The purified enzyme, which was pink in colour, was stable and could be stored in the frozen state for a week with no loss of activity. It was precipitated by ammonium sulfate between 70 to 100% saturation at pH 5.5 and was not sedimented by centrifugation for one hour at 80,000 *g*. There was no hydrogen uptake in the absence of enzyme or methylene blue. Riboflavin-5'-phosphate was also active as a hydrogen acceptor, but the rate of hydrogen uptake was less than 5% of that obtained with methylene blue. Ferricyanide, sulfite and sulfate were active as hydrogen acceptors with the crude enzyme preparations, but not with the purified enzyme.

Electrophoretic and ultracentrifugal studies on the enzyme are in progress.

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THE VISCOSITY AND STREAMING BIREFRINGENCE OF CONCENTRATED SOLUTIONS OF SODIUM DESOXYRIBONUCLEATE

by

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PETERLIN[1] has recently proposed a semi-empirical theory of the viscosity and streaming birefringence of concentrated solutions of macromolecules, which led to the relationships

$$\lim \frac{(\pi/4 - \chi)c}{(\eta - \eta_0)G} = \frac{\beta M}{2RT} \qquad (1)$$

($\beta = 1$ for flexible, 3 for rigid molecules)

$$\text{and } \frac{\Delta n}{n} = \frac{4\pi}{5}\left(\frac{n^2+2}{3n}\right)^2 \frac{(\alpha_1 - \alpha_2)}{kT}(\eta - \eta_0)G \qquad (2)$$

(where χ is the orientation angle, G the gradient, M the molecular weight, η and η_0 solution and solvent viscosities, $(\alpha_1 - \alpha_2)$ the optical factor, Δn the birefringence, and c the concentration in grams per ml).

These equations agree well with existing data for several uncharged polymers.

The solution properties of sodium desoxyribonucleate (DNA) are extremely dependent on concentration and gradient, and extrapolation to zero concentration and gradient, previously necessary to obtain molecular parameters from viscosity and birefringence measurements, is often difficult. This, together with its polyelectrolyte properties, makes DNA a very interesting substance with which to test PETERLIN's theory.

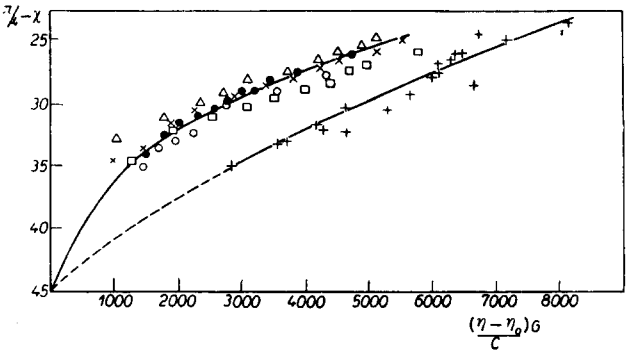

Fig. 1. Extinction angle χ vs. $(\eta - \eta_0)G/c$ for DNA in 0.2 *M* salt (upper curve) and in aqueous solution (lower curve). DNA in 0.2 *M* salt:
○, 0.0190% DNA;
●, 0.0384%;
□, 0.0582%;
×, 0.0768%;
△, 0.0960%. DNA in aqueous solution:
+, 0.00900–0.0177% DNA.

We have made a detailed study of the viscosity and streaming birefringence of calf thymus DNA[2] in aqueous solution and in the presence of salt over a range of concentration of 0.0003% to 0.10% and from zero gradient to 400 sec^{-1} in the same Couette-type apparatus. These results, which will be reported in detail elsewhere, allow PETERLIN's theory to be applied to DNA. A plot of χ against $\frac{(\eta-\eta_0)G}{c}$ is shown for various concentrations of DNA in 0.2 *M* NaCl, at 25° C and shows excellent agreement with the theory. In aqueous solution the viscosity of DNA is not very reproducible[3,4], especially in the more concentrated region, but nevertheless agreement with the theory is found. Plots of Δn against $(\eta-\eta_0)G$ show equally good agreement for the solutions in 0.2 *M* NaCl.

Values of the molecular weight of the DNA in 0.2 *M* NaCl calculated from equation (1) are $1.5 \cdot 10^6$ for a rigid molecule and $4.5 \cdot 10^6$ for a flexible molecule. Other evidence[4,5], which shows that the DNA molecule possesses a limited degree of flexibility, suggests the molecular weight lies somewhere between these two values. This compares well with the values of the molecular weight of this sample of DNA determined in dilute solution by other methods, and shown in the table.

TABLE I

Measurements employed	*Theory*	*Molecular weight* $\times 10^{-6}$
η and χ in concn. soln.	PETERLIN[1]	1.5–4.5
Sedimentation (S) and diffusion at zero concn.*	SVEDBERG[6]	7.9
$[\eta]$** and rotary diffusion (θ) at zero concn.	KUHN AND KUHN[7]	3.0
$[\eta]$** and S at zero concn.	SCHERAGA AND MANDELKERN[8]	2.2
$[\eta]$** and θ at zero concn.	SCHERAGA AND MANDELKERN[8]	6.6

* data of G. J. HOWARD, Thesis, Nottingham, 1953; ** at zero shear.

Molecular weights of different samples of calf thymus DNA prepared by similar procedures, determined by light scattering[9], generally lie in the range $4–8 \cdot 10^6$.

Comparison of the results in aqueous solution and in 0.2 *M* salt reveals that an apparently different value of the molecular weight is obtained from the two sets of measurements. This will presumably be due to a decrease in the value of β in aqueous solution from that which applies in 0.2 *M* NaCl, since it is likely that the molecular weight is not sensibly different in the two systems. Hence the molecule of DNA is presumably more flexible in aqueous solution than in 0.2 *M* salt.

It is concluded that solutions of DNA obey the predictions of the semi-empirical theory of PETERLIN and that this theory gives values of the molecular weight in agreement with those obtained by other methods in dilute solution. Also, the range of molecular weights predicted, which depends on the model assumed for the molecule, is narrower than that observed with other theories.

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