

## LETTERS TO THE EDITORS

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## Molecular Weights and Physical Properties of Deoxyribonucleic Acid

IN the measurements of the molecular weight from light scattering and of other physical properties of deoxyribonucleic acid, an extraordinary variety of results has been obtained<sup>1</sup>, even with samples prepared from a single source, namely, calf thymus. To some extent this might be accounted for as a result of fractionation of the material resulting from its physical heterogeneity, which has been demonstrated by Shooter and Butler<sup>2</sup>, and by Schachman *et al.*<sup>3</sup>. It may also arise from small amounts of protein impurity, which have been shown<sup>4</sup> to affect the physical properties to a marked extent. In order to reduce the sources of discrepancy, it has been thought desirable to determine (1) light scattering, (2) intrinsic viscosity, (3) sedimentation coefficients, on identical solutions of calf thymus deoxyribonucleic acid treated in various ways. It has been found that centrifuging as a means of clarifying the solutions is made much more effective by reducing the concentration from 0.1 to 0.04 per cent. Table 1 shows the effect of centrifuging for 18 hr. at 20,000*g* at these two concentrations. The difference must be due to the greater viscosity of the stronger solution. The light-scattering molecular weight, especially of the more turbid samples, is markedly reduced by centrifuging in this way at the lower concentration, without any great change of either the intrinsic viscosity or of the sedimentation coefficients. This must mean that the component which gives rise to high turbidities in the unclarified solutions does not contribute appreciably to the viscosity and sedimentation coefficient. That the high turbidities of the unclarified solutions is not due to deoxyribonucleic acid has been demonstrated by the fact that the action of deoxyribonuclease on a clarified solution (*J40*) with molecular weight  $4 \times 10^6$  reduced the light scattering to 20 per cent of its original value; while on a turbid solution (*J38*), having an apparent molecular weight of  $17 \times 10^6$ , the light scattering value was reduced by only 40 per cent, that is, to  $10 \times 10^6$ .

The results obtained with a number of preparations when treated in this way now agree very well with each other in most cases (Table 2), and in the exception (*J38*) it has been shown that the high value is not due to deoxyribonucleic acid itself. It will be seen, however, that the molecular weight determined from light scattering in the final solutions is less by a factor of 3 than that determined from the intrinsic viscosity and mean sedimentation coefficient by the equation of Mandelkern *et al.*<sup>5</sup>. The cause of this

Table 1. EFFECT OF CENTRIFUGING DEOXYRIBONUCLEIC ACID SOLUTIONS FOR 18 HR. AT 20,000*g* AT DIFFERENT CONCENTRATIONS

Sample	Concentration (per cent)	Yield of deoxyribonucleic acid supernatant	$M_L \times 10^6$	$[\eta]$	$S_0$
<i>J 31 C</i>	0.1	88	>20	35	27
	0.04	66	3.0	35	27
<i>J 34</i>	0.1	92	12.2	34	25
	0.04	61	3.3	34	25

Table 2. PHYSICAL CONSTANTS OF DEOXYRIBONUCLEIC ACID SOLUTIONS AFTER CENTRIFUGING FOR 18 HR. AT 20,000*g* AT 0.04 PER CENT

Specimen of deoxyribonucleic acid	$M_L \times 10^6$	$[\eta]$	$S_0$	$M_{S,\eta} \times 10^6$
<i>31C</i>	3.0	35	27	10
<i>33</i>	3.3	29	25	8.5
<i>36</i>	3.3	33.4	28	10.8
<i>40</i>	4.0	50	22	9.0
<i>34</i>	3.3	34	25	9.0
<i>38</i>	17	47	23	9.5

discrepancy may be (1) failure of the light scattering theory when applied to very long rods; (2) the effect of the heterogeneity weighting the intrinsic viscosity in favour of the larger particles. These possibilities require further study.

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J. A. V. BUTLER  
D. J. R. LAURENCE  
A. B. ROBINS  
K. V. SHOOTER

Chester Beatty Research Institute,  
London, S.W.3.  
Oct. 1.

<sup>1</sup> Reichmann, M. E., Rice, S. A., Thomas, C. A., and Doty, P., *J. Amer. Chem. Soc.*, **76**, 3047 (1954). Sadron, C., Rep. 3rd Int. Cong. Biochem. (Brussels), 210 (1955). Brown, G. L., McEwen, M. B., and Pratt, M. I., *Nature*, **176**, 161 (1955).

<sup>2</sup> Shooter, K. V., and Butler, J. A. V., *Trans. Farad. Soc.*, **52**, 734 (1956).

<sup>3</sup> Schumaker, V. N., and Schachman, H. K., *Biochim. Biophys. Acta*, **23**, 628 (1957).

<sup>4</sup> Butler, J. A. V., Phillips, D. M. P., and Shooter, K. V., *Arch. Biochem.* (in the press).

<sup>5</sup> Mandelkern, L., Krigbaum, W. R., Scheraga, H. A., and Flory, P. J., *J. Chem. Phys.*, **20**, 1392 (1952).

## Optical Rotation and Infra-Red Spectra of some Polypeptide and Protein Films

It is well known that the  $\alpha$ -helix configuration of Pauling and Corey<sup>1</sup> is associated with a carbonyl stretching mode in the neighbourhood of 1,652–1,655  $\text{cm}^{-1}$  in films of simple enantiomorphic polypeptides<sup>2,3</sup>. Since this frequency is also found in the infra-red spectra of films prepared from a number of native proteins<sup>4–6</sup> it is of interest to know whether other configurations besides the  $\alpha$ -helix are associated with it, for some X-ray diffraction photographs of fibrous proteins suggest a mixture of crystalline and amorphous forms. In the case of water-soluble films of *Bombyx mori* silk fibroin<sup>7</sup>, a completely amorphous X-ray diffraction pattern can be obtained, along with a carbonyl band in the infra-red spectrum at 1,660  $\text{cm}^{-1}$ . It is difficult to think that an  $\alpha$ -helix arrangement would not produce sufficient order to reveal the characteristic intensity diffraction pattern of this helix.

The dispersion of optical rotation has recently been shown to be a very useful method for investigating