was present up to 50% of the material. Sedimentation in alkaline caesium chloride gave a sharp band at $s_{0, \text{obs.}}$ 20S with some trailing material, and denatured mitochondrial DNA sedimented at $s_{20, \text{w}}$ 97S in neutral solvent. These results are consistent with a linear DNA of approx. 30×10^6 daltons, without single-strand interruptions. Examination of the DNA in the electron microscope showed only linear filaments of $15 \mu\text{m}$, a size consistent with this and thus the same length as the genome, as previously determined (Flavell & Jones, 1970).

To test for cohesive ends, DNA ($10 \mu\text{g/ml}$) was heated to 70°C in 0.5M-sodium chloride, cooled slowly to 50°C and incubated for 3h, followed by sedimentation analysis. Annealed DNA sedimented at 33.8S and the control DNA (unincubated) sedimented at 35.0S. A circular DNA produced by self-annealing should have sedimented at approx. 38S. Hence no evidence for cohesive ends was obtained.

The possibility also remained that the DNA existed as circles which were broken by one double-stranded break on isolation. Renatured DNA should thus show circles in the electron microscope. The examination of this DNA is at present under investigation and will be discussed.

R.A.F. thanks the Medical Research Council for a postgraduate studentship.

Borst, P. & Kroon, A. M. (1969). *Int. Rev. Cytol.* **26**, 107.
Flavell, R. A. & Jones, I. G. (1970). *Biochem. J.* **116**, 811.

Hollenberg, C. P., Borst, P., Thuring, R. W. J. & Van Bruggen, E. F. J. (1969). *Biochim. biophys. Acta*, **186**, 417.

Suyama, Y. & Miura, K. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 235.

Vinograd, J., Bruner, R., Kent, R. & Weigle, J. (1963). *Proc. natn. Acad. Sci. U.S.A.* **49**, 902.

The Effect of Substitution of Deuterium Oxide for Water on Very Lysine-rich Histone-Deoxyribonucleic Acid Complexes

By S. LEWIN and M. N. ROSMARIN.* (*Physical Biochemistry Laboratory, North East London Polytechnic, Waltham Forest Precinct, Forest Road, London E.17, U.K.*)

The substitution of D_2O for water is known to result in elevated 'melting'-temperature values and therefore in correspondingly increased helical stability of synthetic polynucleotides and nucleic acids (for references see Lewin, 1967a,b). The enhanced stabilities are interpretable in terms of

* Present address: Department of Chemistry, Birkbeck College, Malet Street, London W.C.1, U.K.

substitution of heavy-water bridges for ordinary water bridges and in terms of substitution of $-\text{O}-\text{D}\cdots\text{O}$ for $-\text{O}-\text{H}\cdots\text{O}$; the former bond is comparatively stronger than the latter (Lewin, 1969). The association of histones with DNA is also known to enhance the helical stability of DNA; this enhancement has been attributed to the histones bridging the two complementary DNA strands (for references see de Reuck & Knight, 1966). The association of histones with DNA via the ionic linkages involving the histone positively charged guanidinium (arginine) and ϵ -ammonium (lysine) groups has been computed to result in a significantly decreased extent of hydration directly involved in ionic linkages (Lewin, 1969, 1970); this applies also to displacement of water bridges as a result of direct hydrogen-bonding between the hydroxyl groups of the histone serine and threonine to DNA loci. Substitution of D_2O for water in aqueous solutions of histone-DNA would not be expected to result in increased helical stability if water bridges did not contribute effectively. We therefore investigated the variations in E_{255} of DNA and of lysine-rich histone F1-DNA associations with temperature, in both water and D_2O media. With a calf thymus histone F1-DNA ratio of 1:1, our results showed that at low ionic strength (1.5×10^{-3}) and pH 7 the curves were practically the same (and the resulting 'melting'-temperature value was $78.5 \pm 0.5^\circ\text{C}$). Simultaneous experiments under otherwise identical conditions confirmed the increase in 'melting' temperature of DNA on D_2O substitution for water (from $51.5 \pm 0.5^\circ\text{C}$ to $57.2 \pm 0.2^\circ\text{C}$).

Lewin, S. (1967a). *J. theor. Biol.* **17**, 181.

Lewin, S. (1967b). *Studia biophys.* **4**, 29.

Lewin, S. (1969). *Abstr. FEBS 6th Meet., Madrid*, p. 328.

Lewin, S. (1970). *J. theor. Biol.* **26**, 481.

de Reuck, A. V. S. & Knight, J. (1966). *Histones*.

London: J. and A. Churchill Ltd.

The Mechanism and Energetics of Histone Associations with Deoxyribonucleic Acid

By S. LEWIN. (*Waltham Forest Precinct, North East London Polytechnic, Forest Road, London E.17, U.K.*)

I have used correctly proportioned space-filling molecular models (Courtauld) and energetic considerations in evaluating the potential of association of histones with DNA. The selective removal of histones from chromatin requires increasingly higher electrolyte concentrations (for references see Busch, 1965), from which computations can be made as to the relative energy values