while 2-methyl-3-methoxy-1,4-naphthoquinone with the $-OCH_3$ group in that position is not, lends support to the hypothesis that the 3 position on the quinone ring is important in the inhibition of bacterial growth by these compounds. The antibacterial activity of the methoxy quinone, however, even in the presence of sulfhydryl compounds, suggests that the foregoing may be only one of the mechanisms involved.

Details of these studies will be reported in a subsequent communication.

SUMMARY

Thioglycolic acid neutralized with sodium carbonate, sodium thioglycolate (Eastman), ethyl mercaptan, cysteine hydrochloride and certain sulfur-containing reducing agents (sodium bisulfite and sodium hydrosulfite) antagonize the antibacterial action of 2-methyl-1,4-naphthoquinone on *Escherichia coli* in a synthetic medium. Other reducing agents such as stannous chloride, potassium formate and sodium thiosulfate, show no such antagonism. The antibacterial activities of 2-methyl-3-chloro-1,4-naphthoquinone and 2,6-dimethyl-1,4-naphthoquinone are also abolished by excess thioglycolate and cysteine, while that of 2-methyl-3-methoxy-1,4-naphthoquinone with $-OCH_3$ instead of –Cl or –H in the 3 position on the quinone ring, is not. These findings suggest that the mode of antibacterial action of 2-methyl-1,4-naphthoquinone is by blocking essential enzymes through combination with sulfhydryl groups, or through combination with sulfhydryl groups of essential bacterial metabolites. This combination may take place in the 3-position on the quinone ring. This mode of action is similar to that suggested by other investigators for several antibiotic agents including penicillin. The antibacterial activity of the methoxy quinone, however, even in the presence of sulfhydryl groups, suggests that the foregoing explanation may not be the complete one.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ELECTRON SHADOW MICROGRAPHY OF THE TOBACCO MOSAIC VIRUS PROTEIN[1,2]

WE have recently shown[3] that electron micrographs of tobacco mosaic and influenza viruses shadowed by the oblique deposition of a very thin evaporated metal film reveal many details that have not otherwise been seen. For this earlier work the evaporated film was chromium in a calculated thickness of *ca.* 80A. This procedure is satisfactory as long as one is concerned with objects like bacteria[4] and most viruses that are large compared with the thickness of the metallic layer deposited on them during shadowing. In attempts to detect very small particles it has been found that they may be recorded, though smaller in diameter than the thickness of chromium they accumulate when shadowed. Though this does not in itself interfere with their detection, it gives an obviously false impression of shape and renders difficult and inaccurate any measurements of their true size. We have accordingly sought a metal of higher electronic scattering power than chromium which would give continuous films upon evaporation and could therefore be used in much thinner layers for shadowing. Gold in a calculated thickness of 5 to 10A meets these requirements. It replaces chromium to great advantage in the photography of the smallest viruses and of other particles of macromolecular size.

An electron micrograph, made with an RCA Type EMB instrument, of a gold-shadowed tobacco mosaic virus preparation[5] is shown in Fig. 1. Compared with our previously published picture of this substance[3] there is a clear improvement in the delineation of the tobacco mosaic fibrils, though it should be emphasized that when allowance is made for the different thicknesses of the coating metals the same general idea of fibril-shape emerges from a consideration of each photograph. There is also a reduction in the texture of the background detail which has its origin in the ultimate structure of the collodion substrate. This reduction is important for work with other macromolecules because such molecules can be studied on collodion substrates only as long as a discrimination can be made on the photographs between the macromolecules and the structural details of collodion.

Since, as the figure indicates, collodion has an ultimate structure approaching in dimensions the width of the tobacco mosaic virus molecule and since far smaller particles can be recognized through shadow-casting, it has been important to find and utilize a

[1] From the Department of Physics and the Virus Laboratory, Department of Epidemiology, School of Public Health, University of Michigan.

[2] Supported in part by a grant from the National Foundation for Infantile Paralysis, Inc.

[3] R. C. Williams and R. W. G. Wyckoff, *Proc. Soc. Exp. Biol. and Med.*, 58: 265, 1945.

[4] R. C. Williams and R. W. G. Wyckoff. In press.

[5] We are indebted to W. M. Stanley for the purified tobacco mosaic virus protein used in this work.

smoother substrate. Polished glass has such a smoothness, and we have found that replica preparations made in the following fashion reproduce particles of macromolecular dimensions spread upon its surface. To make these replicas of purified tobacco mosaic protein, a drop of an appropriate dilution in distilled water is first placed on a chemically cleaned microscope slide. This slide, when thoroughly dry, is placed in the vacuum chamber of the evaporating outfit and coated with a calculated average thickness of *ca.* 8A of gold, the angle of deposition being such that the lengths of the shadows are several times the heights of the objects causing them. On removal from the vacuum chamber the regions of the metal-coated slide intended for study are immediately covered with a thin (*ca.* 500A) layer of collodion. As soon as it is dry, this film and its adhering gold is stripped from the glass onto the customary wire specimen screen with the help of "Scotch tape," following procedures commonly employed in handling replicas for metallographic examination. Because of the predominant scattering power of the gold, it makes little difference whether the small organic particles actually leave the glass when the replica is stripped. The collodion film now shows no structure when examined in the microscope because its fine structure has not been brought out by the shadow-cast gold. Fig. 2 is a photograph of such a stripped gold replica of tobacco mosaic fibrils lying on a glass surface.

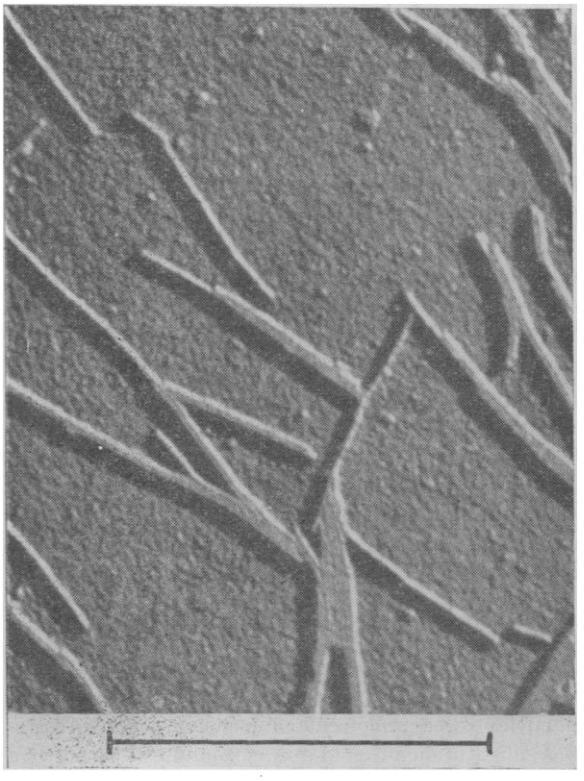

FIG. 1. An electron micrograph of fibrils of the tobacco mosaic virus protein on collodion and shadowed with *ca.* 8A of gold. The lines drawn at the bottom of this and the next figure are each one micron long. This magnification has been established by the photography of an all-metal replica of a 15,000 line per inch diffraction grating. These two figures are negatives; they have been prepared for reproduction by making contact positives from the original negatives on medium lantern-slide plates and printing from them onto No. 3 Bromide paper.

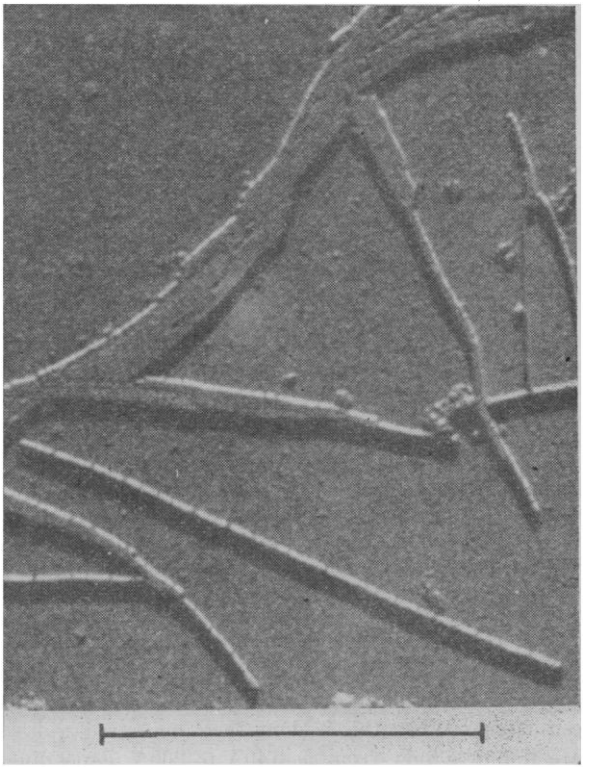

FIG. 2. An electron micrograph of a gold replica of fibrils of the tobacco mosaic virus protein deposited on glass. The same details of fibril structure appear on the two photographs, but the greater background smoothness of the glass surface is apparent.

A careful comparison of these two photographs and of others like them has demonstrated that there is no detectable difference in appearance or dimensions between the tobacco mosaic fibrils seen in the replicas or shadowed directly on collodion. The fine details of structure of the tobacco mosaic rods, as exemplified by their parallel groupings and by the segmentation of their separate elements, appear at least as well in the replica as in Fig. 1. This apparent faithfulness of the replica and the far greater smoothness of its background make certain that it can be used to reveal macromolecules much smaller than those of the tobacco mosaic protein.

Photographs such as those reproduced above pro-

vide a measure of both the breadths and the heights of individual tobacco mosaic fibrils. Since the separate rods are easily distinguished from one another as they lie parallel and closely packed in bundles, they can be counted and their total width measured. This can be done with accuracy by photometering across the parallel clusters of fibrils that appear in both figures. Such a photometric traverse is given in Fig. 3. Disregarding small irregularities intro-

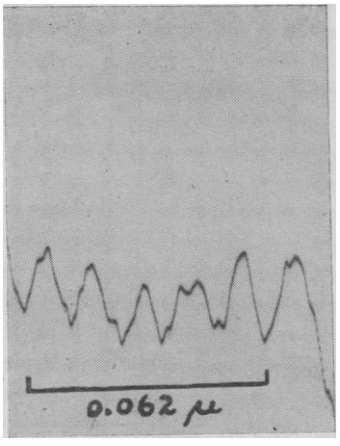

FIG. 3. A microphotometric record of a traverse normal to the length of a group of five parallel fibrils. As the appended scale of magnification indicates, this corresponds to a linear magnification of about half a million.

duced by photographic grain at this high magnification, it is seen that the fibres have a uniform width of 125A. The breadth of isolated fibrils can also be estimated. When this is done their measured widths are found to vary from *ca.* 140A to 200A, depending on their orientation with respect to the direction of shadowing. As would be expected, the apparent breadth is greatest for those that lie about normal to the direction of metal evaporation. Rods that lie parallel to this direction cast no lateral shadows and are so poorly visible that their widths can not be measured accurately; but upon plotting apparent widths as a function of angle of deposition for the other rods, it is found that the unshadowed fibrils must have a breadth close to *ca.* 120A. Hence the width of a fibril is substantially the same, whether it is lying alone or as a member of an oriented bundle. This has importance in connection with problems involving the changes in shape and size that these and other macromolecules may suffer during desiccation. The breadths we have measured above are comparable with previous estimates of 150A based on direct electron micrography.[6]

The height of a tobacco mosaic fibril can be deduced from a knowledge of the length of the shadow it casts and the angle between the shadowing atomic beam and the shadowed surface. Collodion films mounted on the wire screens used for electron micrography are not flat, and this has made it difficult to know accurately the angle of shadowing at a given point on them; but this uncertainty no longer exists when dealing with replicas taken from a flat glass surface. The replica of Fig. 2 was made so that shadow lengths are five times the heights of the objects causing them. The lengths of the shadows of the tobacco mosaic fibrils are best measured from the shadowed edge of groups of rods lying normal to the direction of evaporation. Such shadows prove to be *ca.* 600A long. The fibrils then are one fifth of this, or 120A, high; they are thus of equal width and height.

Individual fibrils, of which the one lying horizontally at the bottom of Fig. 2 is a good example, often appear segmented along their lengths. It has been suggested[6] that the lengths of individual tobacco mosaic rods as observed in the electron microscope are integral multiples of some unit that could be considered as the fundamental molecular length. We have examined many of the segments seen in our photographs to determine whether their lengths are either uniform or simple multiples of an underlying unit. They vary in an apparently continuous manner between *ca.* 200A and *ca.* 1,000A.

SUMMARY

Two improvements are described in the use of shadow electron micrography for the observation of particles of macromolecular dimensions. One involves the substitution of gold for chromium as shadowing metal. The thinner gold film that can be employed gives a truer representation of the shape of particles so small that shape and size are significantly altered by the thickness of the shadowing metal deposited on them. The other consists in metal-shadowing small particles deposited on a very smooth surface such as that of polished glass and the handling of this metal film as a replica of the glass surface and the particles resting on it. This technique permits the photography of particles whose direct observation is disturbed by the fine structure that is brought out by shadowing a collodion substrate. Application of these methods to the electron micrography of the tobacco mosaic virus protein shows that its fibrils are rods about 125A both in height and breadth. Though the rods appear segmented, these segments have not been found to have a length that is constant or a small integral multiple of an underlying unit.

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[6] W. M. Stanley and T. F. Anderson, *Jour. Biol. Chem.*, 139: 325, 1941.