PREPARATION OF MICROORGANISMS FOR ELECTRON MICROSCOPY

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Electron microscopic examination of purified viruses, bacteria, and bacterial cell walls is often made difficult by undesirable aggregation which occurs during evaporation of aqueous suspensions upon specimen-supporting membranes. The aggregation effect is especially troublesome when microorganisms are suspended in distilled water or in neutral solutions containing low concentrations of electrolyte; under these conditions the microorganisms are negatively charged, and the suspensions usually do not wet nitrocellulose or Formvar membranes. Electron microscopic studies of colloidal cellulose crystallites (Ribi and Rånby, 1950) and spontaneous mouse encephalomyelitis virus (Leyon, 1951) have shown that the phenomenon of aggregation depends upon the relations between electrical charges on the supporting membrane and on the particles in suspension. A technique which includes evaporation of beryllium or aluminum layers upon the plastic membranes has been described (Ribi and Rånby, 1950). These metal layers, oxidized by air, are positively charged and adsorb negatively charged particles such as cellulose crystallites (Ribi and Rånby, 1950) or virus particles (Leyon, 1951) from aqueous solution without aggregation.

This report describes a rapid method for the preparation of nitrocellulose and Formvar membranes overlaying highly oxidized Phosphor bronze grids which avoids or minimizes aggregation of viruses, bacteria, and bacterial cell walls.

Strips of Phosphor bronze cloth purchased from the Cambridge Wire Cloth Company, Cambridge, Maryland, (mesh 150 X 150, wire 0026, Catalog no. 67070) were cleaned by scrubbing with detergent and water and then were flattened by placing them between two clean 21-gauge stainless steel plates and running them through a sheet metal roller. Batches of 200 to 300 disks punched from these strips were placed in a 250-ml Erlenmeyer flask containing 100 ml 6 N HCl. The acid solution containing the disks was agitated until it began to turn yellow; it was then heated and agitated until it boiled (formation of stannous chloride and hydrogen). The boiling was continued for 15 to 30 sec after the solution became colorless, the acid was poured off, and 100-ml warm distilled water was added to the acid-wet grids, which were shaken vigorously until they turned dark brown. The grids were then thoroughly rinsed with fresh cold water and covered with plastic membranes. It was essential to discontinue oxidation before the grids turned black.

The mechanism of the procedure may be explained as follows. Dilute hydrochloric acid at room temperature in the presence of air converts some of the copper component of the Phosphor bronze to a yellow cupric complex. Following the increase in temperature, a portion of the tin dissolves to form stannous chloride. Sn++ reduces Cu++ to metallic copper, and the latter is precipitated upon the grids. The resulting reddish layer of freshly deposited copper is highly active and is readily oxidized by the dissolved oxygen when shaken with water in the presence of the residual acid.

Membranes supported by these oxidized grids are wetted when completely covered with a drop of aqueous microbial suspension and some of the organisms are adsorbed. After a short period of time, varying from a few seconds to several minutes, depending upon the nature and concentration of the specimen, the excess liquid is sucked off with a fine capillary, leaving a very thin fluid layer which dries rapidly. The specimen is then evenly dispersed or the formation of aggregates is minimized.

The technique described here has recently been successfully employed in electron microscopic studies of whole cells and of cell wall material of Salmonella enteritidis (Ribi et al., 1959), Bordetella pertussis (Munoz et al., 1959), and Mycobacterium tuberculosis (figure 1) suspended in distilled water.
Figure 1. Cell walls of *Mycobacterium tuberculosis* (BCG) on a Formvar membrane supported by an oxidized Phosphor bronze grid.
Similar results were obtained with purified viruses such as tobacco mosaic (figure 2), poliomyelitis (Hoyer et al., 1959), and psittacosis (Beaudette, 1958). It is known that the addition of a small amount of salt (0.005 to 0.01 M) is required to keep purified animal viruses from crystallizing in distilled water. The salt residues were not detectable when adsorption techniques described here were applied (Leyon, 1951).

**SUMMARY**

A rapid method for preparation of specimens for electron microscopy which eliminates or minimizes the aggregation of viruses, bacteria, and bacterial cell walls during evaporation of aqueous suspensions upon Formvar and nitrocellulose membranes is described.

**REFERENCES**


