ELECTRON MICROGRAPH STUDIES ON SODIUM DESOXYRIBOSE NUCLEATE

by

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INTRODUCTION

Estimates of particle weight, size and shape of nucleic acids and nucleates from different sources vary considerably depending on the method of preparation[1]. Viscosity and streaming double refraction of desoxyribose nucleate extracted by neutral salt[2, 3] indicate the possible presence of particles of sizes approaching those resolvable with the electron microscope. Thus far, no electron micrographs of nucleic acids have, to the author's knowledge, been published, though CLARK, QUAIFE and BAYLOR[4] showed electron micrographs of nucleoprotein prepared in the presence of high salt concentration. In the present paper electron microscope studies of nucleic acid obtained from rat liver are described.

MATERIALS AND METHOD

Sodium desoxyribose nucleate was isolated from the liver of adult, female, albino, SPRAGUE-DAWLEY rats following the methods of MIRSKY and POLLISTER[3]. The nitrogen, phosphorus, molecular extinction coefficient and ultraviolet absorption spectrum were determined. The biuret and MILLON tests for protein were negative as was the spectrophotometer test for protein as outlined by MIRSKY and POLLISTER[3]. The BIAL[8] test was negative while the KILIANI[5, 6] and DISCHE[7] tests were positive.

Solutions of the isolated material in concentrations of $1 \cdot 10^{-3}$ g/ml in double distilled water show negative streaming birefringence. Such a solution is water clear and exhibits properties of a gel.

Dilutions were prepared to yield concentrations of from *ca* $1\text{–}50 \cdot 10^{-6}$ g/ml in double distilled water with a final p_H of 6.5–7.0. Specimens from these dilutions were prepared in several ways for examination with the electron microscope.

In one series the solution was placed on the collodion supporting membrane, allowed to remain two minutes, and then withdrawn by touching the edge of the grid with a piece of filter paper. In a second series a drop was placed on the film and allowed to evaporate without blotting. A third series was prepared by freezing and drying the solution from the frozen state on the grid by a method similar to that of WYCKOFF[9]. The specimens thus prepared were examined without further treatment or after shadowing with a layer of chromium 40 A thick at an angle of from 6–9°. Shadowed and unshadowed specimens showed similar structures. Since it was not possible to see the

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unshadowed material on the fluorescent screen of the microscope, shadowed specimens were used for the most part.

All photographs were done with an R.C.A., model EMB electron microscope.

RESULTS

In relatively high concentrations the nucleate formed a more or less continuous layer over the surface of the film, although fiber patterns were sometimes seen. At lower concentrations a network of laterally branching and uniting fibers, which, in a few instances, showed evidence of overlaying of the fiber structures, was most commonly seen (Figs. 1 and 3). In the lowest concentrations employed a dendritic appearance was predominant. This variation in appearance with changing concentration was found with all three methods of drying the material. As can be seen in the figures, the lengths and widths vary widely. In specimens prepared from the most dilute solutions by draining before drying, extremely long and very narrow structures with little branching were observed (Fig. 4), the lengths of which commonly exceeded 3 μ.

DISCUSSION

The possibility that the structures are caused by surface tension effects of the water drying on the hydrophobic supporting film has been considered. However, since essentially the same structures were seen in the frozen-dried material (Fig. 2) as in the specimens prepared by drying from the liquid state (Fig. 1) and since overlay of the filaments was observed on material allowed to dry without blotting, it is considered unlikely that the structures result entirely from the conditions of preparation.

From the data of ASTBURY[10], TENNENT and VILBRANDT[11], SIGNER, CASPERSSON and HAMMARSTEN[12], PETERSEN[13], and SCHMIDT, PICKLES and LEVENE[14], particle widths ranging from 13–22 A and lengths varying from 2720–5200 A may be expected. Considered in this light, it is obvious from the lengths and widths of the filaments in the shadowed and unshadowed preparations that they represent a lateral and longitudinal aggregation of nucleic acid columns. It was suggested by GREENSTEIN and JENRETTE[15] that such lateral interaction might take place between the phosphate and purine amino groups of the adjacent polynucleotide chains.

Further experiments are in progress designed to define the unit of the polymer and the nature of the interaction between the units themselves and with other compounds.

The author is indebted to Dr B. S. GOULD for the phosphorous determination and to Mrs H. M. BEUMER and Mrs R. BEST for technical assistance.

SUMMARY

The electron micrographs of sodium desoxyribose nucleate are described and typical photographs are reproduced. Comparison of the existing data in the literature regarding particle lengths and widths with these micrographs indicates the occurrence of lateral and longitudinal aggregations of the nucleic acid columns.

RÉSUMÉ

On décrit les micrographies électroniques du sel sodique de l'acide desoxyribonucléique et on

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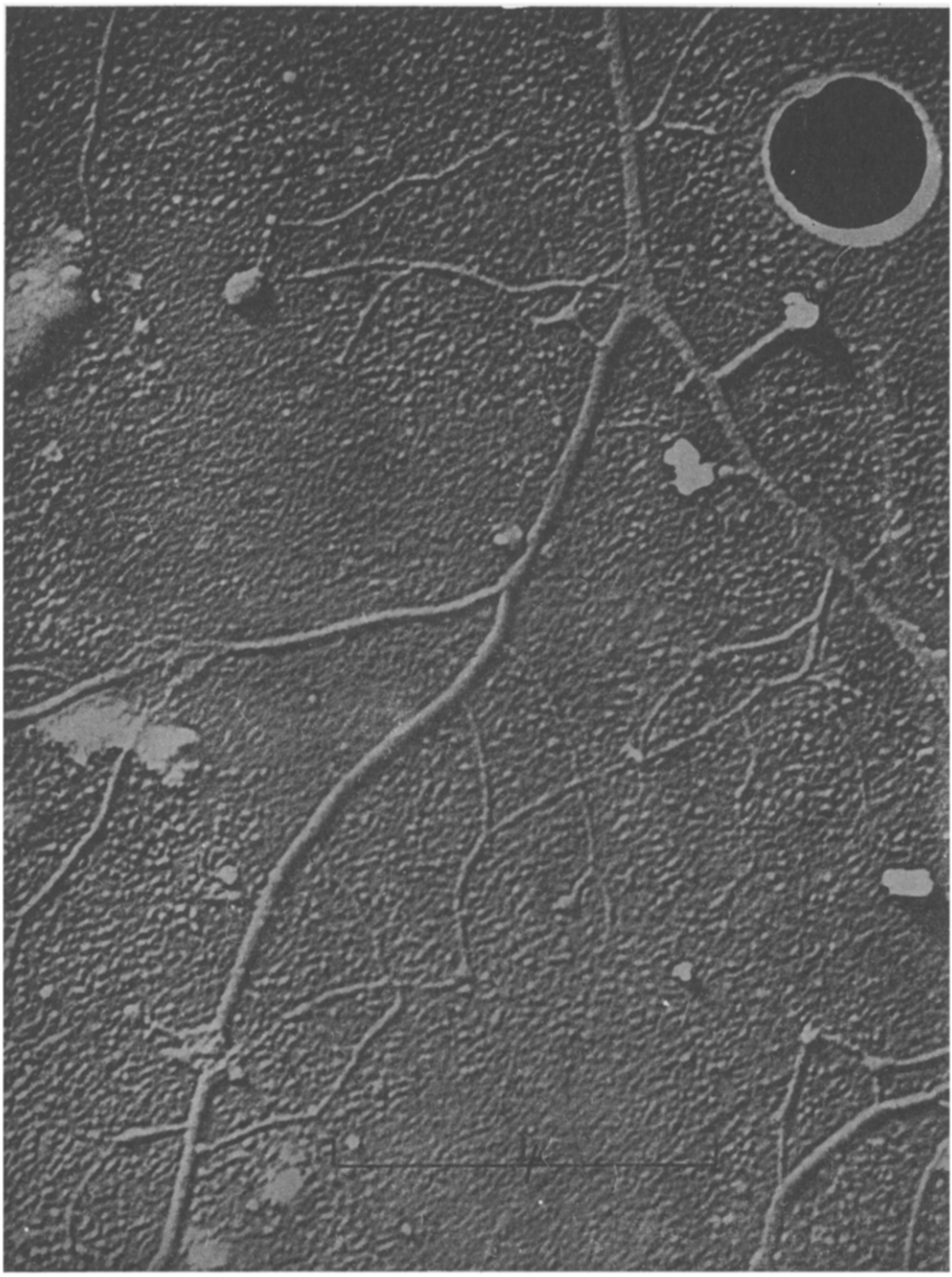

Fig. 1. Nucleate prepared by drying a solution containing $40 \cdot 10^{-6}$ g/ml on the grid without blotting. Shadow cast with chromium. Showing filament overlay. 53 000 ×.

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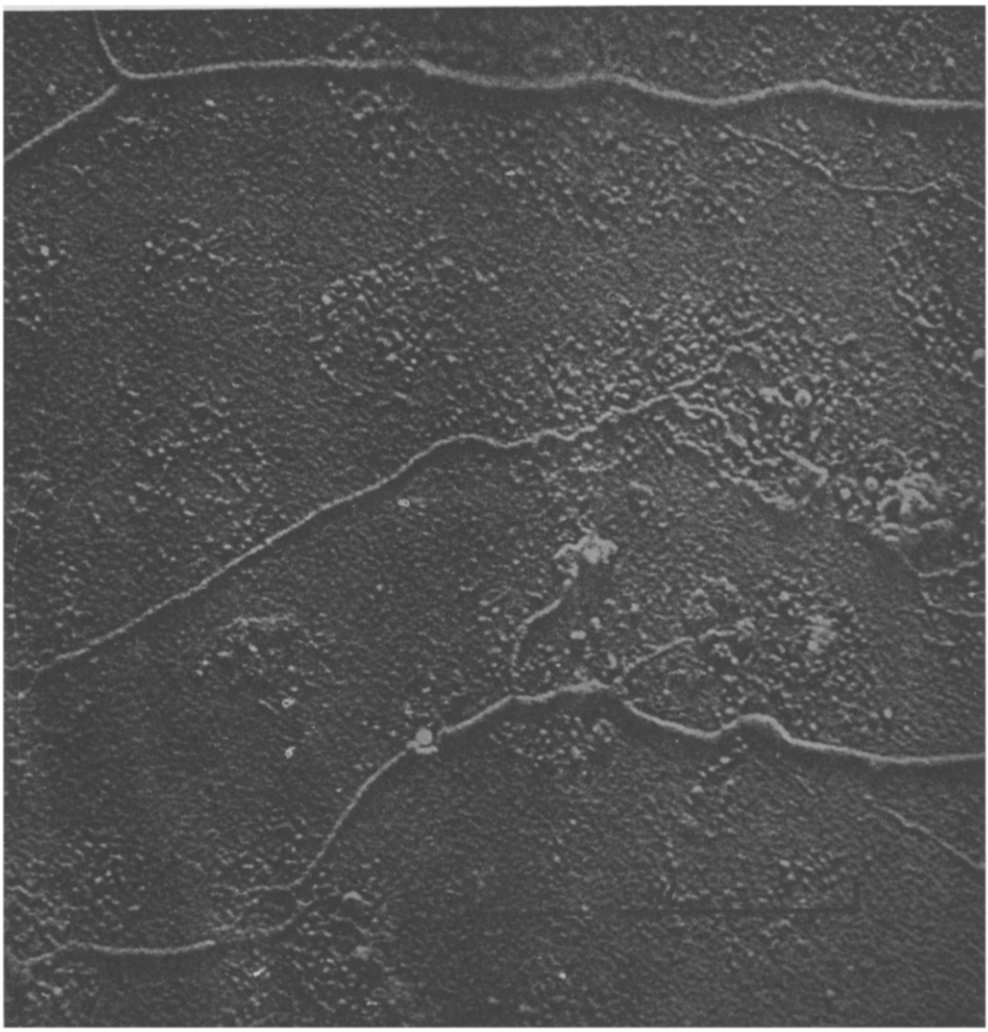

Fig. 2. Nucleate prepared by freezing and drying a solution containing $8 \cdot 10^{-6}$ g/ml on the grid. Shadow cast with chromium. 51 000 ×.

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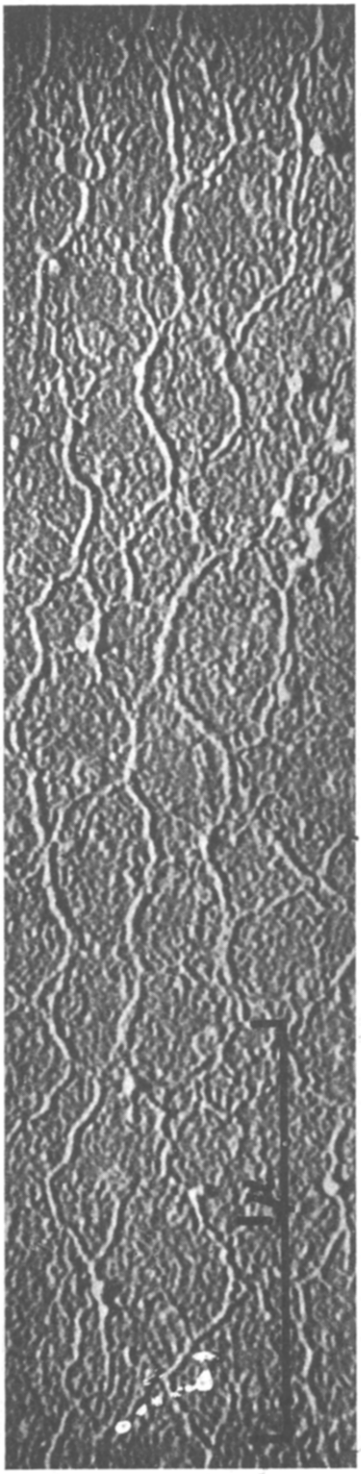
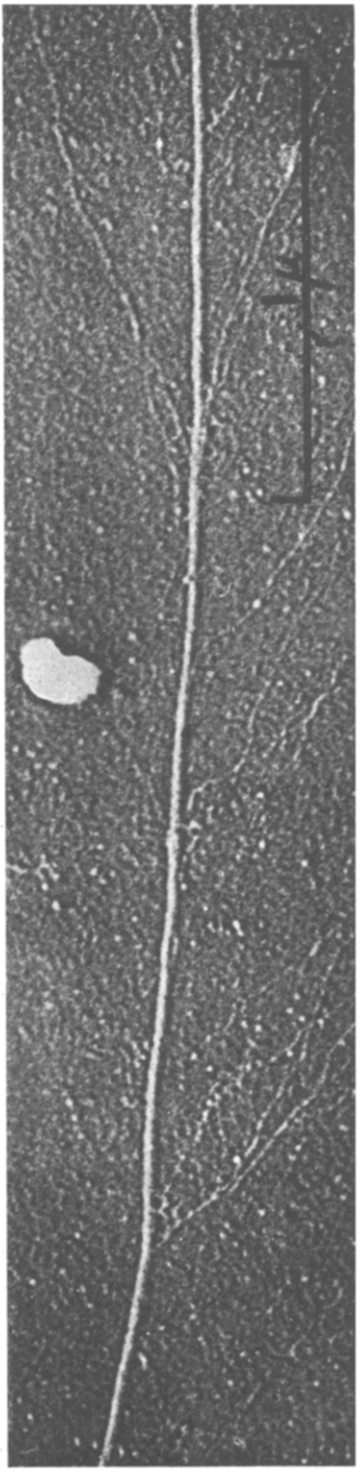


Left:

Fig. 3. Nucleate prepared by drying a solution containing $20 \cdot 10^{-6}$ g/ml on the grid without blotting. Shadow cast with chromium. 55 000 ×. Showing the network appearance described in the text.



Right:

Fig. 4. Nucleate prepared by draining a solution containing $6 \cdot 10^{-6}$ g/ml on the grid before drying. Shadow cast with chromium. 58 000 ×. Showing the appearance when the polymer is oriented by flow.

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reproduit quelques photographies typiques. La comparaison des données existantes dans la littérature concernant les longueurs et les largeurs des particules avec les micrographies reproduites ici indique la présence d'aggrégations latérales et longitudinales des colonnes de l'acide nucléique.

ZUSAMMENFASSUNG

Elektronenmikroskopische Aufnahmen von Na-Desoxyribo-Nukleat werden reproduziert und beschrieben. Ein Vergleich mit den in der Literatur vorhandenen Angaben über die Länge und Breite dieser Teilchen zeigt das Vorhandensein einer Verdichtung in der Länge- undue Qrrichtung dieser Nukleinsäuresäulen.

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Received November 21 st, 1947