Brief Notes

The Use of Novel Supporting Films for the Electron Microscopy of Deoxyribosenucleic Acid and Other Macromolecules.

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Earlier attempts at studying with the electron microscope the long chain polar polymers, of which deoxyribonucleic acid (DNA) is probably the most important example, appear to have been frustrated for want of a suitable substrate. On cast films of the conventional materials, formvar and nitrocellulose, the greater part of the DNA is gathered together in a mass at the centre of the drop as it dries. The effect is probably due to the hydrophobic character of the supporting film and attention has therefore been directed to the use of more hydrophilic surfaces. Hall (1) has avoided this difficulty by spraying the DNA solution on to the very hydrophilic surface of mica which is subsequently shadowed and then replicated by a stripping technique.

The method we wish to report consists of the use of a specially prepared polymer supporting film which, though it can be cast from chloroform, is both hydrophilic and basic. Therefore, the drop of DNA solution wets the film and the basic charge of the film attracts the negatively charged DNA macromolecules in the drop. The particular polymer used for the supporting film is a co-polymer of styrene and 2-vinyl pyridine. The pyridine group is slightly ionised in water and confers a small degree of basicity to the film while still being soluble in chloroform. The styrene is used as a diluent because pure polyvinyl pyridine is too water-soluble and its films adhere to glass too strongly to be stripped. Since this pair of monomers have very similar reactivity ratios, the polymer formed has practically the same composition as the monomer mixture, and can therefore be readily made in a wide range of compositions. The co-polymerisation is carried out at 40°C., with 0.1 per cent benzoyl peroxide as initiator. Because the polymerisation of vinyl pyridine is very sensitive to oxygen, the monomer must be freshly redistilled and oxygen must be rigourously excluded during polymerisation (2). A solvent is unnecessary during polymerisation, although toluene may be used. The product is dissolved in chloroform and purified by precipitation with ether. A co-polymer containing 40 per cent vinyl pyridine has been found to have the most useful properties for studying proteins and nucleic acids.

Supporting films are prepared by casting on glass from a 0.1 per cent solution of the polymer in chloroform. Although the film adheres more strongly and is mechanically less strong than formvar, it is possible to float satisfactory films on to a water surface. These films are then transferred to electron microscope specimen grids. Herring sperm DNA is dissolved at a concentration of 10^{-6} to 10^{-7} gm./ml. in M/100 ammonium acetate. This solution is sprayed on to the coated grids so as to give a convenient distribution of drops about 50 μ diameter. The specimens are shadowed with platinum at an angle of approximately $\tan^{-1}1/4$ and photographed in a Siemens (Elmiskop 1) electron microscope.

The electron micrographs of DNA, Figs. 1 and 2, show long filaments (up to $> 4\ \mu$ in length) with a shadow width of $\sim$ 100 A. This is in reasonable agreement with the expected width ($\sim$ 20 A) found from the x-ray data (3) and also with that already reported by Hall (1). On the co-polymers of low vinyl pyridine content, the DNA is not strongly attracted to the film and the filaments may be seen (Fig. 1) stretched out pointing towards the centre of the drop. With more concentrated DNA solutions, there is a striking tendency for the filaments to form lengthwise aggregates. Using greater concentrations of vinyl pyridine, a better dispersion, in which largely single molecules are seen, is obtained. An attempt to estimate the polydispersity and molecular weight of DNA is under way.

These films have also shown themselves to be very suitable for studying proteins, again because the hydrophilic surface allows a better dispersion. It would seem that the risk of surface denaturation is also greatly reduced.

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Explanation of Plate 80

FIG. 1. An electron micrograph of herring sperm DNA on 40 per cent PVP/60 per cent polystyrene. The specimen is shadowed with Pt and photographically reversed. The single molecule, with ends marked by arrows, has a length of about 1.7 μ and a corresponding molecular weight of about 3×10^6. $\times$ 64,000. The line in the lower left hand corner of Fig. 1 represents 1 micron.

FIG. 2. A higher power micrograph of parts of several molecules. The central filament, in the upper part of the micrograph, is presumably two molecules aggregated side by side. $\times$ 160,000. The line in the lower left hand corner of Fig. 2 represents $\frac{1}{10}$ micron.

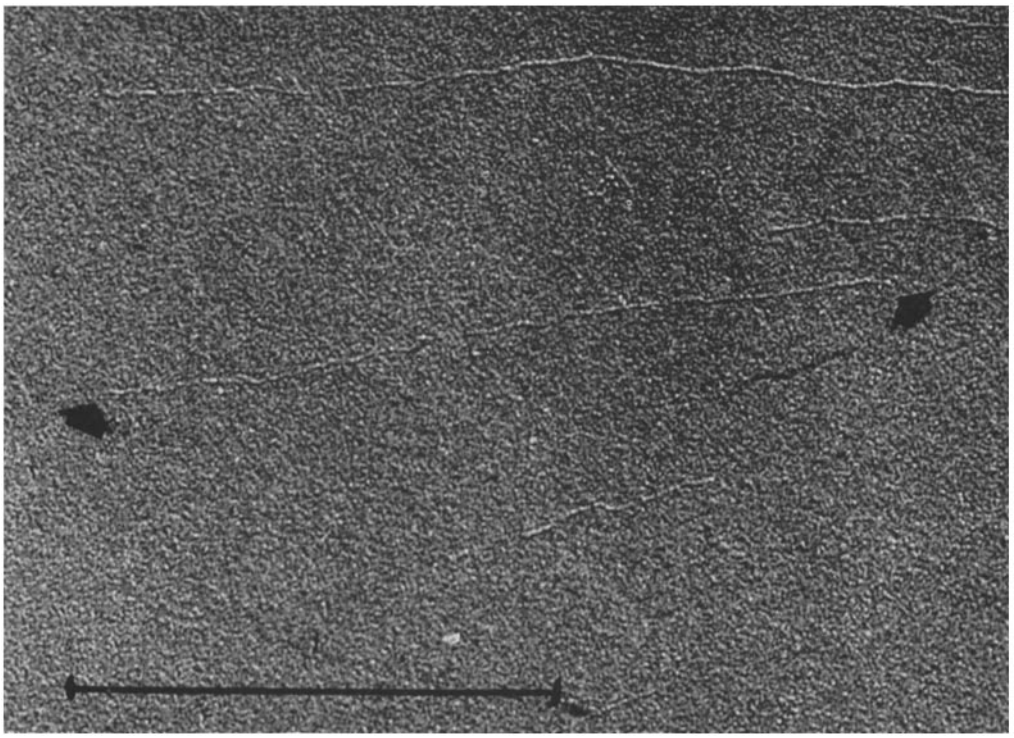

FIG. 1

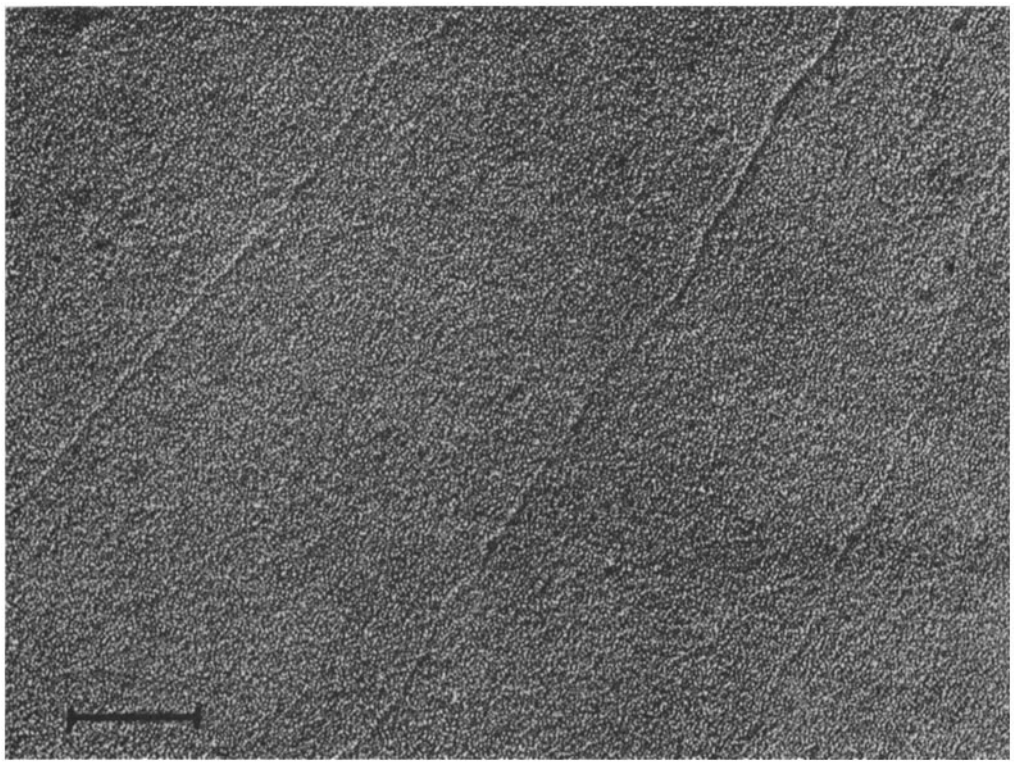

FIG. 2

(Birbeck and Stacey: Electron microscopy of DNA)