APPENDIX

Sedimentation Properties of the Deoxyribonucleic Acid Component of Rat-Thymus Deoxyribonucleoprotein

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An attempt was made to relate the properties of types I and V dNP to those of their constituent DNA by measuring the sedimentation coefficients of the separated DNA. DNA was separated from protein by using density-gradient equilibrium centrifugation. Samples of dNP solution were added to saturated caesium chloride to make final solutions containing 30–35 $\mu\text{g.}$ of DNA/ml. and 56–57% (w/w) caesium chloride. The solutions were centrifuged in a Spinco model L ultracentrifuge at 33500 rev./min. in an SW39 rotor for 65–70 hr. at about 25°. Fractions were obtained by piercing the tube bottoms and collecting drops (0.20–0.25 ml./fraction). After dilution with distilled water, the extinction at 260 $m\mu$ was measured; fractions in the region of the DNA band were then pooled and dialysed for 16–18 hr. at 4° against

0.1 M-sodium acetate in 0.01 M-phosphate buffer, pH 7.0. Velocity sedimentation experiments were then conducted on dilutions of a fraction (generally four) with a Spinco model E ultracentrifuge, equipped with absorption optics, at a rotor speed of 42040 rev./min. Sedimentation distribution curves were prepared and the sedimentation coefficient was measured at the 50% concentration by using standard procedure. On extrapolating the plot of $1/S$ versus C to zero concentration, the sedimentation coefficient, $S_{20,w}^0$, was found to be 28.5 s.

Similar measurements were made on type V dNP. Table 1 gives the results and shows that no significant difference in polydispersity and S exists between the DNA from the two types of dNP. This suggests that the DNA from these preparations

Table 1. *Velocity sedimentation of the DNA component of dNP*

Expt. no.	dNP type	Concn. of DNA ($\mu\text{g./ml.}$)	Range $S_{20,w}$ (s)	Average $S_{20,w}$ (s)
1	I	29	2–42	23.1
		42	10–44	21.8
		52	8–40	20.0
2	I	14	8–50	25.8
		17	6–48	25.5
3	I	13	13–48	27.2
		22	12–40	24.7
		37	12–38	24.9
		46	11–38	23.6
3	V	12	7–44	27.1
		22	12–44	27.3
		40	14–42	25.1
		47	16–54	30.0
4	I	13	6–44	27.4
		20	10–40	24.0
		48	12–40	23.5
		54	10–40	22.4
4	V	13	12–40	24.5
		14	13–44	29.0
		19	8–46	26.6
		40	8–46	27.2

is similar, i.e. that type V dNP does not contain degraded DNA. However, the ultracentrifuge cells were filled with a syringe, so if any material of very high molecular weight were present in either or both types of dNP this would be degraded by shear; thus any difference in molecular weight at this level would not be detected.

Additional experiments on the isolated DNA with analytical equilibrium density-gradient centrifugation in caesium chloride showed that there was

no essential difference in density and density distribution of the DNA from types I and V dNP.

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