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Sedimentation Behavior of Desoxypentose Nucleic Acids from Normal and Pathologic Human Spleens. (22356)

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Previous work in this laboratory has demonstrated that the desoxynucleic acids (DNA) of normal and leukemic human cells differ in some physico-chemical characteristics(1,2). Interest in this study of macromolecular structures of normal and malignant cells has been greatly stimulated by the "template hypothesis"(3-5). According to this hypothesis, neoplastic growth would be the consequence of an abnormal event in the self duplication of some macromolecules in the cell(6), resulting in a malignant transformation of some cytoplasmic and/or nuclear structures.

Although the "template hypothesis" concerns primarily the role of ribonucleic acid (RNA) in the mechanism of specific protein synthesis, it could be extended to the role of DNA in mitosis and gene duplication. For this reason the sedimentation constant of DNA from normal and leukemic cells was studied.

Material and methods. DNA was prepared from calf thymus, normal human spleen removed surgically after traumatic rupture, human spleens from cases of multiple myeloma, Brill-Simmers disease, myeloid leukemia and hereditary spherocytosis. The organs were frozen with dry ice immediately after removal and powdered in a high speed mixer with additional dry ice. Thirty grams of powdered tissue were suspended in 100 ml of NaCl-Na Citrate solution (0.14 M NaCl; 0.01 M Na Citrate) and homogenized for 2 minutes at 12,000 r.p.m. The material was then filtered through 3 layers of gauze, centrifuged, and the sediment washed 3 times. The sediment was then resuspended in 20 volumes of 1.7 M NaCl-0.01 M Na Citrate solution and homogenized again for 30 seconds at a lower speed. DNA sodium salt was extracted according to the method of Gulland (7) as modified by Chargaff *et al.*(8), then dialyzed against distilled water for 72 hours at 0-4°C and lyophilized. The N/P ratio as well as other chemical properties of the obtained DNA, correspond closely to the theoretical values and have been published elsewhere(2). DNA was dissolved in cacodylate buffer of ionic strength 0.3 and pH 7(9) by gentle overnight stirring in the cold room (0-4°C). The DNA concentration of the solution was calculated from its phosphorus content (P DNA) determined with the method of Fiske and Subbarow, after conversion of the arsenic from the penta- to the trivalent form(10). The samples were analyzed at room temperature within 24 hours of being dissolved. Sedimentation data were obtained in the Spinco Model E analytical ultracentrifuge. Sedimentation constants were corrected for a solvent having viscosity and density of water at 20°C; the partial specific volume of DNA was assumed to be 0.55(11,12).

Results. Typical schlieren photographs of DNA sedimentation boundaries are seen in Fig. 1. The data obtained with DNA from a series of normal and pathological spleens are shown in Fig. 2 where the S_{w20} values are plotted against the P DNA concentration of the samples. It can be seen that points fall on the same curve, regardless of the source of the DNA samples.

Discussion. The spread of values obtained with any given P DNA concentration seems to indicate a variability within each determination rather than differences in the sedimentation constant of DNA samples of dif-

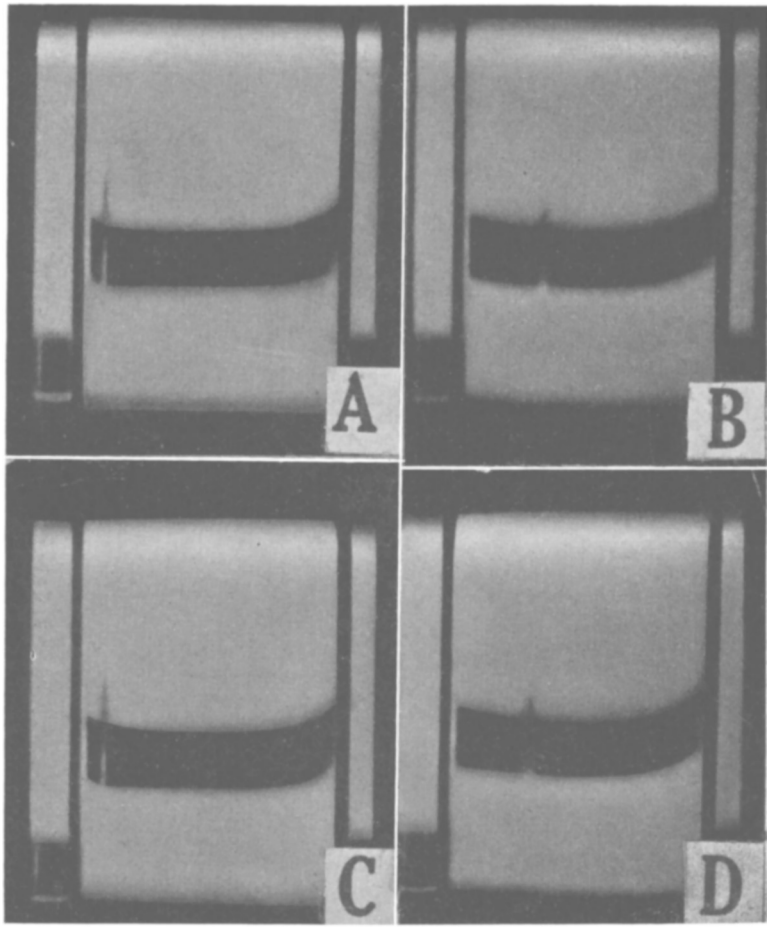

FIG. 1. A and B. Schlieren photographs of sedimenting boundaries of DNA from spleens of patients with multiple myeloma. Speed, 59,780 rpm. Bar angle, 45°. Time, 16 min. Concentration, 1.7 mg P DNA/100 ml. C and D. Schlieren photographs of sedimenting boundaries of DNA from spleens of patients with myeloid leukemia. Speed, 59,780 rpm. Bar angle, 45°. Time, 16 min. Concentration, 1.9 mg P DNA/100 ml.

ferent origin. These results do not support the contention that DNA from normal and pathological human cells have different sedimentation constants. Extrapolation of the data to concentration zero indicates an S_{w20} value of about 20 to 21, in agreement with the data of Peacocke and Schachman(9) and of Doty(12). Other authors(12-16) have reported lower S_{w20} values at zero concentration although at a concentration of DNA of 9-18 mg %, the sedimentation coefficients reported by them are in agreement with ours. This difference in the extrapolated values may be due to the different concentration range of the samples and to the different ionic strength of the buffer solutions used by various investigators. Sadron(17) and Jordan (18) have shown that some physical properties of DNA, such as viscosity, are strongly influenced by the ionic concentration of the solution, and that reproducible data can be obtained only when all free negative charges of DNA are saturated. Furthermore Peacocke and Schachman(9) have demonstrated that, when DNA is sedimented in distilled water at a concentration below 60 mg %, it behaves like degraded DNA and that the sedimentation constant increases up to an ionic strength of 0.3. Further addition of electrolytes has no greater effect.

Very strong intermolecular forces act between DNA molecules at relatively high con-

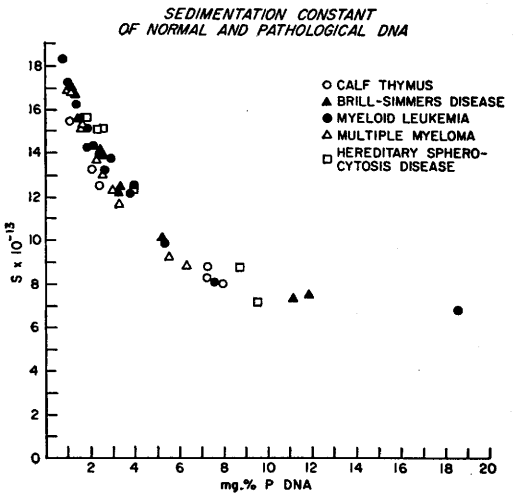

FIG. 2.

centrations, and may disturb the sedimentation rate even at the lowest concentrations used in this work. This may make the demonstration of heterogeneity in various DNA samples difficult. While this suggests the need for further studies of the sedimentation behavior of DNA at low concentrations with more sensitive recording systems (such as the optical absorption method used by Butler and Shooter(19)), the results reported in this paper indicate that DNA samples from normal and tumoral cells give identical sedimentation curves over the large range of concentrations studied.

Summary. 1. Sedimentation curves of DNA from spleens of normal individuals and patients with diseases causing a leukocyte infiltration in the spleen were obtained by means of the ultracentrifuge. 2. DNA was dissolved in cacodylate buffer (pH 7; u 0.3) at concentrations range 1-20 mg % of P DNA. No differences in sedimentation curves of DNA from normal and infiltrated spleens were noted.

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