# ELECTRON MICROSCOPY OF SODIUM DESOXYRIBONUCLEATE BY USE OF A NEW FREEZE-DRYING METHOD*

by

ROBLEY C. WILLIAMS

*Virus Laboratory, University of California, Berkeley (U.S.A.)*

A new method of freeze-drying for the preparation of biological specimens for electron microscopy has recently been developed in this laboratory. Preliminary results indicate that the technique successfully preserves three-dimensional structure in fibrous materials and in particulate objects ranging in size from human red-cells to the smaller viruses. This note briefly describes the technique, and illustrates its application in the electron microscopy of the fibrils of sodium desoxyribonucleate (DNA).

The apparatus for the freezing and vacuum sublimation of specimen material consists primarily of an open-end glass tube, with a removable vacuum-tight lid, and a means for high-vacuum evacuation through a cold trap. In the bottom of the tube is inserted a closely fitting copper support whose upper surface is smooth and coated with a film of collodion. The tube containing the copper support is placed in a Dewar flask, where it is initially cooled to $-78°$ C and subsequently warmed for final desiccation. The specimen material, in aqueous suspension, is sprayed[1] at high velocity through the chilled tube upon the cold ($-78°$ C) collodion-filmed copper surface. The subsequent vacuum sublimation is performed at $-50°$ C for 10 minutes, followed by 10 minutes' sublimation with the copper heated to 60° C. Air is admitted to the tube and the upper support is placed in a shadowing unit. After the collodion film is shadowed it is transferred by a replica technique to microscope grids.

The essential merits of the technique are: (1) the use of micro-volumes of specimen material, in the form of spray droplets, and (2) the rapid cooling of the droplets upon a surface in good thermal contact with a relatively massive thermal reservoir. The average droplet volume is $10^{-9}$ ml, and this small amount of liquid splashes out into a layer about 3 $\mu$ thick before freezing takes place. Calculation shows that the cooling rate of this layer is so great that the water-ice change of phase occurs in less than $10^{-3}$ seconds.

Fig. 1 shows an example of the results of freeze-drying fibrous material. The specimen is a 0.01% water solution of DNA prepared by Dr ARTHUR PARDEE of this laboratory, using the method of SCHWANDER AND SIGNER[2]. Chemical tests show that its protein and ribonucleic acid contents are less than 1%, and its high degree of polymerization is indicated by a reduced viscosity ($\eta_{sp}/c$) of over 4000 ml/g. Fig. 1a is a low magnification micrograph of a portion of a freeze-dried droplet. The most conspicuous aspect of this micrograph is the net-like, three-dimensional structure of the

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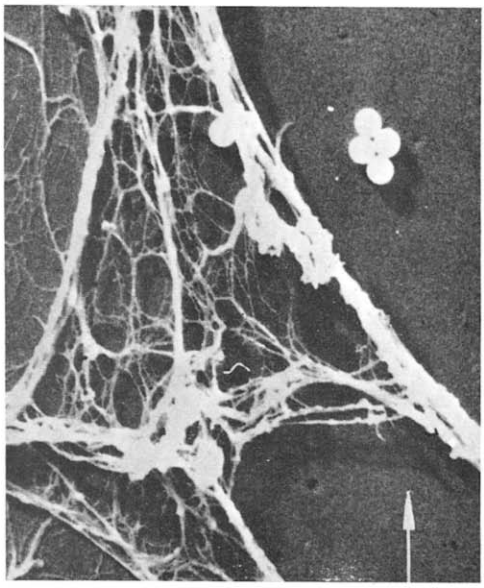

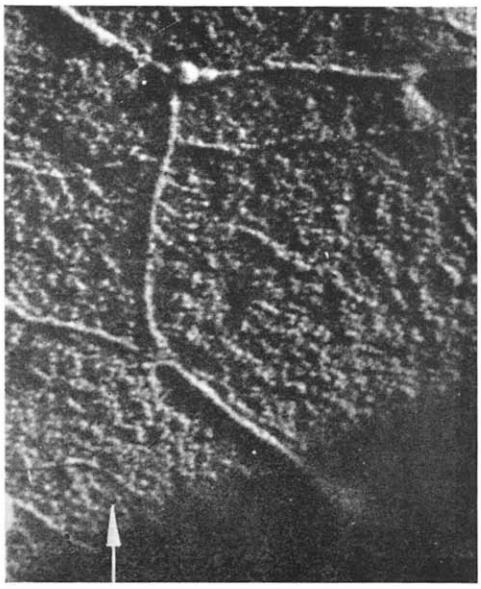

Fig. 1a. Low-magnification (× 13,500) electron micrograph of a freeze-dried water solution of DNA. Three-dimensional character of specimen is well shown by the separation of fibers and their shadows in the region marked with an arrow. The horizontal fibers here are about 0.5 $\mu$ above substrate. Spherical particles are of polystyrene latex.

Fig. 1b. High magnification (× 100,000) electron micrograph of DNA. Region indicated with an arrow shows fibril about 15 Å in diameter.

fibrils, which is unlike earlier micrographs showing material flattened by forces of surface tension. In many places the elementary strands appear aggregated into bundles; probably this occurred during vacuum sublimation.

Fig. 1b is a high-magnification micrograph (× 100,000) of a few fibrils of frozen-dried DNA in contact with the collodion film. The local shadow angle is 10/1, and hence the ratio of shadow width to heights of specimen objects in this micrograph is $10^6$. The fibril in the lower left corner has a shadow approximately 1.5 mm wide (marked with an arrow), and hence it can be estimated that this fibril is approximately 15 Å in diameter. Numerous fibrils of about this diameter are seen in the original micrographs.

A point to observe in the micrographs is the frequent occurrence of places where three fibers come together at a point. It seems most likely that any aggregative effects that might take place during the sublimation of the ice would result in a random tangling of the fibrils, as is seen in concentrated, freeze-dried suspensions of tobacco mosaic virus. The most likely explanation of the fibrillar connections seen in these micrographs is that, even in such a dilute solution, the DNA exists as an extremely tenuous, network gel.

Some electron microscopic observations of DNA have been reported previously. SCOTT's micrographs[3] of air-dried material show branched fibrillar structure obviously affected by surface tension forces, and his micrographs of freeze-dried specimens appear so similar to the air-dried ones as to cast doubt upon the completeness of the freeze-drying. BAYLEY[4] has obtained micrographs of surface replicas of sodium thymonucleate

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which appear to exhibit globules of about 100 A diameter. BAYLEY offers the plausible interpretation that the globules are coiled-up fibers. Recently ROWEN, EDEN AND KAHLER[5] have reported physical studies of sodium thymonucleate, including electron microscopy, and state that the molecular configuration appears to be between that of a rod and of a coil.

The details of the new freeze-drying method are being published elsewhere.


## SUMMARY

Description of a new method of freeze-drying for the preparation of biological specimens for electron microscopy. Micro-volumes of specimen material are used in the form of spray droplets which are rapidly cooled upon a surface in good thermal contact with a relatively massive thermal reservoir. The new technique is illustrated with micrographs of fibrils of sodium desoxyribonucleate.

## RÉSUMÉ

Description d'une nouvelle méthode de séchage à l'état congelé pour spécimènes biologiques destinés à être étudiés au microscope électronique. L'on emploie des micro-volumes de matériel sous forme d'un jet de goutelettes qui sont refroidies rapidement sur une surface en bon contat thermique avec un réservoir thermique relativement grand.

## ZUSAMMENFASSUNG

Beschreibung einer neuen Methode zum Trocknen biologischer zur elektronenmikroskopischen Untersuchung bestimmter Proben. Es werden Microvolumina des Untersuchungsmaterials verwendet, die in Form eines Strahles feiner Tröpfchen auf einer Oberfläche, welche mit einem relativ grossen Wärmereservoir in guter thermischer Verbindung steht, rasch gekühlt werden.


## REFERENCES


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