[8] Sharpe, R., "X-ray Microscopy and Microradiography", 590 (Acad. Press, New York, 1957).
[9] Andrews, K. W., and Johnson, W., "X-ray Microscopy and Microradiography", 581 (Acad. Press, New York, 1957).
[10] Trillat, J. J., *Rev. Sci.*, Paris, **78**, 212 (1940); *Rev. Métall.*, **46**, 79 (1949).
[11] Engström, A., "Physical Techniques in Biological Research", 3, chapter 10 (Acad. Press, New York, 1956).
[12] Engström, A., and Lundberg, B., *Exp. Cell. Res.*, **12**, 198 (1957).
[13] Combée, B., and Engström, A., *Biochim. Biophys. Acta*, **14**, 432 (1954).
[14] Czermak, P., *Ann. Physik, Lpz.*, **60**, 760 (1896).
[15] Sievert, R., *Acta Radiol.*, Stockh., **17**, 299 (1936).
[16] Ardenne, M. von., *Naturwiss.*, **27**, 485 (1939).
[17] Cosslett, V. E., and Nixon, W. C., *Nature*, **168**, 24 (1951); *J. App. Phys.*, **24**, 616 (1953).
[18] Saunders, R. L. de C. H., "X-ray Microscopy and Microradiography", 561 (Acad. Press, New York, 1957); *Nature*, **180**, 1353 (1957).
[19] Barclay, A. E., "Micro-arteriography" (Blackwell, Oxford, 1951).
[20] Bellman, S., *Acta Radiol.*, Stockh., Supp. 102 (1953).
[21] Smith, D. S., "X-ray Microscopy and Microradiography", 492 (Acad. Press, New York, 1957).
[22] Nixon, W. C., *Proc. Roy. Soc.*, A, **232**, 475 (1955).
[23] Ong, S. P., and Le Poole, J. B., *App. Sci. Res.*, **7**, 233 (1958).
[24] Lindström, B., *Acta Radiol.*, Stockh., Supp. 125 (1955).
[25] Röckert, H., *Acta Odont., Scand.*, **16**, Supp. 25 (1958).
[26] Long, J. V. P., *J. Sci. Instr.*, **35**, 323 (1958); thesis, Cambridge (1959).
[27] Mosley, V. M., and Wyckoff, R. W. G., *J. Ultrastructure Res.*, **1**, 337 (1958).
[28] Castaing, R., and Guinier, A., Proc. Conf. Electron Microscopy, Delft, 1949, p. 60 (Martin Nijhoff, Delft, 1950). Castaing, R., thesis, Paris (1951).
[29] Cosslett, V. E., and Duncumb, P., *Nature*, **177**, 1172 (1956); "X-ray Microscopy and Microradiography", 374 (Acad. Press, New York, 1957).
[30] Melford, D. A., and Duncumb, P., *Metallurgia*, **57**, 341 (1958).
[31] Long, J. V. P., and McConnell, J. D. C., *Miner. Mag.* (in the press).
[32] Agrell, S. O., and Long, J. V. P. (unpublished work).
[33] Mulvey, T., and Campbell, A. J., *Brit. J. App. Phys.*, **9**, 406 (1958).
[34] Dolby, R. M., *Proc. Phys. Soc.*, **73**, 81 (1959).

HETEROGENEITY IN DEOXYRIBONUCLEIC ACIDS

I. Dependence on Composition of the Configurational Stability of Deoxyribonucleic Acids

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THE base-pair interactions that unite the two chains of deoxyribonucleic acid in its native, helical configuration are of two kinds: adenine–thymine and guanine–cytosine[1]. Because these pairs involve different numbers of hydrogen bonds[2] and are otherwise dissimilar, it is to be expected that they contribute differently to the stability of the helix. We wish to report here the magnitude of this effect (which is substantial), its use in estimating the extent of heterogeneity in composition within samples of deoxyribonucleic acid and to some degree within its molecules, and its importance in inactivation studies.

Upon heating neutral solutions of deoxyribonucleic acid, striking changes in some properties[3] (for example, viscosity, extinction coefficient, light scattering and optical rotation) are known to occur in a relatively narrow range of temperature. These changes reflect a transition from the double-stranded helical configuration to a disordered coil: the transition is essentially the melting of one-dimensional crystallites and may be characterized by the temperature at the midpoint of the transition, T_m. In earlier studies[4] on calf thymus deoxyribonucleic acid there appeared some indications that the transition consisted of a series of overlapping transitions of individual deoxyribonucleic acid molecules having different melting temperatures.

In particular, the transition was broader than expected for such very long, perfectly formed helices, and at temperatures within the transition region the viscosity would quickly reach a constant value and fall further only if the temperature was increased[5]. Furthermore, electron microscopy revealed that within the transition region only two types of deoxyribonucleic acid molecules could be discerned, namely, completely denatured or native ones[6]. The most reasonable explanation lay in assuming that the two hydrogen-

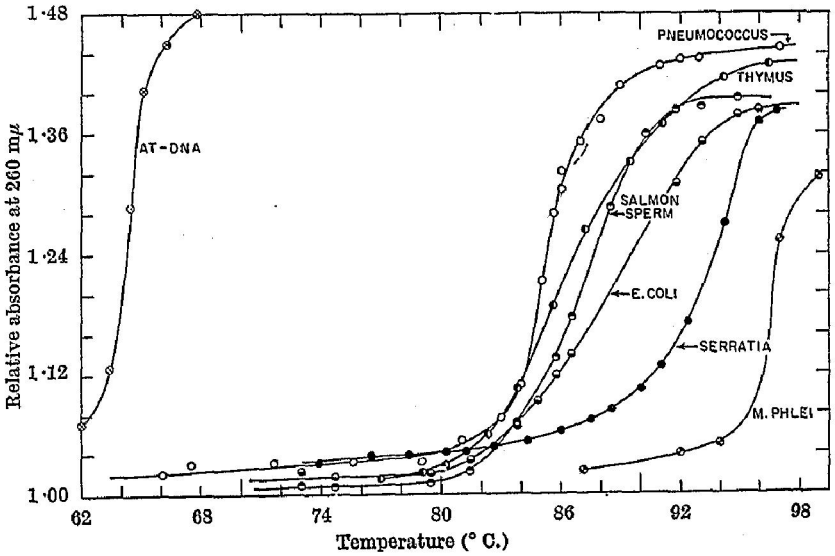

Fig. 1. Variation in absorbance (260 mμ) as a function of the temperature of the deoxyribonucleic acid solution. The result for bacteriophage T_4r deoxyribonucleic acid is not plotted because of serious overlap with *D. pneumoniae*. It was sharper and had T_m equal to 84°. In all cases the solvent was 0·15 *M* sodium chloride plus 0·015 *M* sodium citrate. The samples of bacterial deoxyribonucleic acid were deproteinized by successive mild shakings with chloroform and *iso*amyl alcohol. The samples of animal deoxyribonucleic acid were prepared by the Simmons technique using detergents (see ref. 5). The bacteriophage T_4r deoxyribonucleic acid was a gift from Mr. J. Fleichman

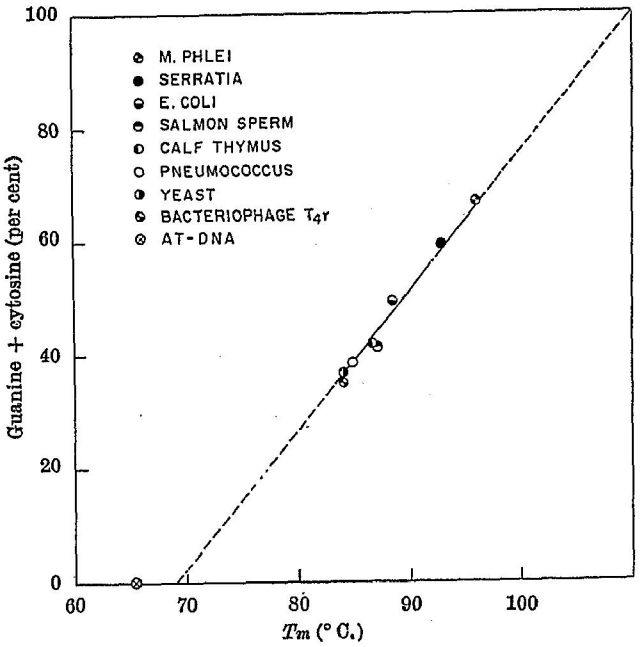

Fig. 2. Dependence of the denaturation temperature, T_m, on the guanine–cytosine content of various samples of deoxyribonucleic acid

bonded base pairs (guanine–cytosine and adenine–thymine) contributed unequally to the stability of the helix, thereby causing T_m to depend on the mean composition of either whole deoxyribonucleic acid molecules or large segments thereof.

In order to test this proposal, we have prepared a number of protein-free samples of deoxyribonucleic acid of different base composition from various sources (see Fig. 1) and determined their transition profiles by measuring the absorbance at 260 mμ as a function of the temperature of the solution. These are recorded in Fig. 1, and the melting temperatures are plotted as a function of the guanine–cytosine content[7] in Fig. 2. In the solvent used (0·15 *M* sodium chloride and 0·015 *M* sodium citrate), it is seen that T_m ranges from 85° C. for deoxyribonucleic acid from *Diplococcus pneumoniae* to 97° C. for that from *Mycobacterium phlei* in a manner directly proportional to the guanine–cytosine content. The T_m values are reproducible to within 0·5 deg. for different carefully prepared samples, and hence they may be considered as intrinsic characteristics of total deoxyribonucleic acid from a given species.

The extrapolation of the results in Fig. 2 permits an estimation of the T_m of deoxyribonucleic acid made exclusively of one base-pair or the other; for adenine–thymine this is 69° C. and for guanine–cytosine it is 110° C. The gift of a sample of adenine–thymine deoxyribonucleic acid[8] allowed us to check one of these predictions. The melting profile is shown at the extreme left of Fig. 1; its T_m value is 65° C. (In contrast to samples of naturally occurring deoxyribonucleic acid, the absorbance–temperature curves of which are far from being reversible, this adenine–thymine deoxyribonucleic acid exhibited complete reversibility. This behaviour would only be possible if the adenine–thymine sequence was strictly periodic.) In addition to verifying the dependence of T_m on composition, the magnitude of the hyperchromicity observed for this sample (52 per cent) supported the trend seen in the naturally occurring deoxyribonucleic acid samples between the hyperchromicity and the composition. In these the hyperchromicity decreased from about 44 to 34 per cent as the guanine–cytosine content increased from 38 to 67 per cent. Thus, in the limit of pure guanine–cytosine deoxyribonucleic acid, the hyperchromicity would probably be only about 25 per cent. Of course, this can only be a rough correlation, because the hyperchromicity in a given sample probably depends on sequence as well as overall composition.

An estimate of the dispersion of the guanine–cytosine content among the molecules of a given sample can be made in the following way. The melting profile of the adenine–thymine deoxyribonucleic acid, since it is of high molecular weight ($S_{20,w}$ = 12·6 *S*.) and with no heterogeneity due to composition, can be assumed to represent the natural transition width due to the intrinsic nature of this co-operative phenomenon. The displacements of this curve from its T_m can then be subtracted from the other melting profiles to give corrected profiles that should reflect only the spread due to heterogeneity of composition. This has been done for the samples studied with the following results. The transition of T_4r bacteriophage deoxyribonucleic acid had the same sharpness as the adenine–thymine deoxyribonucleic acid; hence the deoxyribonucleic acid molecules in this case appear to have essentially identical composition. The profiles for *D. pneumoniae* and *M. phlei* were only slightly broader. The remaining samples of bacterial deoxyribonucleic acid showed increased broadening of the transition, indicating significant heterogeneity in composition. The broadest profile is that from calf thymus; the central part of this transition (50 per cent of the rise in absorbance) covers about 4°. Since the slope of the line in Fig. 2 equates a change of 1 deg. in T_m to a 2·5 per cent change in composition, the half-width of the distribution of guanine–cytosine residues in calf thymus deoxyribonucleic acid can be estimated as extending ± 5 per cent about the mean of 49 per cent guanine–cytosine.

With calf thymus deoxyribonucleic acid appearing to have a relatively broad distribution of composition, an attempt was made to recover undenatured deoxyribonucleic acid from a partially denatured sample and to see if it had a higher melting profile. A sample heated to 90°, corresponding to 80 per cent of the transition, was cooled and carried through two successive precipitations with streptomycin. The resulting sample exhibited a melting curve that was displaced 1·5 deg. higher than the calf thymus curve in Fig. 1. Thus, it is seen that components of different T_m are indeed contained within calf thymus deoxyribonucleic acid.

In a further experiment, calf thymus deoxyribonucleic acid was degraded sonically to one-twelfth of its original molecular weight. Its melting profile was then only very slightly broadened. This indicated that the heterogeneity observed here exists on a scale very much smaller than that of whole molecules.

The relatively small dispersion in the distribution of guanine–cytosine among the samples of bacterial deoxyribonucleic acid, viewed in conjunction with the very broad range of mean composition, leads to the interesting conclusion that there is essentially no overlap in the composition of the deoxyribonucleic acid molecules of many bacteria. This point is confirmed and discussed in the following communication.

The relation between the transition profile and the inactivation of the transforming ability of *D. pneumoniae* deoxyribonucleic acid has been examined in two ways. When this deoxyribonucleic acid undergoes extensive inactivation by ultra-violet irradiation,

no significant changes in its macromolecular properties can be detected. However, the transition profile is relatively sensitive to irradiation, being shifted to successively lower temperatures. For example, at 10 per cent remaining streptomycin transforming activity, T_m is 1·5 deg. lower. Thus, what is generally considered to be a very small amount of chemical alteration can be detected by the alteration of the absorbance–temperature profile.

When the thermal inactivation of various genetic markers is compared with the transition profile, it is found that the inactivation generally lags behind to varying degrees. This may arise either from a partial reversibility of the transition, that is, a re-healing of some molecules that are only partially melted out, or from our happening to select markers that are carried by the deoxyribonucleic acid molecules having higher T_m values.

We wish to thank Miss D. Lane, Mr. J. Kucera and Mr. L. Matthews for essential contributions to experimental work presented here. We are grateful to Drs. J. Fresco and N. Sueoka for helpful discussions. This investigation has been supported by a grant from the U.S. Public Health Service, C-2170.

[1] Watson, J. D., and Crick, F. H. C., *Proc. Roy. Soc.*, A, 233, 80 (1954).
[2] Pauling, L., and Corey, R. B., *Arch. Biochem. Biophys.*, 65, 164 (1956).
[3] See, for example, Doty, P., *J. Cell. Comp. Physiol.*, 49 (1), 27 (1957).
[4] Doty, P., Boedtker, H., Fresco, J. R., Haselkern, R., and Litt, M., *Proc. U.S. Nat. Acad. Sci.*, 45, 482 (1959).
[5] Rice, S. A., and Doty, P., *J. Amer. Chem. Soc.*, 79, 3937 (1957).
[6] Hall, C. E., and Litt, M., *J. Biophys. Biochem. Cytology*, 4, 1 (1958).
[7] Taken from the compilation by Chargaff, E., "The Nucleic Acids", 307 (Academic Press, New York, 1955), and Lee, K. Y., Wahl, R., and Barbu, E., *Ann. Inst. Pasteur*, 91, 212 (1956).
[8] This sample was a gift from J. Adler, I. R. Lehman and A. Kornberg. See Lehman, I. R., *et al.*, *Proc. U.S. Nat. Acad. Sci.*, 44, 1191 (1958).

II. Dependence of the Density of Deoxyribonucleic Acids on Guanine–Cytosine Content

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The discovery by Meselson, Stahl and Vinograd[1] of a technique of banding deoxyribonucleic acids in the density gradient established in the cell of an analytical ultracentrifuge has made possible the precise determination of the density of samples of deoxyribonucleic acid in microgram amounts. With this sensitive method available, it was of interest to see if a variation of density with guanine–cytosine content could be detected that would parallel that found for the denaturation temperature, reported in the foregoing communication. A systematic examination of samples of deoxyribonucleic acid having different guanine–cytosine contents showed that there was indeed a significant dependence. We wish to summarize these results here and to indicate the use of this effect in estimating the heterogeneity of composition in deoxyribonucleic acid samples and in studying the denaturation of deoxyribonucleic acid. The estimates of heterogeneity of composition for samples of bacterial deoxyribonucleic acid are unexpectedly small; the implications of this are briefly discussed.

The experimental technique employed was precisely that described by Meselson *et al.*[1]. About 3 μgm. of deoxyribonucleic acid was added to a concentrated solution of cæsium chloride (about 8 *M*) adjusted to the anticipated density. This was centrifuged in the 'Spinco' Model E ultracentrifuge at 44,770 r.p.m. at 25° C. for 24 hr. By this time the deoxyribonucleic acid had banded and its distribution in the band had become essentially constant. The distribution was recorded by photographing the cell using ultra-violet light and making a microdensitometer trace. As an illustration of the effect observed, Fig. 1 shows a photograph of a solution containing nearly equal amounts of four samples of deoxyribonucleic acid and the corresponding trace. The resolution of the four bands is evident. The results of a systematic study are shown in Fig. 2, where the density is plotted against guanine–cytosine content. It is seen that a linear relation is found; it corresponds to a change of 0·00103 in density per 1·0 per cent change in guanine–cytosine content. Since the uncertainty in determining densities in this way is about 0·001, it follows that the guanine–cytosine content of a sample of deoxyribonucleic acid can be determined from its density with a precision of about 1 per cent.

The extrapolation of the results for naturally occurring deoxyribonucleic acid to the extremes of the composition axis indicates that adenine–thymine deoxyribonucleic acid should have a density of 1·662 and guanine–cytosine deoxyribonucleic acid 1·764. The measured density for the former is 1·680. The difference may be attributed to the adoption of a slightly more compact structure when guanine–cytosine pairs need not be accommodated[2].

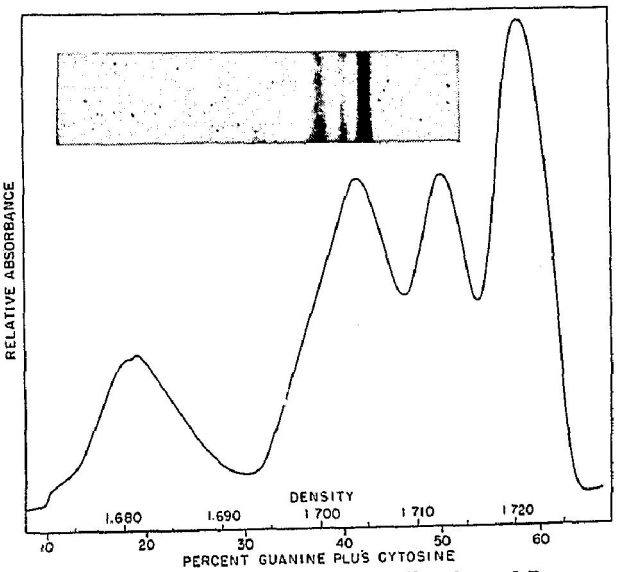

Fig. 1. Resolution of polydeoxy adenine–thymine and *Pneumococcus*, *E. coli* and *Serratia marcescens* deoxyribonucleic acids by density-gradient centrifugation. The photograph was taken after centrifugation at 44,770 r.p.m. for 24 hr. The tracing was taken with a microdensitometer in the region of the four bands. (The composition scale does not apply to the adenine–thymine deoxyribonucleic acid)