Durch Einsetzen der Zahlen $L' = 3 \cdot 10^{-4}$, $T = 300°$ abs., $\eta_0 = 10^{-2}$, $r = 10^{-7}$, $q = 3{,}4 \cdot 10^{-7}$ erhält man hieraus die für den konkreten Fall im Text genannte Zeit von $1{,}3 \cdot 10^7$ s oder 150 Tagen.

Summary

The time requirement is treated for a longitudinal fission by Brownian movement of a very long particle consisting of two or more filaments twisted a great number of times round each other to form a double spiral. It is shown that a comparatively swift disintegration is obtained by partial rotation or torsion round the axis of the spiral, resulting in a loosing of the spiral structure and subsequent separation of the constituents by translational Brownian movement. The time required to separate a double spiral consisting of about 900 turns of a height of $3{\cdot}4 \times 10^{-7}$ cm and a radius of 10^{-7} cm, thus having a length of 3×10^{-4} cm being realized approximately by deoxyribonucleic acid is found by this mechanism to be about 50 to 80 s. The time required to undo the same spiral by unwrapping it turn by turn would be about 150 days. The result of the considerations is related to observations published by ALEXANDER and STEACY on deoxyribonucleic acid. An additional remark stresses the importance of stereochemical asymmetry for the practicability of the mechanism and therefore the importance of optical activity for the time requirement of such disintegrations or transformations of high polymer material occurring in living organisms.

The Influence of Concentration on Dissociation of Deoxyribosenucleic Acid by 4 Molar Urea

In earlier publications[1] we had shown that the molecular weight of deoxyribonucleic acid (DNA) as determined by light-scattering fell to one half its value in dilute salt solution when sufficient urea was added to bring the final concentration to 4 *M*. This and the associated changes in the shape of the molecule were consistent with the view that a longitudinal splitting of the double molecule had occurred. In all these experiments concentrated urea was added to solutions containing less than 0·04% of DNA since these were most convenient for light-scattering experiments. When urea was added to solutions containing more than 0·08% DNA dissociation no change was observed in molecular weight within 3 h (Fig. 1). Since it was not possible to measure molecular weights directly at these concentrations the experiments were carried out as follows.

Equal volumes of the initial DNA solution and a solution of 8 *M* urea were mixed and allowed to stand. They were then diluted four times with dilute salt solution so that the final urea concentration was 1·0 *M* and the light scattering measurements made. A parallel experiment was made in which the final concentration of DNA and urea were the same but the dilutions were made in such a way that the DNA was never exposed to urea of more than 1·0 *M*. This concentration is not capable of dissociating DNA and a molecular weight of 6×10^6 ions was found in this solvent (Fig. 2). If DNA at a concentration of 0·04% or less was treated with 4 *M* urea and subsequently diluted with water to 1·0 *M* urea the molecular weight was found to be 3×10^6 (Fig. 2), but, if the same experiment is done with more concentrated DNA solutions (> 0·08%) then a molecular weight of 6×10^6 is found on a subsequent dilution, proving that 4 *M* urea was not able to split DNA at these higher concentrations. No reliable data could be obtained for DNA solutions in between the two ranges of DNA concentration studied (Fig. 1) but this is not surprising since the situation in this intermediate range (0·05 to 0·08%) would be expected to be very complex.

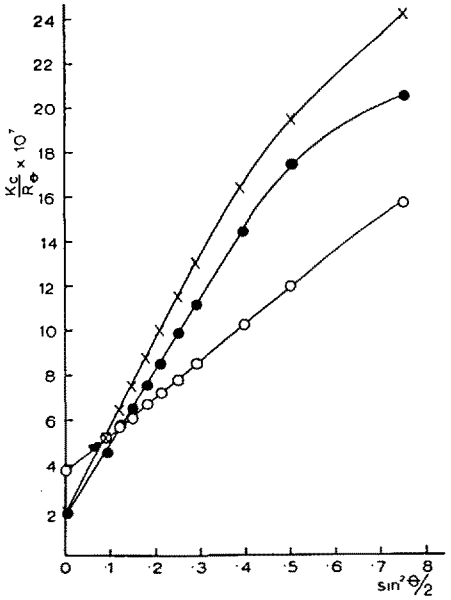

Fig. 1. — Limiting lines of Zimm plots of light scattering data of DNA from herring sperm (for definition of symbols see [1]). The intercept is inversely proportional to the weight average molecular weight of the dissolved DNA.

-•-•- 0·01% DNA containing 1·0 *M* urea and 0·2 *M* NaCl (Molecular weight 6×10^6).

-o-o- To 0·08% DNA an equal volume of 8 *M* urea was added (i.e. solution was 0·04% DNA in 4 *M* urea) and diluted 2 h later to finish up as 0·01% DNA containing 1·0 *M* urea and 0·2 *M* NaCl (Molecular weight $\sim 3 \times 10^6$).

-x-x- To 0·16% DNA an equal volume of 8 *M* urea was added (i.e. solution was 0·08% DNA in 4 *M* urea) and diluted 2 h later to finish up as 0·01% DNA containing 1·0 *M* urea and 0·2 *M* NaCl (Molecular weight 6×10^6).

The reason for this remarkable concentration dependance of the dissociation of the twin molecule can not be found in a mass action phenomenon: since the urea is present in vast excess, there can be no question that the equilibrium is completely on the side of the dissociated molecule. An explanation may be found in Professor W. KUHN's suggestion[2] that length-wise association of the double molecules would prevent the rotational movement of the two threads relative to one another and thus make the relatively facile disentangling process envisaged by KUHN[2] impossible. Electron micrographs of DNA (Fig. 3), show that it is possible for these long molecules to aggregate in parallel and in fact under the conditions of drying on the supporting film a whole range of aggregates have been observed.

[1] P. ALEXANDER and K. A. STACEY, Biochem. J. *60*, 194 (1955).

[2] W. KUHN, Exper. *13*, 301 (1957).

Temporary contact of this kind in urea solution would completely inhibit the torsional motion necessary for the untwisting, and clearly the chance of its happening would rapidly increase with increasing concentrations. In order to prevent this aggregation during the drying of the specimen on the E. M. grid it is necessary to dilute the solution 100 fold from that used to prepare the specimen shown in Figure 3. So it is clear that there exists, despite the repulsive action of the ionized phosphate groups, strong attractive forces between DNA molecules. The failure to observe any aggregation by light scattering shows that this can only be of a very temporary nature although the small value of the second virial coefficient may be regarded as suggestive of interparticle attraction.

So far we have been unable to demonstrate a similar splitting by calf thymus nucleic acid as it is ordinarily prepared[3]. After prolonged exposure to a relatively concentrated solution of a sequestering agent, sodium ethylene-diamine tetracetate (versene), it was found that in 4 *M* urea this too slowly split into two half molecules of molecular weight 3×10^6. Since JUNGNER[4] had reported the presence of a surprisingly large amount of magnesium in calf thymus DNA it seemed possible that these bivalent ions might be responsible for the greater stability of DNA from this source. But we have been unable by trace metal analysis to confirm JUNGNER's findings and can detect no significant amounts of magnesium or any other trace metal in DNA obtained from either herring sperm or calf thymus. There is, however, a marked difference between the two in the amount of residual protein.

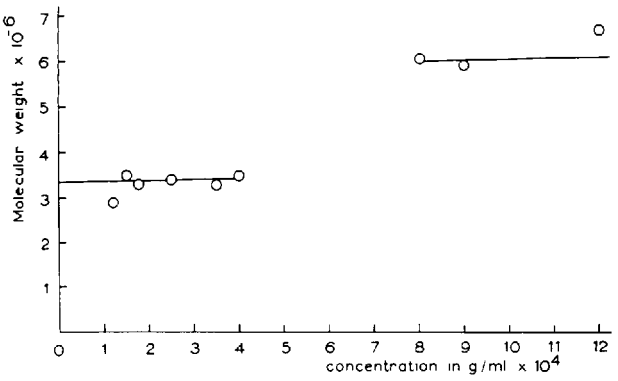

Fig. 2. – Molecular weight determined from light scattering data of DNA from herring sperm after standing at different concentrations in 4 *M* urea.

To show this effect clearly the concentration of versene required is 0·1 *M* which is the strength at which this and other chelating agents are effective in the method KIRBY has developed for the separation of DNA from protein[5]. It is clear from the great difference in size, a factor over a hundred, that a trace of protein contaminant of this order, could provide sufficient polypeptide chains adhering to those of nucleic acid to inhibit the unfolding in urea. It seems possible therefore that the action of versene on DNA from calf thymus consists of the removal of this trace of protein impurity which may interfere with the disentangling process.

[3] P. ALEXANDER and K. A. STACEY, Nature *176*, 162 (1955).
[4] G. JUNGNER, Science *113*, 378 (1951).
[5] K. S. KIRBY, Biochem. J. (in press).

The electron micrograph was taken on a Siemens electron microscope (Model UM 100) by Mr. M. S. C. BIRBECK, for whose cooperation we are most grateful. We

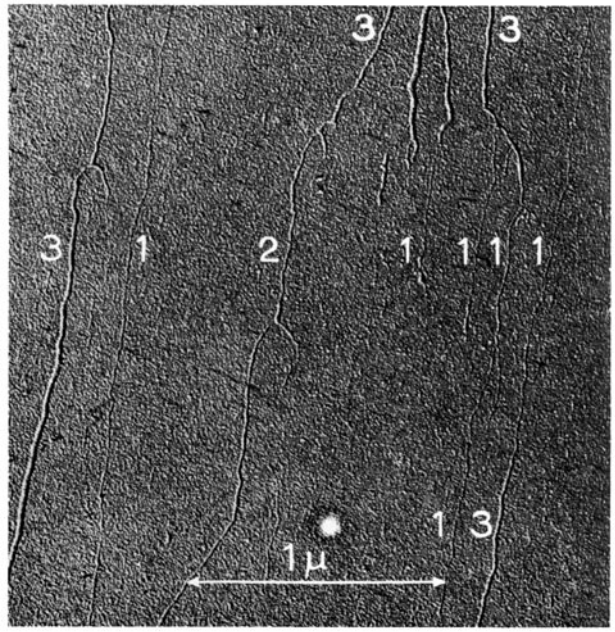

Fig. 3. – Electron micrograph of DNA from herring sperm; magnification 96,000. The width of shadow (platinum) of the thinnest fibres is 100 *A* which corresponds to a fibre width of 20 *A* under the condition of shadowing used. The numerals written in the fibres indicate the estimated number of double-helix threads which lie side by side to make up the fibre.

have developed a new technique for the preparation of DNA specimens which will be described in a future publication.

We would also like to thank Dr. K. S. KIRBY for several protein analyses and for valuable discussions and Mr. A. S. NICKELSON of Atomic Energy Authority for the very accurate trace metal analyses.

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P. ALEXANDER and K. A. STACEY

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, February 4, 1957.

Zusammenfassung

DNA spaltet in 4-*m*-Harnstofflösung, wenn die DNA-Konzentration weniger als 0,04% beträgt, in 2 gleich grosse Teile auf. Elektronenmikroskopische Aufnahmen zeigen, dass das Ausbleiben einer Dissoziation in höher konzentrierten Lösungen durch Parallelzusammenlagerung der Fäden bedingt sein dürfte, eine Zusammenlagerung, welche den von W. KUHN in der vorangehenden Mitteilung für die Längsteilung vorgeschlagenen Mechanismus behindern würde.