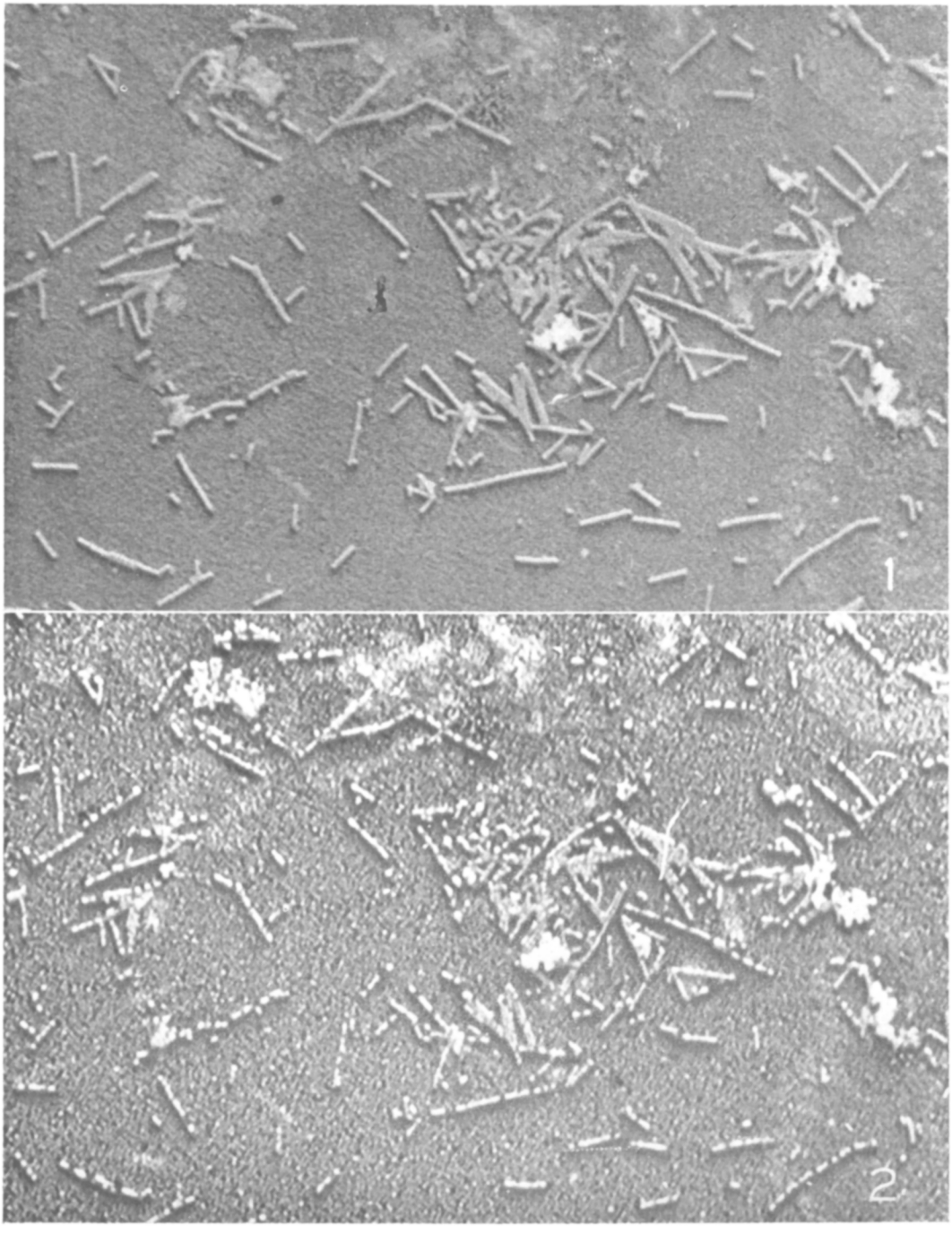

FIG. 1. Electron micrograph taken at low intensity of tobacco mosaic virus prepared with gold by the shadow-casting technic.

FIG. 2. The same specimen as that shown in Fig. 1 following exposure to a high intensity electron beam for 3 minutes. Many of the virus rods appear segmented.

mosaic virus rod. However it is obvious from Fig. 1 that the appearance of segmentation in Fig. 2 is an artifact resulting from exposure to the electron beam. It seems likely

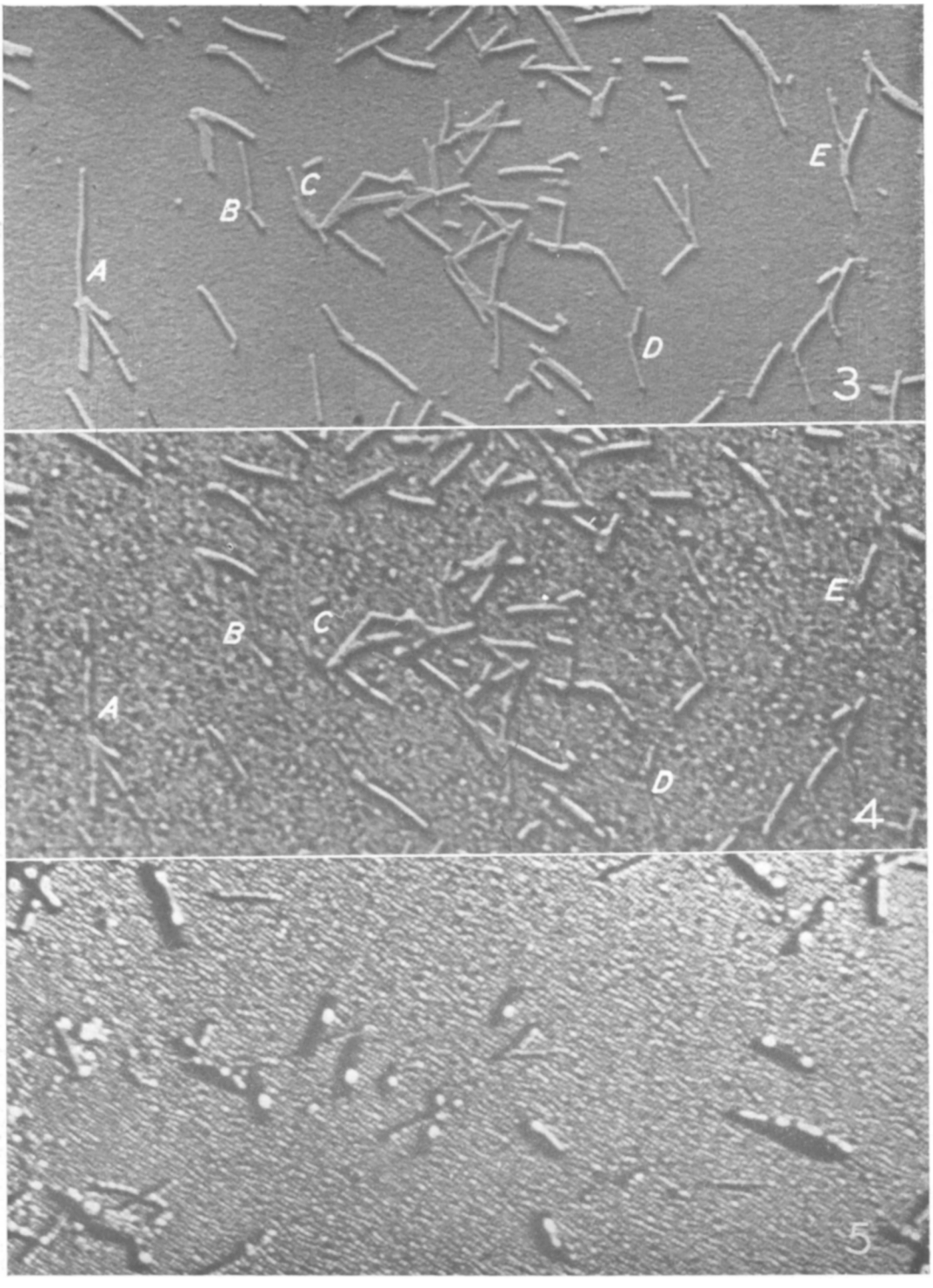

FIG. 3. Electron micrograph taken at low intensity of tobacco mosaic virus prepared with gold by the shadow-casting technic. Letters indicate identical rods in Fig. 4.

FIG. 4. The same specimen as that shown in Fig. 3 following exposure to a high intensity electron beam for 10 minutes. The exposure has caused the background to become granular and several of the particles are missing or not well defined.

FIG. 5. Tobacco mosaic virus prepared with gold by the shadow-casting technic after prolonged exposure to an electron beam at high intensity. The picture shows an extreme case of segmentation or bead formation.

that the segmentation of tobacco mosaic virus described by Williams and Wyckoff[6] was caused in a similar manner.

An effort was made to ascertain whether or not segmentation could be produced in tobacco mosaic virus preparations before shadow casting with gold or in preparations stained with phosphotungstic acid. No evidence for segmentation following prolonged exposure to a high intensity electron beam was obtained in either case. The results confirm the statement by Stanley and Anderson[8] for unshadowed material that "The fact that a micrograph taken with the first flow of electrons through a specimen does not appear to differ from subsequent micrographs taken after longer exposure to the electron beam makes it seem unlikely that gross changes are caused by the electrons."

The results of the present study indicate that in order to reduce or eliminate granulation and other obvious artifacts, which can occur when the shadow-casting technic with gold is used, it is necessary to avoid prolonged exposure of the specimen to the intense electron beam.

Summary. Electron micrographs of gold shadow-cast specimens of tobacco mosaic virus show that artifacts, consisting of segmented or even globular particles, are formed when the specimen is exposed to the beam of electrons at high intensity. It is suggested that these are caused by the thin film of gold becoming fluid and forming globules on the surface of the virus rods and supporting membrane. Tobacco mosaic virus not treated with gold did not show any change after having been subjected to the same treatment. The results indicate that gold covered specimens should not be subjected to prolonged exposure to an electron beam of high intensity.

It is a pleasure to acknowledge my indebtedness to Dr. W. M. Stanley for his active interest and helpful guidance.

8 Stanley, W. M., and Anderson, T. F., *J. Biol. Chem.*, 1941, **139**, 325.

15795

Hypersensitivity to Egg White in the Rat.*

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Injection of egg white into previously sensitized rats elicits symptoms which have been taken as proof of an anaphylactic reaction.[1,2] Among these symptoms are hyperemia, cyanosis and edema. One author[1] mentioned that egg white has no primary toxicity. On the other hand it has been reported[3] that egg white administered parenterally is toxic and that sensitized and unsensitized rats reacted with almost identical symptoms. Among the symptoms there is a marked edema of the face, tongue, clitoris and paws preceded by hyperemia.[4] It seems therefore that the rat is naturally hypersensitive to egg white and that the symptoms attributed to anaphylaxis may be the result of this hypersensitivity. Since hypersensitivity is very seldom observed in animals from the first

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