Brief Notes

Method for the Observation of Macromolecules with the Electron Microscope Illustrated with Micrographs of DNA.*,‡

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The theoretical limit of resolving power for transmission electron microscopes is in the range 5 to 10 A and it is not unusual for resolutions in the range 10 to 20 A to be reported in practice. This means that the dimensions of a large class of biologically important molecules and synthetic polymers should be directly observable if contrast and other limitations could be overcome. For example, a spherical protein molecule with a diameter of 20 A would have a molecular weight of only about 3500. Except for a few isolated instances, however, electron microscopy as a means for determining molecular size and shape has largely been confined to the study of very large macromolecules which have molecular weights of several millions and dimensions of several hundred angstroms. The reasons for the relative ineffectiveness of the electron microscope for the study of the smaller organic particles are their low electron-scattering power, the difficulty of distinguishing them from their surroundings or substrate, and in many instances the destruction or distortion of structure resulting from the removal of water. The shadow-casting process introduced by Williams and Wyckoff (1) has long been recognized as an ideal method for enhancing the visibility of isolated small particles but a series of technical difficulties have been encountered in attempts to exploit the potentialities of the process. These have included difficulties in finding a sufficiently smooth substrate, granularity of the shadowing metal, difficulties in stripping the metallized deposit from the substrate, and the presence of contaminating deposits. An extensive study of metals and the problems of stripping them from glass was carried out by Williams and Backus (2) and later Williams (3) concluded that fine detail of surfaces was obscured by a film of pump oil that covered all surfaces placed in a conventional shadow-casting apparatus. No evidence for such an oil film has been found in the course of the work to be described but whether or not such a film occurs may well be a function of the nature of the surface. The methods proposed in this paper represent refinements and improvements in an approach that has long been recognized in principle.

The procedure is as follows: (*a*) The substrate used is freshly cleaved mica which is presumably smooth to atomic dimensions and has failed to show by this method any observable structure other than an occasional step. Such surfaces are highly hydrophilic which is an extremely desirable property and they

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remain hydrophilic after being exposed to the residual vapors in a conventional shadow caster. (*b*) Materials are suspended in liquids containing volatile buffers or salts and are sprayed on to the surface of the mica from a commercial throat sprayer.[1] In most cases an aqueous suspension of polystyrene spheres of average diameter 880 A is added to the solution to aid in locating drops, as an aid to focusing and to provide an internal standard for the shadow length-to-height ratio. Depending on the material, concentrations range from about 0.01 to 0.1 per cent which is sufficiently high that residual impurities in water and reagents do not appear to be serious. (*c*) The sprayed surfaces are shadow-cast with platinum (4) usually at an angle of 5:1 in a conventional apparatus pumped with octoil. The grain of such films is quite fine as compared with other common materials used for shadow casting but some improvement would be desirable since the granularity of the metal is apparently the limiting factor in the method at present. The granularity becomes worse as the shadow length-to-height ratio increases and sometimes an apparent coarseness in the metal is actually due to an overlying deposit of particulate impurities rather than to the metal itself. Since apparent granularity is also a function of focus, proper interpretation requires that all micrographs be recorded in a through-focus sequence. (*d*) To provide additional support for the Pt, a film of about 25 to 50 A of SiO is evaporated normal to the surface though this is not absolutely necessary. After removal from the vacuum chamber the surfaces are coated with collodion. The film is scored into convenient squares for the specimen grids and the films are floated off on water—an operation which proceeds easily owing to the fact that the mica is hydrophilic.

Examples of the results obtainable by this method are provided by the micrographs in Figs. 1 to 4. Fig. 1 shows segments of DNA molecules together with polystyrene spheres of average diameter 880 A which had been mixed in with the water solution of DNA. Measurements of the lengths of the shadows from this and similar micrographs yield a figure of 20 ± 5 A for the height of the threads above the surface in agreement with the x-ray value for the DNA double helix. (5) Measurements of filament widths parallel to the plane of the substrate making allowance for the thickness of the metal deposit in this direction (about 25 A) indicate that in most of the micrograph the strands are 1 molecule wide but in some places, for example to the lower right of Fig. 1, the strand is several molecules wide indicating a degree of folding or coiling.

It is rare to find the isolated end of a molecule in these micrographs. Except where they terminate or join up with a polystyrene sphere to which they seem to adhere strongly, the threads terminate in flat patches such as are shown in Fig. 2. Such patches indicate a collapse or folding of the molecule although the patches are very thin normal to the substrate, probably only one or two molecules thick. To the left in Fig. 2 is one or more folded molecules without any strand protruding from the mass. These are frequently seen unattached to threads or polystyrene spheres. Estimates of the molecular weight represented by such masses made from a measurement of the area and an estimate of the thickness yield figures ranging upward from 5,000,000. Also visible in Fig. 2 are some

[1] Vaponefrin vaporizer.

small particles having an apparent diameter of about 100 A. These do not always appear and may represent some impurity or fragments of denatured DNA which have coiled up into spherical particles before being deposited on the mica.

Fig. 3 shows an area of a sprayed surface where a relatively high concentration of DNA has formed a typical gel-like network. Under these circumstances, most of the interconnecting filaments are several molecules wide although a few of the finest are estimated to be only one molecule wide.

Fig. 4 shows a thread from a synthetic polymer of adenosine monophosphate made by Dr. R. F. Beers (6) in this laboratory. These threads tend to show shorter extended lengths in electron micrographs and are also thinner on the average than natural nucleic acid. The thinnest observed threads from the synthetic polymer are estimated to be about 10 A wide and are barely discernible. Otherwise the morphological appearances of the two materials are interestingly similar.

This method has also been used for the observation of molecules of collagen, fibrinogen, and other materials which will be reported later. The results indicate that the smoothness of the mica substrate and the grain of the Pt film are sufficiently fine to permit efficient use of the shadow-casting method for the observation of molecules with widths in the 10 to 20 A range. The principal difficulties at present are those associated with obtaining an appropriate distribution of the individual particles on the surface free of salts, buffers, and drying artifacts.

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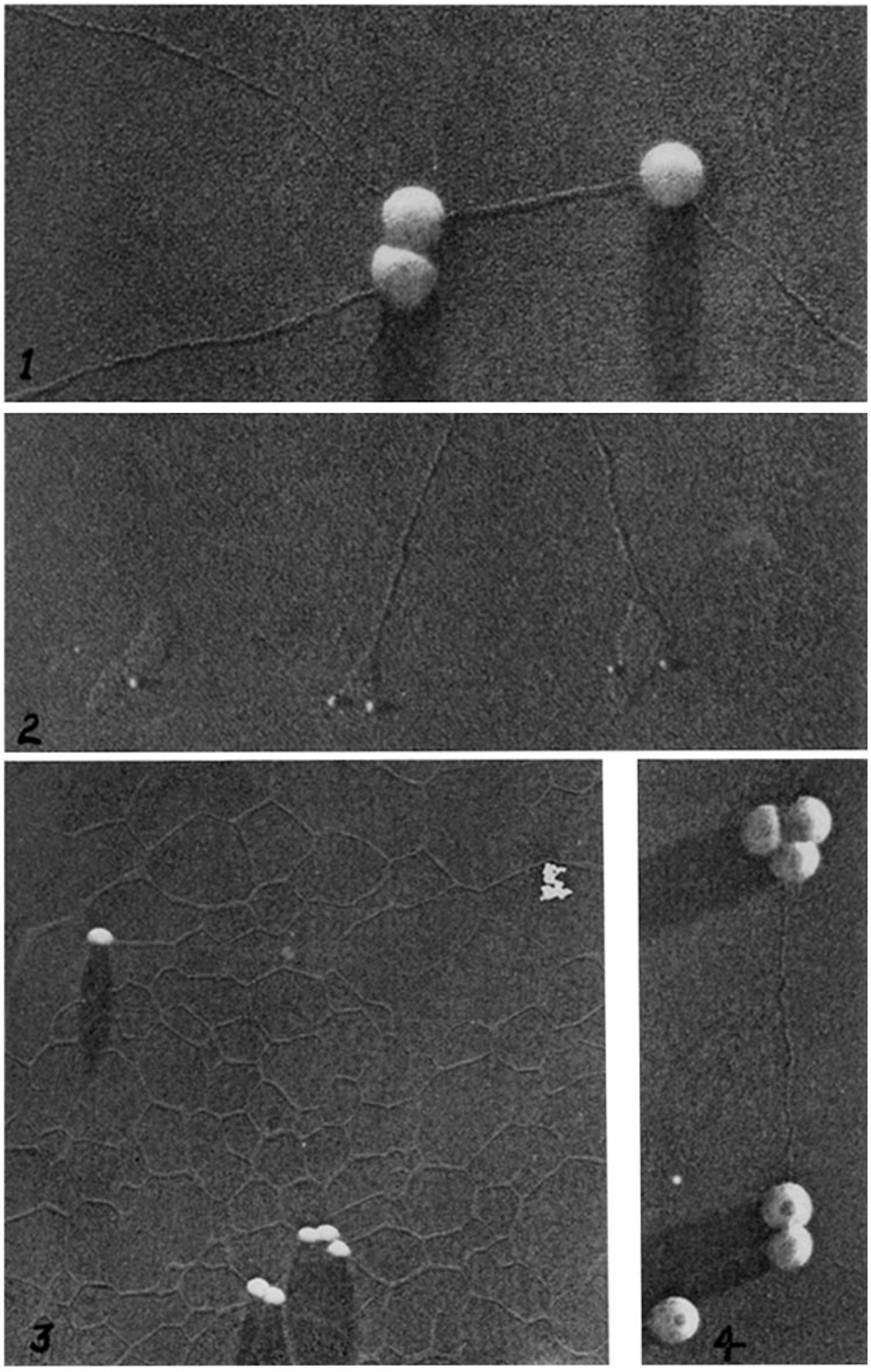
EXPLANATION OF PLATE 160

FIG. 1. Desoxyribose Nucleic Acid from calf thymus deposited from a water suspension. The admixed polystyrene spheres in this and other figures have an average diameter of 880 A. × 112,000.

FIG. 2. Same as Fig. 1 showing coiled-up molecules. The small spherical particles are either an impurity or possibly denatured fragments of DNA. × 112,000.

FIG. 3. DNA sprayed from a relatively concentrated aqueous solution, showing a network of gel-like structure. × 44,000.

FIG. 4. Threads of synthetically polymerized adenosine monophosphate. × 83,000.



(Hall: Method for observation of macromolecules)