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THE HELIX-COIL TRANSITION IN DNA

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"We believe a gene—or perhaps the whole chromosome fibre—to be an aperiodic solid."
Erwin Schrödinger, What is Life? The Physical Aspect of the Living Cell, Macmillan, New York, 1945 (Russ. Transl., IL, M., 1947, p. 88).

I. INTRODUCTION

As we know, the genetic information that comprises the overall construction plan of a living organism is coded in the sequence of monomer links of a giant molecule of deoxyribonucleic acid (DNA). Figure 1 shows the structure of DNA. The DNA molecule is a complex of two polymer chains bound together by intermolecular forces. Each chain in the complex forms a right-hand helix, and it consists of a sugar-phosphate "backbone" having nitrogenous bases of four types attached to it: adenine (A), guanine (G), thymine (T), and cytosine (C). The repetitive element of the chain (nitrogeneous base + sugar + phosphate) is called a nucleotide. Thus, DNA consists of two mutually-twisted polynucleotide chains. While the bonds between the nucleotides within each of the chains are rigid and covalent, and have an energy of about 60 kcal/mole (3 eV), it is an essential fact that the bonds between the polynucleotide chains are at least an order of magnitude weaker. These chains obey a strict complementarity (correspondence) rule. Namely, thymine is always found opposite adenine, and cytosine opposite guanine. The complementarity is determined by the steric correspondence of the bases. Here the complementary base pairs are stabilized both by hydrogen bonds (shown by the dotted line in Fig. 1), and simply by electrostatic and London forces. The interaction between neighboring base pairs in the double helix is essential for the stability of DNA. The structural parameters of DNA are the following: the diameter of the molecule is ≈ 20 Å; the distance between adjacent base pairs is ≈ 3.4 Å; and 10 base pairs occur per turn of the helix, so that adjacent pairs are twisted with respect to one another by 36° (see Note 1 at the end of the article). As a rule, DNA consists of 10^4–10^5 links (see Note 2). Thus, the macromolecule of DNA is a one-dimensional aperiodic solid (or crystal). This solid is one-dimensional because each unit cell (base pair) interacts only with two adjacent cells (the coordination number if two).

DNA is an aperiodic crystal because it consists of a sequence of unit cells of two types: the nucleotide pairs AT and GC. This sequence cannot be periodic, just as

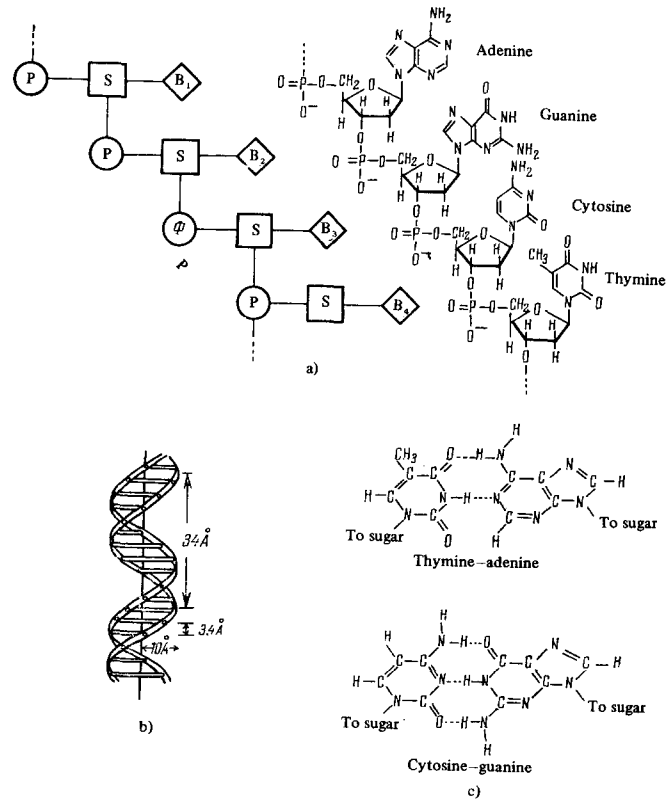


FIG. 1. Structure of DNA. a) A fragment of a single polynucleotide chain: at left—schematic diagram (P denotes phosphate, S is sugar (deoxyribose), and B_1, B_2, etc. are different nitrogenous bases), and at right—the chemical structure (the carbon atoms in the deoxyribose ring and in the nitrogenous bases, as well as the hydrogen atoms on the carbon atoms of the bases, are not depicted); b) approximate parameters of the double helix of DNA in the B-form; c) combination of the complementary bases by hydrogen bonds (dotted lines).

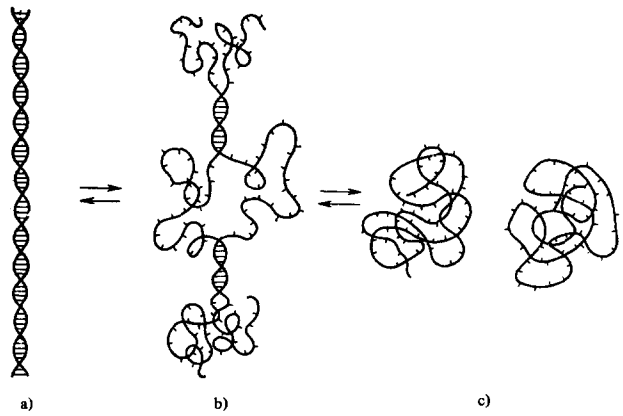


FIG. 2. Schematic diagram of the helix–coil transition in DNA. a) Completely helical state (low temperature); b) partly melted state (a temperature in the transition region); c) completely melted state with separated strands.

the sequence of letters in the meaningful text of a book is not periodic, since genetic information is contained in the order of succession of the nucleotides.

Study of both the experimental and theoretical properties of such a one-dimensional aperiodic crystal is of undoubted interest for physics. Physical studies are also important for understanding the genetic function of DNA, which consists in preservation, reproduction, and realization of the genetic information (see Note 3).

Among the physical processes that occur in DNA, an especial place is occupied by the relatively well-studied process of melting of the DNA molecule, which is called the helix-coil transition. Figure 2 depicts the helix-coil transition schematically. The single-stranded DNA that is formed in the helix-coil transition is a flexible polymer chain. Both the sugar-phosphate skeleton and the nitrogeneous bases acquire great freedom of rotation with respect to the single bonds. The statistical segment of the single-stranded molecule (the distance between links in single-stranded DNA is 6.8 Å) is about 7 links (see Note 4). This means that the single-stranded polynucleotide in solution behaves like a chain of freely-jointed segments containing seven nucleotides each. Such a chain has a Gaussian distribution of distances between the ends, and is called a Gaussian or random coil. The different states of a Gaussian chain are equivalent to the trajectories of a Brownian particle that has a free-path length equal to the length of the segment.

The helix-coil transition is reversible. Upon cooling, the complementary chains again form hydrogen bonds with one another. As before, the distance between bases along the sugar-phosphate chain is 6.8 Å. At the same time, direct van der Waals contact between neighboring base pairs at a distance of 3.4 Å is energetically favorable. In order that neighboring base pairs can approach to this distance, they must rotate by 36° with respect to one another. This requirement dictates the stereochemistry of the sugar-phosphate skeleton, and the rotation occurs in such a way that a right-hand DNA helix is always formed. DNA has a highly rigid structure in the helical state. Freedom of rotation about the single bonds is almost completely

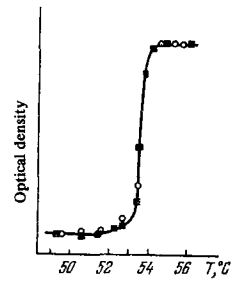


FIG. 3. Melting curve of a homopolynucleotide. [2]

ruled out. Nevertheless, the chain still keeps a certain flexibility, and two-stranded DNA is a coil having the anomalously large statistical segment of about 300 base pairs. This size of the statistical segment arises from continuous flexibility of the helix, rather than from local breakdown of the helical structure. That is, DNA is a wormlike (persistent), rather than a zigzag chain (see Note 4). Thus, the helix-coil transition is a reversible transition from the energetically favorable highly-ordered helical "crystalline" state to the disordered, "liquid" random-coil state. It is essential to emphasize that the sequence of bases in the chain remains just as strictly fixed in the coil as in the helix, since only the weak intermolecular forces are destroyed upon melting, while the covalent bonds within the sugar-phosphate chains remain unaffected. The problem of reversibility of the helix-coil transition is complicated by the fact that mutual searching of complementary regions is hindered after complete separation of the filaments, and kinetic factors enter in, so that hysteresis is often observed experimentally. However, one can avoid hysteresis by not carrying the melting process to the end, so that the strands remain attached at several points.

This review is concerned with the thermodynamic theory of the helix-coil transition in DNA. However, before we proceed to presenting the theory, we shall give very briefly the fundamental experimental data that must be adduced, both for selecting the theoretical model, and for formulating the question that the theory must answer.

II. EXPERIMENTAL DATA

The helix-coil transition can be observed by various methods: optical (absorption, optical activity), microcalorimetric, etc. The most widespread has been the method of measuring the absorbance of a DNA solution in the near ultraviolet (near 2600 Å). It is based on the hyperchromic effect, which is an increase in the absorbance of DNA upon helix-coil transition (see, e.g.[1]). This increase in absorbance is due to loss of the interaction of neighboring base pairs in the transition. If D is the absorbance of the solution, and D_{min} and D_{max} are its values that correspond to the completely helical and completely coil-like states of DNA, then the quantity $(D - D_{min})/(D_{max} - D_{min})$ gives the fraction of links $1 - s$ that occur in the coil-like (melted) state. Figure 3 gives as an example the melting curve for a two-stranded homopolynucleotide* (i.e.,

*Different two-stranded homopolynucleotides (both ribo- and deoxyribo-) have melting curves completely analogous in form. They differ from one another only in the melting temperature. Chamberlin [3] has made the most complete study of the melting curves of different two-stranded homopolynucleotides.

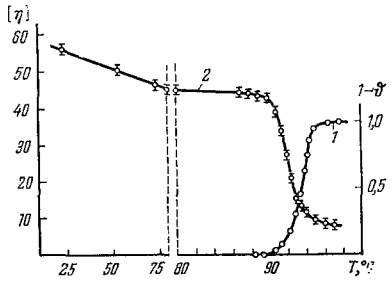


FIG. 4. The melting curve (1) as measured by the optical absorption, and the temperature-dependence of the characteristic viscosity (2) for T2 phage DNA. [4]

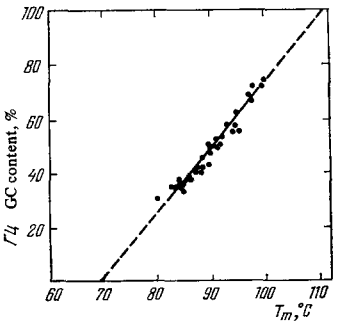


FIG. 5. Relation between the GC content of DNA and its melting point. [8]

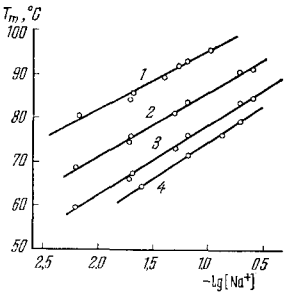


FIG. 6. Relation of the melting point T_m of DNA to the logarithm of the sodium ion concentration in the solution. The points are the experimental data: [9] 1—M. lysodeikticus (x_0 = 0.72); 2—E. coli (x_0 = 0.50); 3—S. saprophyticus (x_0 = 0.33); 4—M. mycoides var. capri (x_0 = 0.24). The straight lines are plotted according to Eq. (2) for the appropriate values of x_0.

a polynucleotide consisting of identical base pairs), and Fig. 4 shows the same for the DNA of T2 phage. The hydrodynamic characteristics, the sedimentation constant, viscosity, etc. (see Fig. 4, curve 2) also vary during the helix-coil transition. These quantities characterize the variation in dimensions of the macromolecule during the transition. Parallel measurements of the helicity by the optical method and of the molecular dimensions by the hydrodynamic method make it possible to find the mean length ν per helical region in the DNA molecule. Such measurements have been recently performed in[4,5] (see below, Fig. 19). The specific volume effects found in[4] proved to interfere with recalculation of the $\nu(\vartheta)$ relation from the experimental data. Hence, reliable enough values could be obtained only for $\vartheta > 0.8$. Finally, people have studied the helix-coil transition by microcalorimetry. The latter permits one not only to observe the helix-coil transition from the change in heat capacity of the solution, but also to determine the heat of transition.[6,7] However, a unique experimental technique is required for performing microcalorimetric studies. This greatly limits the possibilities of using the method.

The melting curve is conveniently characterized by two parameters: the melting point T_m, which corresponds to the inflection point of the curve, and the width ΔT of the melting range, which is equal to the difference between the temperatures at which the tangent at the inflection point intersects the levels $\vartheta = 0$ and $\vartheta = 1$ (ϑ is the helicity, i.e., the fraction of the links in the helical state). Thus,

$$\Delta T = \frac{1}{\left|\frac{\partial \vartheta}{\partial T}\right|_{\max}}. \qquad (1)$$

The varieties of DNA existing in nature differ rather widely in their relative content of AT and GC pairs. The melting temperature of the DNA increases with increasing concentration x_0 of GC pairs (x_0 = (G + C)/(A + T + G + C) (Fig. 5). As we see from the diagram, the dependence of T_m on x_0 is strictly linear. The melting point of DNA depends considerably on the medium in which the molecules are dissolved. Usually the DNA is kept in salt solution, sodium salts being used as a rule. At neutral pH, the melting point of DNA obeys the empirical formula

$$T_m = 176.0 - (2.60 - x_0)(36.0 - 7.04 \lg [Na^+]), \qquad (2)$$

where T_m is the melting point in °C, and $[Na^+]$ is the molecular concentration of Na ions. Figure 6 shows the experimental data obtained in[9] (points) for four different DNA specimens, and the straight lines are drawn according to Eq. (2), Eq. (2) holds up to $[Na^+]$ concentrations of the order of 1 M. As the salt concentration is increased further, a decrease in the rise in T_m is observed, while T_m begins to fall at very high salt concentrations. This phenomenon depends also on the anions of the dissolved salt.

The melting temperature also depends on the pH of medium, and drops considerably at pH values below 5 or above 9.

The width of the melting range of DNA is about 3°. On the other hand, $\Delta T \approx 0.5°$ for homopolynucleotides.

We should note that the various properties of DNA have been studied reliably at present primarily only under conditions in the medium close to standard. The standard conditions corresponding to those in the cell are: pH = 7, $[Na^+]$ = 0.195 M. Henceforth, unless expressly specified, we shall assume that DNA exists under these conditions.

Substances that bind strongly to the DNA molecule exert an especially strong effect on the melting curves. Below, we shall call molecules of such substances stabilizers. In contrast to agents that alter the properties of the medium as a whole (such as salts or the pH), stabilizers bind strongly to DNA and can substantially change the melting curves when their molar concentration D in the solution is considerably smaller than the molar concentration P of the bases contained in the DNA. This type of substances includes: heavy-metal ions (Cu, Fe, etc.), certain antibiotics (actinomycin)$_X$ and dyes (acridine orange, proflavin), and certain proteins.

As an example, Fig. 7 gives the relation of ΔT to the concentration of the protein ribonuclease. It is essential to emphasize that stabilizers are bound to DNA by intermolecular forces, and they redistribute themselves on the DNA molecule during the time of experiment. At each temperature, they occupy the thermodynamically most favorable state.

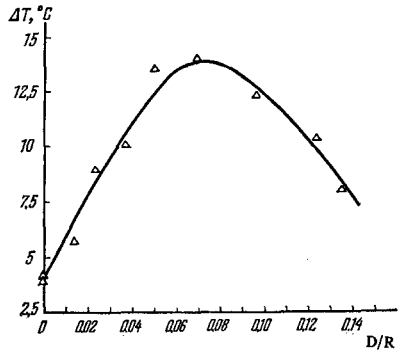


FIG. 7. Relation of the width of the melting range of DNA to the concentration of ribonuclease in the solution. [10]

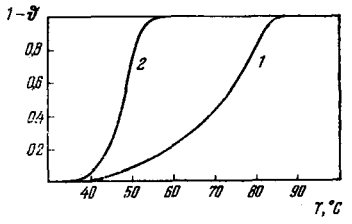


FIG. 8. Melting curves of annular, closed polyoma DNA (1), and of the same DNA after one of the strands has been broken (2), in 7.2M $NaClO_4$ solution. [12]

The given experimental data pertain to the linear, unclosed DNA that is usually studied in solution. A special annular, closed form of DNA is found in a number of viruses, and also in bacterial cells at certain stages in their development.

In this DNA, which exists as an ordinary double helix, each of the complementary strands is continuous and closed on itself. Hence, the total number of turns of one strand with respect to the other cannot vary under any changes in conditions that maintain the integrity of the sugar-phosphate skeleton of the two strands. The experiments that have been performed have shown[11] that at room temperature the double helix of annular DNA is twisted as a whole into a superhelix. The latter has a density of one turn of superhelix per 200 base pairs and is of opposite sign to the double helix (i.e., left-handed). Upon heating, the DNA "crystal" undergoes thermal expansion, and the degree of twisting of the double helix decreases. This reduces the amount of superhelix formation. At some temperature (about 40°C) that corresponds to the temperature at which the closed annular molecule was originally formed in the cell, the superhelix formation totally vanishes. Upon further heating, the double helix untwists, and a superhelix of the same sign (right-handed) is formed. The melting characteristics of annular closed DNA also differ.[12] The melting temperature of this DNA is approximately 20° higher than for the linear molecule (Fig. 8). This happens because the melted strands in the annular molecule remain twisted about each other, and the entropy of the melted state is lower than for the linear molecule. Moreover, the width of the melting range of closed annular DNA is 2–3 times as large as that of the linear molecule.

The presented data pertain to the melting of DNA of high molecular weight. The melting curves do not depend on the chain length over a wide range of variation of the length. However, if the molecule is fragmented strongly enough, the melting curves are affected: the

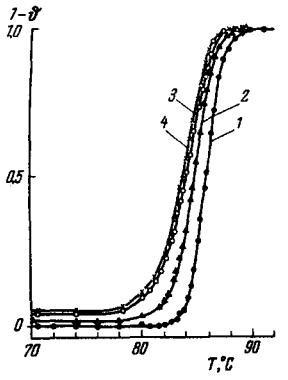


FIG. 9. Melting curves of T2 phage DNA for different values of the number of links in the molecule: [13] 1–3.3×10^4; 2–9.0×10^2; 3–4.5×10^2; 4–2.9×10^2.

melting point decreases and the width of the melting range increases (Fig. 9).

These are the fundamental facts concerning the helix-coil transition in DNA. We shall cite further experimental information as needed during the following discussion. The problems of the theory are evident from what we have presented: 1) The choice of a model. 2) Devising a theory of the helix-coil transition for homopolynucleotides. 3) Devising a theory of the helix-coil transition in heteropolynucleotides, with account taken of the difference in stability of the different monomer links (AT and GC pairs). This theory must explain the substantial increase in ΔT in going from a homopolymer to a heteropolymer and from linear to closed DNA. Furthermore, the theory must explain the relation of the parameters of the melting curves to the chain length.

III. THE HELIX-COIL TRANSITION IN HOMOPOLYMERS

1. A Single-stranded Homopolymer (the Ising Model)

We shall start the discussion with a very simple model of a homopolymer, the Ising model. The one-dimensional Ising model has been studied from all sides, and is widely applied in the most varied branches of physics (see, e.g., the review[14]). We shall give below an elementary solution of the model and derive formulas to be used in the theory of helix-coil transitions.

Let the given linear chain consist of N links, each of which can occur in two states: melted (1) and helical (2). The macroscopic state of the system is completely determined by assigning the values of three variables: the number of links in state 1 (N_1), the number of links in state 2 (N_2), and the number n of regions consisting of links of type 1 (or 2).*

The number of microstates that corresponds to the given values of N_1, N_2, and n equals the number of ways in which one can arrange N_1 identical elements in n groups and simultaneously arrange the other N_2 identical elements in n groups. This is equal to W_1W_2, where

$$W_1 = \frac{(N_1-1)!}{(n-1)!\,(N_1-n)!}, \tag{3}$$

$$W_2 = \frac{(N_2-1)!}{(n-1)!\,(N_2-n)!}. \tag{4}$$

*We assume that $N \to \infty$ and that we can neglect end effects.

Since we are considering the limiting case as $N \to \infty$, we can neglect the number unity. We can find the equilibrium values of the quantities N_1, N_2, and n from the condition of minimum "non-equilibrium" free energy*

$$F = N_1F_1 + N_2F_2 + nF_s - T(\ln W_1 + \ln W_2) \quad (5)$$

under the condition that $N_1 + N_2 = N$. Here F_1 and F_2 are the (free) energies of links in the melted and helical states, respectively. The difference $F_1 - F_2 \equiv \Delta F = U - T\Delta S$. The heat of melting $U \approx 7-9$ kcal/mole (3500–4500°K)[6,7] involves both rupture of the hydrogen bonds between the bases of the complementary strands and loss of the "interplanar" interaction between consecutive base pairs along the chain. The entropy difference ΔS between the melted and helical states involves the increase in the number of degrees of freedom of each of the nucleotides in passing to the melted state. We can estimate ΔS roughly by taking into account the fact that each nucleotide acquires six rotational degrees of freedom in the melted state (see Fig. 1a). Correspondingly, a pair of nucleotides acquires 12, while there are 2–3 energy minima for each rotation. Roughly speaking, this gives $\Delta S \approx 12$. The melting point T_m of the polymer is determined by the condition $\Delta F = 0$, which gives

$$T_m = U/\Delta S.$$

Hence, T_m should be of the order of 300–400°K, which corresponds approximately to the experimental value of 340–380°K. The quantity F_S is the additional (free) energy that arises upon forming a single melted region within a helical region. Roughly speaking, this additional energy arises from the fact that $\nu + 1$ interplanar interactions are disrupted when ν base pairs are broken. In the more general case, introduction of the quantity F_S reflects the fact that links can occur in certain intermediate states in the boundary region between the melted and helical regions. In order that Eq. (5) and the entire ensuing discussion may be valid, the dimensions of these boundary regions must necessarily be considerably smaller than those of the melted and the helical regions that arise in the melting interval. Upon applying Stirling's formula, we get

$$F = N_1F_1 + N_2F_2 + nF_s - T[N_1 \ln N_1 - n \ln n - (N_1 - n)\ln(N_1 - n) + N_2 \ln N_2 - n \ln n - (N_2 - n)\ln(N_2 - n)].$$

We obtain from the condition $(\partial F/\partial n)_{N_1N_2} = 0$:

$$\left(\frac{N_1}{n} - 1\right)\left(\frac{N_2}{n} - 1\right) = \frac{1}{\sigma}, \quad (6)$$

where $\sigma = \exp(-F_S/T)$. From the condition $(\partial F/\partial N_1)_n = 0$, we get:

$$\frac{1 - \frac{n}{N_2}}{1 - \frac{n}{N_1}} = s, \quad (7)$$

where $s = \exp[(F_1 - F_2)/T]$.

The derived equations give the relation of N_1, N_2, and n to s, which is unambiguously related to the temperature. Thus, for example, we obtain the following equation for the fraction of the links $\vartheta = N_2/N$ occurring in the helical state:

$$\frac{1 - 2\vartheta}{\sqrt{\vartheta(1-\vartheta)}} = \frac{1}{\sqrt{\sigma}}\frac{1-s}{\sqrt{s}}. \quad (8)$$

The mean number of links per helical region is (when $\sigma \ll 1$)

$$\nu_2 = \frac{N_2}{n} = \frac{1}{\sqrt{\sigma}}\left(\frac{\vartheta}{1-\vartheta}\right)^{1/2}.$$

Equation (8) gives a simple expression for the width of the melting range:

$$\Delta T = 4\sqrt{\sigma}\frac{T_m^2}{U}.$$

We note that Eq. (8) goes over into a Boltzmann distribution when there is no interaction between the links ($\sigma = 1$, $F_S = 0$).

This limiting case corresponds to lack of cooperativity: the energy of formation of a boundary between a melted and a helical region is zero (i.e., $F_S = 0$), and the helical and melted links are "mixed" completely randomly. As the surface energy increases (σ decreases), the cooperativity of the system increases: the mean length of each helical or melted region grows, and the width of the melting range declines correspondingly. In the limiting case as $\sigma \to 0$, we have a fully cooperative, absolutely sharp transition.

The discussed model corresponds physically to the case of a single-stranded homopolymer (see Note 5). In fact, in a single-stranded polymer, the number of states of the ith melted region, which consists of ν_i segments (freely jointed links) is

$$w_i = w_0^{\nu_i},$$

where

$$\sum_{i=1}^{n} \nu_i = N_1.$$

Hence, the additional contribution to the entropy of the system will be

$$\sum_{i=1}^{n} \ln w_i = N_1 \ln w_0.$$

The free energy will be expressed by Eq. (5), with F_1 replaced by $F_1 - T \ln w_0$.

2. A Two-stranded Homopolymer

The discussed model is not appropriate for the case of a two-stranded polymer such as the polynucleotides, since then the entropy of the melted part of the polymer is not an additive quantity. In fact, the melted regions in a two-stranded polymer are closed polymer chains. The number of states of a closed polymer chain equals the number of states of the opened chain $w_0^{\nu_i}$ multiplied by the probability of closure of the chain (i.e., that its beginning and end points will occur within some small volume δV):

$$w_i = \frac{w_0^{\nu_i}}{\nu_i^{3/2}}\left(\frac{3}{2\pi}\right)^{3/2}\frac{\delta V}{l^3}, \quad (9)$$

Here l is the length of a segment of the chain (see Note 6). Eq. (9) is often called the Jacobson-Stockmayer formula.[15] We should emphasize the fact that Eq. (9) gives the total number of states of the closed chain, including the states which the chain must intersect itself to attain. In fact, both states without and with knots were taken into account in deriving Eq. (9). Evidently, a physical transition from an unknotted to a

*Here and below, we assume as usual that the energy and the temperature are measured in the same units.

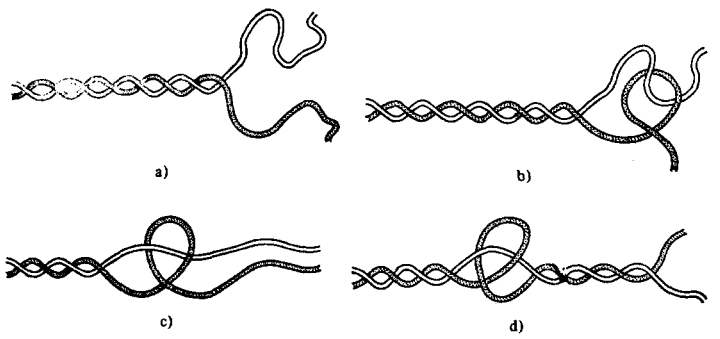


FIG. 10. Formation of a knot and its migration into the molecule.

knotted state is impossible without breaking the chain. Hence, the number of states of a closed loop is smaller than that given by Eq. (9).

In order that Eq. (9) should hold, knot formation must be possible. This possibility exists in a polymer, owing to presence of free ends. In executing their complicated motion, the free ends can give rise to knots in the outer melted region, and the knots can then migrate into the polymer (Fig. 10). Thus, in order to permit applying a formula like (9), relaxation with respect to the number of knots must set in within the time of experiment (topological relaxation). It is very hard to estimate the time of this relaxation. Hence, we shall treat the problem of the helix-coil transition in general form by assigning the arbitrary function $f(\nu)$ as a coefficient to the number of states of a melted region. Here we assume that it has the asymptotic form $\nu^{-\alpha}$ (the problem of the true value of α will be discussed below). A number of studies[16-23] have been concerned with treating the helix-coil transition in homopolymers with account taken of the coefficient $\nu^{-\alpha}$. We shall give a general treatment of this problem below by a method differing from those applied in the cited papers.

The number of states of a two-stranded polymer made of N_1 melted links, N_2 helical links, and n helical and coil-like regions is W_1W_2. As before, W_2 is given by (4), while

$$W_1=\sum_{\{\nu\}=1}^{\infty}\sum_{\{\mu\}=1}^{\infty}\prod_{i=1}^{n} f(\nu_i+\mu_i)\,\delta\Big(N_1-\sum_{i=1}^{n}\nu_i\Big)\,\delta\Big(N_1-\sum_{i=1}^{n}\mu_i\Big); \qquad (10)$$

Here $\{\nu\}$ and $\{\mu\}$ are the numbers of links in segments of the first and second strands, respectively, and

$$\delta(k)=\begin{cases}1 & k=0,\\ 0 & k\neq 0.\end{cases}$$

In order to perform the summation in Eq. (10), we shall use a mathematical procedure that was first applied by Darwin and Fowler (see, e.g.[24]). Let us substitute for the δ-symbols in Eq. (10) their integral representations

$$\delta(k)=\frac{1}{2\pi i}\oint_C z^{-k-1}\,dz,$$

where C is any contour in the complex plane surrounding the origin. We get

$$W_1=\Big(\frac{1}{2\pi i}\Big)^3\oint_{C_1}\oint_{C_2} dz_1\,dz_2\,z_1^{-N_1-1}z_2^{-N_1-1}\,[\Phi(z_1,z_2)]^n, \qquad (11)$$

where

$$\Phi(z_1,z_2)=\sum_{\nu=1}^{\infty}\sum_{\mu=1}^{\infty} f(\nu+\mu)\,z_1^{\nu}z_2^{\mu}=\sum_{\nu=1}^{\infty}\Big(\frac{z_1}{z_2}\Big)^{\nu}\sum_{\mu=1}^{\infty} f(\nu+\mu)\,z_2^{\nu+\mu}.$$

If we make use of the identity

$$\sum_{n=1}^{\infty}a_n\sum_{m=n+1}^{\infty}b_m=\sum_{n=2}^{\infty}b_n\sum_{m=1}^{n-1}a_m$$

and carry out the summation, we get

$$\Phi(z_1,z_2)=\frac{z_1z_2}{z_1-z_2}\sum_{\nu=1}^{\infty} f(\nu+1)\,(z_1^{\nu}-z_2^{\nu}). \qquad (12)$$

We shall calculate the double integral in (11) by the method of steepest descent, while assuming that $N_1 \gg n$. Analysis shows that the saddle point is found at $z_1 = z_2$. That is, it can be found from the condition

$$dg(z)/dz=0,$$

where

$$g(z)=-2(N_1+1)\ln z+n\ln\Phi(z,z). \qquad (13)$$

Here,

$$\ln W_1=g(z_0).$$

We can easily see that

$$\Phi(z,z)=\sum_{\nu=1}^{\infty}\nu f(\nu+1)\,z^{\nu+1}, \qquad (14)$$

and here the saddle point lies near $z = 1$.

In order to calculate W_1 in explicit form, we must treat separately the different regions of α values.

1) $\alpha < 2$. Since z is close to unity, we can replace the summation in Eq. (14) by integration:

$$\Phi(z,z)=\Big(\ln\frac{1}{z}\Big)^{\alpha-2}\int_0^{\infty}x^{-\alpha+1}e^{-x}\,dx=\Big(\ln\frac{1}{z}\Big)^{\alpha-2}\Gamma(2-\alpha).$$

If we substitute this into Eq. (13) and equate the derivative to zero, we find the saddle point:

$$z_0=\exp\Big(-\frac{n}{N_1}\frac{2-\alpha}{2}\Big).$$

Finally, we have

$$\ln W_1=2n\beta\Big(1-\ln\beta-\ln\frac{n}{N_1}\Big)+n\ln\Gamma(2\beta), \qquad (15)$$

where

$$\beta=(2-\alpha)/2.$$

2) $2 < \alpha < 3$. We cannot transform Eq. (14) into an integral in this case, since the obtained integral diverges at the lower limit. However, this summation can be transformed into the form

$$\Phi(z,z)=A-B\Big(\ln\frac{1}{z}\Big)^{\alpha-2},$$

where

$$A=\sum_{\nu=1}^{\infty}\nu f(\nu+1),\quad B=\int_0^{\infty}x^{-\alpha+1}(1-e^{-x})\,dx.$$

The saddle point is

$$z_0 = \exp\left\{-\left(\frac{B}{A}\frac{\alpha-2}{2}\frac{n}{N_1}\right)^{\frac{1}{3-\alpha}}\right\},$$

Hence,

$$\ln W_1 = n\left[\ln A - C\left(\frac{n}{N_1}\right)^{\frac{\alpha-2}{3-\alpha}}\right], \quad (16)$$

where

$$C = (3-\alpha)\left(\frac{B}{A}\right)^{\frac{1}{3-\alpha}}\left(\frac{\alpha-2}{2}\right)^{\frac{\alpha-2}{3-\alpha}} > 0.$$

Let us proceed to calculate the equilibrium values of N_1, N_2, and n. They are found from the condition that the "non-equilibrium" free energy of (5) should be a minimum. Here W_2 is given by Eq. (4), and W_1 by Eqs. (15) and (16) for the cases $\alpha < 2$ and $2 < \alpha < 3$, respectively. Let us treat these two cases separately again.

1) $\alpha < 2$. Minimization of Eq. (5) leads to the equations

$$\left(\frac{N_1}{n}\right)^{2\beta}\frac{N_2}{n} = \frac{\beta^{2\beta}}{\Gamma(2\beta)}\frac{1}{\sigma}, \quad (17)$$

$$\left(1 - \frac{n}{N_2} + 2\beta\frac{n}{N_1}\right) = s. \quad (18)$$

We get the following equation for the dependence of the helicity ϑ on the temperature (i.e., on s) (upon substituting $\beta = (2-\alpha)/2$):

$$\frac{1-(3-\alpha)\vartheta}{(1-\vartheta)^{\frac{1}{3-\alpha}}\vartheta^{\frac{2-\alpha}{3-\alpha}}} = \frac{1-s}{\left[\sigma\Gamma(2-\alpha)\left(\frac{2-\alpha}{2}\right)^{\alpha-2}\right]^{\frac{1}{3-\alpha}}}. \quad (19)$$

The mean length of a helical region $\nu_2 = N_2/n$ is

$$\nu_2 = \left[\frac{1}{\sigma}\frac{\left(\frac{2-\alpha}{2}\right)^{2-\alpha}}{\Gamma(2-\alpha)}\right]^{\frac{1}{3-\alpha}}\left(\frac{\vartheta}{1-\vartheta}\right)^{\frac{2-\alpha}{3-\alpha}}. \quad (20)$$

Equation (19) implies that the point s = 1 (i.e., $F_1 = F_2$) does not correspond to the value $\vartheta = 1/2$, as in a single-stranded homopolymer, but to the value $\vartheta = 1/(3-\alpha)$. Here,

$$\nu_2 = [\sigma 2^{2-\alpha}\Gamma(2-\alpha)]^{-\frac{1}{3-\alpha}}. \quad (21)$$

Upon comparing Eq. (19) with Eq. (8), we see that the case of the single-stranded homopolymer is formally encompassed by Eqs. (19) and (20) if we set $\alpha = 1$ therein, and replace 2σ by σ.

In order to determine the width ΔT of the melting range, we must calculate $|\partial\vartheta/\partial s|_{max}$, which is, to a good accuracy,

$$\left|\frac{\partial\vartheta}{\partial s}\right|_{max} = \frac{1}{8}\frac{3-\alpha}{2-\alpha}\left[1 + \frac{1}{4}\left(\frac{1-\alpha}{3-\alpha}\right)^2\frac{1}{1+2\frac{2-\alpha}{(3-\alpha)^2}}\right] \times\left[\sigma\Gamma(2-\alpha)\left(\frac{2-\alpha}{2}\right)^{\alpha-2}\right]^{-\frac{1}{3-\alpha}}. \quad (22)$$

2) $2 < \alpha < 3$. If we substitute (4) and (16) into (5), we get

$$F = N_1F_1 + N_2F_2 + nF_s^* - Tn\left[1 - \ln\frac{n}{N_2} - C\left(\frac{n}{N_1}\right)^{\gamma}\right], \quad (23)$$

where

$$F_s^* = F_s - T\ln A, \quad \gamma = \frac{\alpha-2}{3-\alpha}.$$

The condition $\partial F/\partial n = 0$ gives

$$F_s^* + T\ln\frac{n}{N_2} + TC(\gamma+1)\left(\frac{n}{N_1}\right)^{\gamma} = 0. \quad (24)$$

We recall that the treatment has been conducted under the assumption that $n \ll N_1$ and $n \ll N_2$. That is, $\sigma^* = \exp(-F_S^*/T) \ll 1$. In this approximation, the solution of Eq. (24) for n has the form

$$n = \sigma^* N_2\left[1 - C(\gamma+1)\left(\sigma^*\frac{N_2}{N_1}\right)^{\gamma}\right]. \quad (25)$$

If we substitute (25) into (23), we get

$$F = N_1F_1 + N_2F_2^* + DN_1\left(\frac{N_2}{N_1}\right)^{\gamma+1}, \quad (26)$$

where

$$F_2^* = F_2 - T\sigma^*, \quad D = TC(\sigma^*)^{\gamma+1} > 0.$$

We see from Eq. (26) that the derivatives of F with respect to N_1 can become discontinuous as $N_2/N_1 \to 0$, while the free energy F itself remains continuous. That is, a second-order phase transition will occur in the vicinity of this point. We can set $N_1 \approx N$ in the vicinity of the transition, and upon introducing the variable $\eta^2 = N_2/N$, we get (omitting the term that does not depend on η):

$$\frac{F}{N} = \Delta S(T - T_0)\eta^2 + D\eta^{2(\gamma+1)}, \quad (27)$$

Here ΔS is the entropy difference per link between the melted and helical states of the homopolymer.

As we see from Eq. (27), the phase transitions in the treated model fit into Landau's generalized scheme.[25] When $T > T_0$, the minimum is attained when $\eta^2 = 0$, i.e., in a state corresponding to the fully melted homopolymer. When $T < T_0$, the minimum in F is reached at a fraction of helical links that differs from zero, being

$$\frac{N_2}{N} = \eta^2 = E(T_0 - T)^{\frac{1}{\gamma}}, \quad (28)$$

where

$$E = \left[\frac{\Delta S}{D(\gamma+1)}\right]^{\frac{1}{\gamma}}.$$

It is interesting to note that γ varies from 0 to ∞ as the parameter α varies from 2 to 3. This makes the type of transition depend very strongly on α. The relation $\eta \sim |T_0 - T|^{1/2}$ derived in the ordinary Landau theory arises in the discussed model at $\alpha = 5/2$.

Thus, the system being treated behaves substantially differently at different values of α. When $\alpha < 2$, the melting curve is a function that is continuous along with all of its derivatives. Hence, even at a temperature appreciably exceeding the "melting point" T_m, a certain finite concentration of helical links remains in the polymer. However, if $\alpha > 2$, melting of the polymer acquires the nature of a phase transition: at temperatures above the critical value T_0, the whole polymer proves to be completely melted. In the interval $2 < \alpha < 3$, the order of the derivative that is discontinuous at the point T_0 gradually declines with increasing $\alpha > 3$, even the melting curve itself is discontinuous.[19]

The question of the true value of α for a two-stranded homopolymer remains open as yet. The value $\alpha = 3/2$ is obtained under conditions of complete topological relaxation. However, in this case helical regions formed by the strands from different melted regions can arise in principle (Fig. 11). It is very hard to take such states into account because the problem

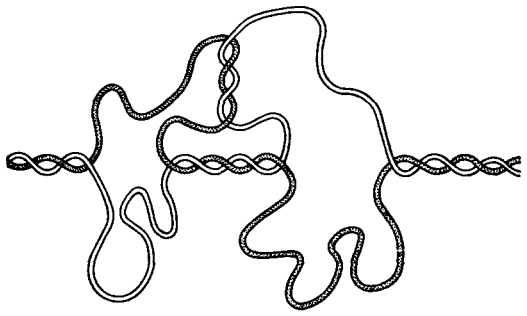


FIG. 11. Two melted regions of a homopolynucleotide that are twisted about each other.

ceases to be one-dimensional. However, one can show (A. M. Dykhne, unpublished results) that phase transitions do not occur in the case of such a complete topological relaxation. Treatment of the actual situation is complicated by the fact that the helical regions formed by twisting together two different coil-like regions cannot arise by diffusion of these elements from the ends of the chain, as happens with knots (see Fig. 11), since slippage of strands into helical regions is impossible. Hence, the relaxation time in the formation of such helical regions must increase exponentially with increasing chain length. On the other hand, helical regions can be formed between two melted regions under conditions in which topological relaxation is absent. However, then the remaining parts of the melted regions that didn't enter into the helical region must be twisted about each other by the same number of turns as the helical region has, but in the opposite sense (to the left, since the DNA helix is right-handed). This leads to additional entropy effects, and accounting for them complicates solution of the problem greatly. Finally, we must take into account excluded-volume effects, which can affect the value of α.[21]

In the ensuing situation, the most reasonable course would be to treat α as a parameter of the theory, and try to determine it by comparing the formulas derived above with experiment. Applequist[26] has made such an attempt for the case of double-helical poly-A. Applequist thinks that his experimental data show that melting of poly-A exhibits a second-order phase transition under certain conditions. However, in our opinion, more painstaking design of the experiments is required to permit drawing such an important conclusion. Mainly, one should perform control experiments on the high-molecular-weight character and homogeneity of the preparations. The data obtained by other authors[2,3] indicate that the melting curve is smooth in most cases. We should emphasize as a whole that there have been too few papers concerned with experimental study of the helix-coil transition in homopolynucleotides, and hence, one cannot yet find the value of α by experiment.

In line with this situation, we cannot deem unreasonable an attempt to estimate σ that starts with the assumption that $\alpha = 3/2$, as Crothers and Zimm[18] have done for the first time. Since ΔT is about 0.5° for homopolymers, the obtained values of σ lie in the range 10^{-4}–10^{-5}. We note that this estimate agrees in order of magnitude with an estimate of σ obtained from completely different data: from the kinetics of interaction of DNA with a slowly reacting agent (formaldehyde).[27] We should emphasize that neither method of estimating σ is completely satisfactory. Hence, these estimates must be refined further. However, the fact that the two methods give similar results permits us to use the estimate until a more accurate value has been found.

In addition to the problem of melting of two-stranded homopolymers, melting of three-stranded homopolymers has also been treated in the literature,[28,29] as well as melting of heteropolymers having strictly alternating sequences of links.[30-32] The former problem has been treated in connection with the helix-coil transition in three-stranded complexes of poly-A + 2 poly-U, and the latter in connection with melting of the polymer poly-AT (i.e., a polymer with the sequence of links ...ATATATAT...). However, in these cases the adequacy of the treated models to the actual situation is even more questionable than in the case of the homopolynucleotides discussed above.

3. Melting of Complexes of Polynucleotides with Stabilizers

Now we shall discuss a polymer that forms a complex with molecules of a substance of low molecular weight (stabilizers). Here we are considering a reversible complex, so that the stabilizers can attain an equilibrium distribution between the helical and coil-like portions of the polymer within the time of experiment at each temperature. In terms of phase systems, such a "solution" of the stabilizers in the polymer corresponds to a binary solution. As we know, a phase transition is smeared out in such binary systems, and it covers a broad temperature range because the concentration of the solute in the liquid phase varies as crystallization proceeds. Thus, the redistribution of the solute between the solid and liquid phases leads to radical changes in the melting of the phase system. If redistribution does not occur (which happens at the saturation concentration), then the system undergoes an ordinary phase transition. Figure 12 gives some data on the temperature-dependence of the heat capacity of pure water and of a salt solution that graphically illustrate the aforesaid.

Equations describing the properties of a binary phase system can be derived from the condition that the chemical potentials of the solvent and the solute should be equal. For weak solutions, it has the form (see, e.g.,[25]):

$$\frac{U}{T_0}(T-T_0)=(C_1-C_2)T_0, \tag{29}$$

$$\frac{C_2}{C_1}=p, \tag{30}$$

$$C_1(1-\vartheta)+C_2\vartheta=C, \tag{31}$$

Here U and T_0 are the heat of melting and the melting

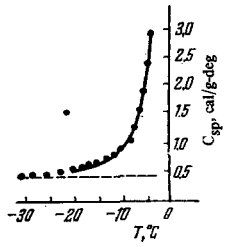


FIG. 12. Temperature-dependence of the partial heat capacity of water in 0.15N NaCl solution (points). [33] The solid curve is plotted according to Eq. (32).

point of the solvent, C_1 and C_2 are the concentrations of the solute in the liquid and solid phases, ϑ is the fraction of the material in the solid phase, and p is the distribution coefficient of the solute between the phases.

For a solution of salt in water, in which $C_2 = 0$, we have

$$1-\vartheta=\frac{T_0^2}{U}\frac{C}{T-T_0}.$$

The heat capacity is

$$c_{sp}=T\frac{\partial S}{\partial T}=-T_0\Delta S\frac{\partial\vartheta}{\partial T},$$

where $\Delta S = U/T_0$ is the entropy of melting. Finally, we get

$$c_{sp}=\frac{T_0^2}{(T-T_0)^2}C. \quad (32)$$

This formula describes well the temperature-dependence of the heat capacity in the interval between the melting point T_0 and the eutectic point (Fig. 12).* For an arbitrary p, we can easily derive the following expressions for the temperature shift and the width of the melting range:

$$\delta T_m=2\frac{p-1}{p+1}\frac{T_0^2}{U}C; \quad (33)$$

$$\Delta T=4\left(\frac{p-1}{p+1}\right)^2\frac{T_0^2}{U}C, \quad (34)$$

Here $\delta T_m = T_m - T_0$, the melting point T_m being defined as the temperature at which $\vartheta = 1/2$, and ΔT is defined by the relation $\Delta T = 1/|\partial\vartheta/\partial T|_{T=T_m}$.

It turned out upon studying the complex of a homopolymer with stabilizers that the results obtained for phase systems fully remain in effect. Thus, Eqs. (33) and (34) have been proved to be strictly valid in this case.[34,22] Here we should take ΔT in Eq. (34) to be $\delta\Delta T$, which is the difference in transition widths between the polymer containing stabilizers and the pure polymer. Detailed analysis shows[22,35] that presence of stabilizers has absolutely no effect on the way in which the polymer breaks down into helical and coil-like regions. Hence, one simply adds a range due to the redistribution of the stabilizers to the melting range of the pure homopolymer, and the redistribution occurs exactly as in a phase system. General formulas were also derived in[22] for δT_m and $\delta\Delta T$ that are valid for any concentration of stabilizers in the polymer. Here the variation in the transition range caused by the stabilizers does not depend on the range for the homopolymer, i.e., on the cooperativity factor, whatever the value of C. Figure 13 shows the variation of the transition temperature and the width of the transition range for p = 5. As we see from the diagram, the theory qualitatively explains the experimental results of Fig. 7. Quantitatively, the theory agreed with experiment in detail for the region of small C (Eqs. (33) and (34)). They found the heat of melting of DNA from these measurements, independently of the microcalorimetric data.[10,36] By analogy with the equilibrium

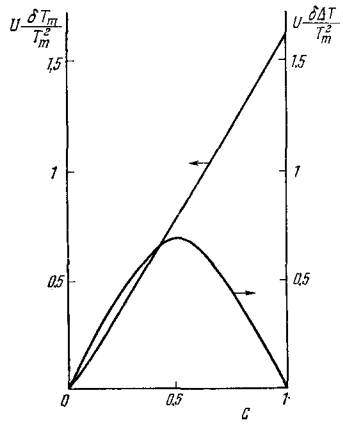


FIG. 13. Theoretical relation of the melting point and the width of the melting range of a complex of a homopolymer with stabilizers to the concentration of the stabilizers. [35,22]

curves of phase systems, we can say that the state diagram for a complex of a polymer with stabilizers is cigar-shaped. Of course, we must take this statement in a qualified sense, since separation into macroscopic phases does not occur here: the "liquid" and "solid phases" remain mixed.

IV. THE HELIX-COIL TRANSITION IN HETEROPOLYMERS

Let us treat the problem of intramolecular melting of real DNA, which consists of two types of links: low-melting AT and high-melting GC. A polymer consisting of only AT pairs (poly-AT) would melt at $T_{AT} \approx 340°K$, and a polymer of only GC pairs (poly-GC) at $T_{GC} \approx 380°K$,* in accord with the theory of melting of a homopolymer presented above. The question arises: how will DNA containing both AT and GC pairs melt? The answer to this question depends on the type of mutual arrangement of the AT and GC links in the DNA. If, for example, the chain is composed of large AT and GC regions, then the melting curve will have the shape depicted by curve 1 in Fig. 14; here the AT regions will first melt independently, and then the GC regions. Melting will follow this type if the length of the AT and GC regions is substantially larger than the mean length of a melted region in a homopolymer. If the DNA contains no large AT and GC regions, then it will melt as a whole near some mean temperature between 340° and 380°K. The melting of DNA having a regular alternation of AT and GC links, periodically repeated AT and GC blocks, can be treated analogously to the treatment of melting of a homopolymer above. For example, a chain of alternating AT, GC, AT, GG, AT, GC links will melt at $T = (T_{AT} + T_{GC})/2$. The width of the melting range will remain very small (Fig. 14, curve 2). However, we are interested in real DNA, in which the sequence of AT and GC pairs can be considered random. The difficulty and peculiarity of the problem of calculating the thermodynamic properties of such a DNA involve the fact that the sequence of AT and GC links is fixed in a definite way, though random. Hence, all the thermodynamic characteristics of the DNA must be calculated for this particular sequence of links. Specifically, this means that we cannot

*For the experimental data shown in Fig. 12, $C \approx 2 \times (0.15/55) = 5.4 \times 10^{-3}$. In order to find c_{sp} in cal/g-deg, we must multiply the right-hand side of Eq. (32) by the gas constant, which is 2 cal/mole-deg, and divide by 18. Then Eq. (32) will give the curve drawn as a solid line in Fig. 12, with the heat capacity referred to the level shown by the dotted line. The sharply discrepant point on the graph at a temperature of about −22°C corresponds to melting of the eutectic.

*Under the so-called standard conditions (SSC), in which 0.15 moles of NaCl is dissolved per liter of water. [Translator's note: This abbreviation is generally used in the literature, e.g., [9] to mean a solution that is 0.15M in NaCl, 0.015M in trisodium citrate, at pH 7.4.]

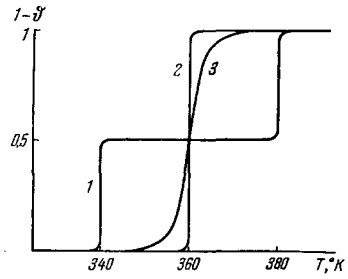


FIG. 14. Melting curves of hypothetical heteropolynucleotides consisting of 50% GC pairs, for different types of distributions of the pairs along the chain: 1—half of the molecule is a GC homopolymer, and half is AT; 2—a strictly alternating sequence of AT and GC pairs; 3—a random sequence of base pairs.

average the partition function over all possible sequences of links having different rupture energies when finding the partition function of the molecule (while maintaining the content of low-melting and high-melting pairs).* In the partition function, we must perform the summation over phase space, while maintaining the fixed sequence of AT and GC links.

From this standpoint, a heteropolymer in solution is a system that is not in complete thermodynamic equilibrium: the DNA "skeleton" undergoes no chemical changes at the temperatures used within the time of experiment. This is, not all the sequences of AT and GC links in the DNA molecules are realized that are permitted by static thermodynamics (and which have equal energies. Rather, only the single sequence exists that the DNA had when originally dissolved. However, this does not prevent us from applying the thermodynamic approach to the helix-coil transition, since weak bonds are broken or reestablished in this transition, and the DNA can reach complete thermodynamic equilibrium with respect to them when the experiment is carried out slowly (within minutes).

In line with what we have said, melting of heterogeneous DNA differs in nature from the melting of DNA containing "stabilizers" (although there are two types of links with differing energies of rupture in both cases). "Stabilizers" redistribute themselves between the solid and liquid "phases" of DNA as melting proceeds, and the process resembles the melting of a solution. However, heterogeneous DNA having a fixed AT—GC sequence melts in a different way: upon heating, first the low-melting portions (with a large content of AT pairs) melt out of it, and then the more high-melting portions. The shape of the melting curve is determined by the fluctuations in AT and GC composition of the DNA in its different regions. Since these fluctuations are microscopic in nature, the discussed melting process of the heteropolymer has no analogy among phase systems: macroscopically, a phase system of this type is homogeneous. Thus, the difference between the problem of melting of a heteropolymer and that of melting of a homopolymer containing stabilizers is manifested especially sharply in the limiting case of a phase system. We shall give below two variants of an elementary semiquantitative theory of the helix-coil transition in a heteropolymer, which are modifications of the treatment carried out in[35].

1. Elementary Theory of the Helix-coil Transition in Heteropolymers

a) Onset of melting.* Let us discuss the very onset of melting, when individual regions of melted links are melting out of the helical DNA, and the fraction of melted links is small. The condition for melting out of a region in heterogeneous DNA that contains ν links and has a concentration of x high-melting GC pairs is that the difference in free energies of all the links of the region in the helical and coil-like states should equal the surface energy F_s of the two boundaries of the melted region with the adjacent helical regions of the DNA:

$$\nu[\Delta F_{AT}(1-x)+\Delta F_{GC}x]=F_s; \quad (35)$$

Here ΔF_{AT} is the difference in free energies per link between the helical and coil-like states of poly-AT; ΔF_{AT} vanishes at the melting point T_{AT} of poly-AT, and near T_{AT} it is given by

$$\Delta F_{AT}=\Delta S_{AT}(T-T_{AT}),$$

where ΔS_{AT} is the entropy of melting of poly-AT. Analogously,

$$\Delta F_{GC}=\Delta S_{GC}(T-T_{GC}).$$

We shall assume that $\Delta S_{GC} = \Delta S_{AT} = \Delta S$ (this is not essential, but it simplifies the formulas). Let us substitute these values of ΔF_{AT} and ΔF_{GC} into (35), and introduce the deviation in concentration of GC pairs in the given region from the mean value x_0 over the molecule: $\Delta x = x - x_0$. Thus we find the melting point of a region having a concentration of high-melting GC links that is reduced by the amount Δx:

$$T=T_0+(T_{GC}-T_{AT})\Delta x+\frac{1}{\nu}\frac{F_s}{\Delta S}; \quad (36)$$

Here we have introduced the notation:

$$T_0=T_{AT}(1-x_0)+T_{GC}x_0. \quad (37)$$

For the entire molecule, ν is very large (10^5—10^7), so that the fluctuations in concentration are very small, and we can neglect the last two terms on the right-hand side of (36). Then (36) implies that the melting point of heterogeneous DNA is given by Eq. (37), in good agreement with the experimental data (cf. the empirical formula (2)).

However, we see from (36) that DNA begins to melt at a lower temperature than T_0: e.g., long regions consisting solely of AT links ($x = 0$, $\Delta x = -x_0$) will have melted at a temperature that satisfies the inequality $T_0 > T > T_{AT}$. Thus, formally speaking, we must assume that melting occurs throughout the interval $T_{AT} < T < T_{GC}$.** In fact, there are few such low-melting regions, and the entire melting process covers

*Such an averaging corresponds to solving the problem of melting of a homopolymer containing stabilizers, as discussed in the preceding section. We should note that the partition function was averaged over different sequences of links in some studies [37,38] treating the helix-coil transition in a heteropolymer. The results obtained in these studies differ shaply from those obtained from a heteropolymer with correct calculation of the partition function. Actually, in these studies they calculated in a complicated way the partition function for a homopolymer containing stabilizers. We can directly convince ourselves of the latter by comparing these studies with the results obtained in [22, 34].

*The completion of melting can be treated analogously.

**As the empirical formula (2) implies, $T_{GC}-T_{AT} = 36.0-7.04 \log [Na^+]$. For the standard salt conditions in which $[Na^+] = 0.195$, this gives the value $T_{GC}-T_{AT} = 41°$. Henceforth in calculations and estimates, we shall use the rounded value $T_{GC}-T_{AT} = 40°$.

a far narrower temperature range of the order of several degrees near T_0. Let us estimate the width of the melting range (i.e., the relation of the number of melted links to the temperature).

We shall assume that the distribution of AT and GC links in any region of the DNA is random. Then the probability that a region of length ν should have a concentration $x = m/\nu$ GC links (when their mean concentration is x_0) is described by the binomial distribution:

$$W_m = \frac{\nu!}{m!(\nu-m)!} x_0^m (1-x_0)^{\nu-m}. \tag{36'}$$

If the region that is melting out is long enough, i.e., $m \gg 1$ (we shall see below that this is exactly the case in the melting of DNA), then the binomial distribution of (36′) goes over into a Gaussian distribution:

$$P_\nu(x) = \frac{1}{\sqrt{2\pi}\,\sigma_\nu} e^{-\frac{(\Delta x)^2}{2\sigma_\nu^2}}, \tag{37'}$$

where $\sigma_\nu^2 = x_0(1 - x_0)/\nu$.

If we find from (36) the fluctuation Δx that is necessary to permit melting of a region of length ν at the temperature T, and substitute it into (37′), we find that the probability that a region of length ν will melt out of helical DNA at $T < T_0$ is proportional to*

$$\exp\left\{-\left(\frac{T_0-T}{T_{GC}-T_{AT}}\sqrt{\bar{\nu}} + \frac{F_s/\Delta S}{T_{GC}-T_{AT}}\frac{1}{\sqrt{\bar{\nu}}}\right)^2 \Big/ 2x_0(1-x_0)\right\}; \tag{38}$$

This same expression also gives the length distribution of the melted regions of the DNA.

As we see from (38), the mean length of a melted region increases as we approach the midpoint T_0 of the melting range:

$$\bar{\nu} = \frac{F_s/\Delta S}{T_0 - T}. \tag{39}$$

If we substitute this value into (38), we can find the fraction of melted links of the heterogeneous DNA:

$$1 - \vartheta \sim \exp\left(-\frac{T_0-T}{\tau}\right); \tag{40}$$

Here, ϑ is the helicity, and

$$\tau = \frac{1}{2} x_0(1-x_0)\frac{(T_{GC}-T_{AT})^2}{F_s/\Delta S}. \tag{41}$$

Analogously, when $T > T_0$, in the region in which the fraction of unmelted regions in the DNA is small, we have instead of (40):

$$\vartheta \sim \exp\left(-\frac{T-T_0}{\tau}\right). \tag{40'}$$

b) Approximate treatment of the entire melting curve. We shall divide the heteropolymer into identical segments containing λ pairs each. If λ is large enough, then the concentration distribution of GC pairs over these segments will be described by Eq. (37′), with ν replaced by λ. Since we have assumed the distribution of base pairs to be random, and the segments do not overlap, there is no correlation at all between the GC content of different segments.

The total number of GC pairs per melted region will be a minimum whenever segments having a concentration of GC pairs below some threshold value x_λ will be melted, while all the segments having $x > x_\lambda$ will be helical. The quantity x_λ is determined by the condition:

$$\int_0^{x_\lambda} P_\lambda(x)\,dx = \frac{N_1}{N}. \tag{42}$$

The mean concentration of GC pairs in the melted part of the molecule is

$$\bar{x}_1 = \frac{\int_0^{x_\lambda} x P_\lambda(x)\,dx}{\int_0^{x_\lambda} P_\lambda(x)\,dx} = \frac{N}{N_1}\int_0^{x_\lambda} x P_\lambda(x)\,dx. \tag{43}$$

Since the compositions of different regions are not correlated, the probability that a melted region will consist of k consecutive segments is simply equal to

$$p_k = \left(1 - \frac{N_1}{N}\right)\left(\frac{N_1}{N}\right)^{k-1}.$$

Hence, the mean number of segments in the melted region is

$$\bar{k} = \frac{1}{1 - \frac{N_1}{N}}.$$

Therefore, the mean number of base pairs in the melted region is

$$\nu_1 = \lambda\bar{k} = \frac{\lambda}{1-\frac{N_1}{N}}, \tag{44}$$

while the number of melted regions in the entire molecule is

$$n = \frac{N_1}{\lambda\bar{k}} = \frac{N_1}{\lambda}\left(1 - \frac{N_1}{N}\right). \tag{45}$$

The free energy of the polymer has the form

$$F(N_1, N_2, n) = N_1[\bar{x}_1 F_1^{GC} + (1-\bar{x}_1)F_1^{AT}] + N_2[\bar{x}_2 F_2^{GC} + (1-\bar{x}_2)F_2^{AT}] + nF_s.$$

If we apply the condition

$$N_1 + N_2 = N, \quad \bar{x}_1 N_1 + \bar{x}_2 N_2 = x_0 N,$$

omit the constant term in the expression for the free energy, and transform from the variable n to the variable λ, we get

$$F(N_1, \lambda) = N_1\bar{x}_1\Delta F_{GC} + N_1(1-\bar{x}_1)\Delta F_{AT} + \frac{N_1}{\lambda}\left(1 - \frac{N_1}{N}\right)F_s, \tag{46}$$

Here, $\Delta F_{GC} = F_1^{GC} - F_2^{GC}$, and analogously for ΔF_{AT}; $\bar{x}_1$ is determined by Eq. (43), using x_λ from (42).

The equilibrium values of N_1 and λ are found from the condition that the expression in (46) should be a minimum in both parameters. In order to find the mean length of a helical region and the width of the melting range of the heteropolymer, we should consider Eqs. (42), (43), and (46) in the neighborhood of $N_1/N = \frac{1}{2}$, i.e., expand them in power series in terms of $\epsilon = (N_1/N) - \frac{1}{2}$.

Upon using Eq. (37′), and again omitting the constant terms, we get, to an accuracy of second-order terms in ϵ:

$$F(N_1, \lambda) = N\left\{\varepsilon\Delta F - \frac{1}{\sqrt{2\pi}}\left[\frac{x_0(1-x_0)}{\lambda}\right]^{1/2}(\Delta F_{GC} - \Delta F_{AT})(1 - \pi\varepsilon^2) + \frac{F_s}{4\lambda}(1 - 4\varepsilon^2)\right\}, \tag{47}$$

where $\Delta F = x_0\Delta F_{GC} + (1 - x_0)\Delta F_{AT}$.

Upon equating to zero the derivative with respect to λ, we get (for $\epsilon = 0$):

*We are carrying out the treatment under the assumption that the fraction of melted regions in the DNA is small, so that the argument of the exponential in (38) is large; we have omitted the coefficients of the exponential, which are inessential under these conditions.

$$\lambda_0 = \frac{\pi}{2}\left(\frac{F_s}{\Delta F_{GC} - \Delta F_{AT}}\right)^2 \frac{1}{x_0(1-x_0)},$$

Using the equality $\Delta F_{GC} - \Delta F_{AT} = (T_{GC} - T_{AT}) \times (U_{AT}/T_{AT})$, we find from this (see (44)):

$$v_i = \pi\left(\frac{F_s}{T_{GC} - T_{AT}}\right)^2 \left(\frac{T_{AT}}{U_{AT}}\right)^2 \frac{1}{x_0(1-x_0)}. \quad (48)$$

Upon equating to zero the derivative with respect to ϵ (at constant λ), and differentiating the expression that we get with respect to T, we obtain

$$\Delta T = \left(2 - \frac{4}{\pi}\right)\frac{U_{AT}}{T_{AT}} \frac{(T_{GC} - T_{AT})^2}{F_s} x_0(1-x_0). \quad (49)$$

Now let us consider how Eqs. (48) and (49) are changed if we take into account the formation of loops in the melting of the heteropolynucleotide. In this case, we must add to the free energy of (46) a term describing the entropy of the loops (see Eq. (9)):

$$-T \ln \prod_{i=1}^{n} (\lambda k_i)^{-\alpha},$$

This gives

$$F'(N_1, \lambda) = F(N_1, \lambda) + \alpha T \frac{N_1}{\lambda}\left(1 - \frac{N_1}{N}\right)\left[\ln\lambda + \gamma\left(\frac{N_1}{N}\right)\right], \quad (50)$$

where

$$\gamma\left(\frac{N_1}{N}\right) = \left(1 - \frac{N_1}{N}\right)\sum_{k=1}^{\infty}\left(\frac{N_1}{N}\right)^{k-1} \ln k.$$

We get for λ_0 the transcendental equation:

$$\lambda_0 = \frac{\pi}{2}\left[\frac{F_s + \alpha T\left(\ln\lambda + \gamma\left(\frac{1}{2}\right) - 1\right)}{\Delta F_{GC} - \Delta F_{AT}}\right]^2 \frac{1}{x_0(1-x_0)}, \quad (51)$$

and the following expression for ΔT:

$$\Delta T = \left(2 - \frac{4}{\pi}\right)\frac{U_{AT}}{T_{AT}} \frac{(T_{GC} - T_{AT})^2}{F_s + \alpha T\left(\ln\lambda_0 + \gamma\left(\frac{1}{2}\right)\right)} x_0(1-x_0). \quad (52)$$

We can easily convince ourselves that $0 < \gamma(1/2) < 1$. On the other hand, λ_0 usually amounts to something of the order of several hundred. Hence, the quantities $\gamma(1/2) - 1$ in Eq. (51) and $\gamma(1/2)$ in Eq. (52) give very small corrections. Thus, the major effect when we take account of loops is to replace F_S by $F_S + \alpha T \ln \lambda_0$. Here we can find λ_0 by Eq. (51), e.g., by the method of successive approximations.

2. Exact Theory of the Helix-coil Transition in Heteropolymers

Let the polymer consist of a definite sequence of N links in which all the links generally differ. The partition function of the polymer has the form

$$z = \sum_{k_1=1,2} \cdots \sum_{k_{N+1}=1,2} \exp\left\{\frac{1}{T}\sum_{i=1}^{N}\left(F^{(i)}\delta_{k_i 2} - F_s^{(i)}\delta_{k_i 2}\delta_{k_{i+1}1}\right)\right\} = \sum_{k_1=1,2} \cdots \sum_{k_{N+1}=1,2} M^{(1)}_{k_1 k_2} M^{(2)}_{k_2 k_3} \cdots M^{(N-1)}_{k_{N-1} k_N} M^{(N)}_{k_N k_{N+1}}.$$

Here the superscript i denotes the serial number of the link along the chain, and δ is the Kronecker symbol. We shall measure the quantities $F^{(i)}$ and $F_S^{(i)}$ with respect to certain standard values F and F_S. Then upon introducing the notation

$$s = e^{\frac{F}{T}}, \quad \sigma = e^{-\frac{F_s}{T}}, \quad \zeta_i = e^{-\frac{F_s^i - F_s}{T}}, \quad p_i = \zeta_i e^{\frac{F^{(i)} - F}{T}},$$

we obtain an expression for the matrix $M^{(i)}$:

$$M^{(i)} = \begin{pmatrix} 1 & 1 \\ \sigma s p_i & \frac{s p_i}{\zeta_i} \end{pmatrix}.$$

We note that the symbol p_i in[39] denotes the quantity $\exp[(F^{(i)} - F)/T]$, i.e., p_i/ζ_i, rather than $\zeta_i \exp[(F^{(i)} - F)/T]$. The new method of defining the quantity p_i is convenient in that here p_i does not depend on the type of the adjacent links, but characterizes only the link i.

As $N \to \infty$, the value of z does not depend on the state of the links at the ends of the chain. Hence, we can attach links in arbitrary states to the beginning and end of the chain. Let these extra links always occur in the melted state, whereby $\zeta_{N\to 1} = 0$. Then the expression for z can be written in the form

$$z = (1\ 1)\begin{pmatrix} 1 & 1 \\ \sigma s p_1 & \frac{s p_1}{\zeta_1} \end{pmatrix} \cdots \begin{pmatrix} 1 & 1 \\ \sigma s p_N & \frac{s p_N}{\zeta_N} \end{pmatrix}\begin{pmatrix} 1 \\ 0 \end{pmatrix}.$$

Let us introduce the vector

$$(g_i\ f_i) = (1\ 1)\begin{pmatrix} 1 & 1 \\ \sigma s p_1 & \frac{s p_1}{\zeta_1} \end{pmatrix} \cdots \begin{pmatrix} 1 & 1 \\ \sigma s p_i & \frac{s p_i}{\zeta_i} \end{pmatrix}, \quad i > 0,$$

$$(g_0\ f_0) = (1\ 1);$$

Then,

$$z = g_N. \quad (53)$$

In order to determine ln z, let us represent it in the form

$$\ln z = \sum_{i=1}^{N} \ln G_i, \quad G_i = g_i/g_{i-1}. \quad (54)$$

We can find G_i by using the equation

$$(g_i\ f_i) = (g_{i-1}\ f_{i-1})\begin{pmatrix} 1 & 1 \\ \sigma s p_i & \frac{s p_i}{\zeta_i} \end{pmatrix}, \quad (55)$$

whence

$$\left.\begin{aligned} g_i &= g_{i-1} + \sigma s p_i f_{i-1}, \\ f_i &= g_{i-1} + \frac{s p_i}{\zeta_i} f_{i-1}, \end{aligned}\right\} \quad (56)$$

$$\left.\begin{aligned} g_{i-1} &= g_{i-2} + \sigma s p_{i-1} f_{i-2}, \\ f_{i-1} &= g_{i-2} + \frac{s p_{i-1}}{\zeta_{i-1}} f_{i-2}. \end{aligned}\right\} \quad (57)$$

If we eliminate f_{i-2} from (57), and substitute f_{i-1} from (57) into (56), we get

$$g_i = g_{i-1}\left(1 + \frac{s p_i}{\zeta_{i-1}}\right) + g_{i-2}\sigma s p_i\left(1 - \frac{1}{\sigma\zeta_{i-1}}\right). \quad (58)$$

If we divide Eq. (58) by g_{i-1}, we get

$$G_i = a_i - \frac{b_i}{G_{i-1}}, \quad (59)$$

where

$$a_i = 1 + \frac{s p_i}{\zeta_{i-1}}, \quad b_i = \sigma s p_i\left(\frac{1}{\sigma\zeta_{i-1}} - 1\right).$$

The helicity is calculated by the formula

$$\vartheta = \frac{1}{N}\frac{\partial \ln z}{\partial \ln s}. \quad (60)$$

If we substitute (54) into (60), we obtain

$$\vartheta = \frac{1}{N}\sum_{i=1}^{N} Q_i, \quad (61)$$

where $Q_i = \partial \ln G_i / \partial \ln s$. We find by direct differentiation that

$$Q_i = \frac{1}{G_i}\left\{G_i - 1 + \frac{b_i}{G_{i-1}} Q_{i-1}\right\}. \quad (62)$$

The number n of the regions in which all the links are in state 2 is determined by the formula

$$n = \frac{\partial \ln z}{\partial \ln \sigma}. \quad (63)$$

Here the mean length per helical region is $\nu = \vartheta N/n$. Upon substituting (54) into (63), we get

$$n = \sum_{i=1}^{N} R_i, \tag{64}$$

where $R_i = \partial \ln G_i / \partial \ln s$. We find by direct differentiation

$$R_i = \frac{1}{G_i G_{i-1}} (c_i + b_i R_{i-1}), \tag{65}$$

where $c_i = \sigma p_i s$.

The recurrent formulas (59), (61), (62), (64), and (65) derived in[39] are very convenient for direct calculations of melting curves on a computer. An analogous, but somewhat more complex, method of numerical solution of the problem was proposed in[40] (see also[41]). In later studies,[42,42] the partition function was calculated by direct matrix multiplication. The results of the calculations performed in the cited studies agree.* The simplest and most convenient formulas for numerical calculations are (59), (61), (62), (64), and (65), since here the problem is reduced to elementary recurrent equations, and the difficulties are eliminated that arise in matrix multiplication or in numerical differentiation. We note that this problem has quite recently been discussed again in[44].

An exact account of loops in treating the helix-coil transition in a heteropolymer is very difficult, and has not been carried out thus far. An approximate method of solving this problem based on the theory presented above has been proposed in[45]

As we have noted in discussing a homopolymer, accounting for loops leads to the appearance of the following coefficient multiplying the term in the partition function corresponding to n melted regions having lengths of $\nu_1, \ldots, \nu_n$:

$$J = \prod_{i=1}^{n} \nu_i^{-3/2}. \tag{66}$$

In order to calculate this partition function by the matrix method, the power-function dependence ν^{α} is replaced by the exponential function q^{ν}/μ. This is done in such a way that the values and the first derivatives of these two functions coincide for the mean length $\bar{\nu}_1$ of a melted region. These conditions give formulas for q and μ:

$$q = e^{\frac{\alpha}{\bar{\nu}_1}}, \tag{67}$$

$$\mu = \frac{1}{\bar{\nu}^{\alpha}} e^{\alpha}. \tag{68}$$

Here the helicity ϑ is calculated by Eqs. (59), (61), and (62), and the mean length $\bar{\nu}_1$ per melted region by Eqs. (59), (64), and (65). Account is taken here of the fact that $\bar{\nu}_1 = (1 - \vartheta)N/n$, and s is replaced by qs, and σ by $\mu\sigma$ in all formulas. In order to get the true values of ϑ and $\bar{\nu}_1$, it is simplest of all to use the method of successive approximations. One starts with arbitrary values of q and μ and calculates $\bar{\nu}_1$ by Eqs. (67) and (68). Then one calculates new values of q and μ, from which ν_1 is again calculated. This is continued until the values obtained in the next step of the procedure no longer differ from those obtained in the preceding step. The basis of this procedure for calculation is given in[45].

a) Analytical solution.[46] One can treat the quantity G as a random quantity, and introduce its distribution function P(G), which is defined as

$$P(G) = \frac{1}{N} \sum_{k} \delta(G - G_k). \tag{69}$$

When there are two types of links, the recurrence formula (59) implies an equation for the function P(G):

$$P(G) = x_1 \frac{b_1}{(a_1 - G)^2} P\left(\frac{b_1}{a_1 - G}\right) + x_2 \frac{b_2}{(a_2 - G)^2} P\left(\frac{b_2}{a_2 - G}\right), \tag{70}$$

where x_1 and x_2 are the fractions of links of types 1 and 2 in the heteropolymer ($x_1 + x_2 = 1$). The partition function is expressed in terms of P(G) as follows (see (54)):

$$\ln z = N \int P(G) \ln G \, dG. \tag{71}$$

It has not been possible to find an exact analytical solution of Eq. (70). Analytical expressions for the sought quantities can be found under the assumption that

$$\frac{1}{2} \frac{F_s}{\Delta S} \gg T_{GC} - T_{AT}. \tag{72}$$

Here it turns out[46] that the helicity ϑ depends on the temperature as follows:

$$\vartheta = \frac{1}{2} - \frac{\operatorname{sh} 2\xi - 2\xi}{4 \operatorname{sh}^2 \xi}, \quad \xi = \frac{T - T_0}{2\tau}, \tag{73}$$

where τ is given by Eq. (41); at the melting point,

$$\bar{\nu} = \frac{1}{(F_s/\Delta S)^2} \frac{1}{(T_{GC} - T_{AT})^2} \frac{1}{x_0 (1 - x_0)}. \tag{74}$$

With decreasing temperature, $\xi = (T - T_0)/2\tau \to -\infty$, $\vartheta \to 1 + 2\xi e^{2\xi}$, so that $1 - \vartheta = [(T_0 - T)/\tau] \exp \times [(T - T_0)/\tau]$. This agrees with Eq. (40), which was derived by qualitative considerations.

As $\xi \to 0$, Eq. (73) gives us $\vartheta = 1/2 - \xi/3$. This applies that the points of intersection of the limiting values of the helicity $\vartheta = 0$ and $\vartheta = 1$ with the tangent to the melting curve at the midpoint of the melting range are separated from one another by the distance $\xi_0 - \xi_1 = 3$. That is, the width of the melting range determined by using the tangent to the melting curve at $\vartheta = 1/2$ is

$$\Delta T = 2(\xi_1 - \xi_0)\tau = 6\tau, \tag{75}$$

where τ is given by Eq. (41).

Thus, the exact theory confirms the correctness of the qualitative picture of the helix-coil transition in heterogeneous DNA outlined above, as based on the idea of stepwise melting (as the temperature rises and the middle of the melting range is approached) of even larger regions in the DNA, which are less and less enriched in the low-melting AT component. The elementary theory and the approximate analytical theory give identical relations of ΔT and $\bar{\nu}$ to all the parameters. However, all these formulas were derived by using various approximations. Hence, the results of the exact calculations that have been performed with the recurrent formulas (59), (61), (62), (64), and (65) are of great interest.

*In [42,43], the method of calculating the partition function by direct multiplication of a random sequence of matrices is called the Monte Carlo method. This terminology is incorrect and conducive to error. Actually, the Monte Carlo method is nowhere used in such calculations, whereas the introduction of a random sequence of links is a necessity involving our ignorance of the true sequence.

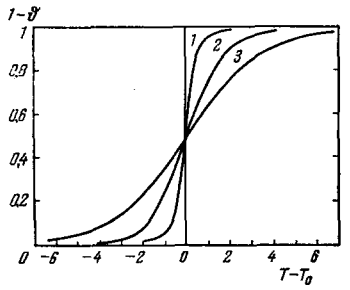


FIG. 15. Melting curves of a one-stranded heteropolymer having a random sequence [39] ($\sigma = 5 \times 10^{-5}$). $1-x_0 = 0$, $T_0 = 340\,^\circ K$, $N = 2 \times 10^4$; $2-x_0 = 0.1$, $T_0 = 344\,^\circ K$, $p = 3.310$, $N = 4 \times 10^4$; $3-x_0 = 0.5$, $T_0 = 360\,^\circ K$, $p = 3.138$, $N = 6 \times 10^4$.

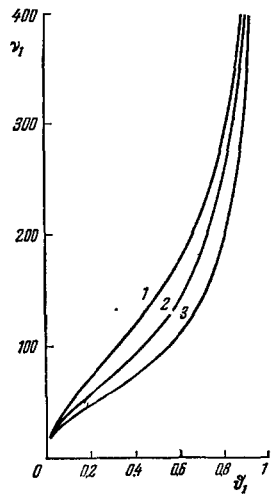


FIG. 16. Relation of the mean length of the melted regions to the fraction of melted links for a one-stranded heteropolymer having a random sequence. [39] Curves 1–3 correspond to the same values of the parameters as the corresponding curves in Fig. 15.

b) Machine calculations of the helix-coil transition in a heteropolymer and comparison with experiment. This section will present the results obtained in[39,45,47]. The independent parameters taken in the calculation were: the fraction x_0 of GC pairs in the DNA, the melting points T_{AT} and T_{GC} of the corresponding homopolymers, the heat of melting U_{AT}, and the cooperativity factor σ. The calculations were run on the M-20 and M-220 computers. In the calculation at an assigned x_0, a sequence of randomly alternating nucleotide pairs of total number N was constructed. The probability of finding an AT pair was taken to be $1 - x_0$, and that of a GC pair was x_0.

First we shall treat the results for the case of melting without formation of loops. For the assigned parameters, the helicity ϑ was calculated by Eqs. (59), (61), and (62), the mean length per helical region by Eqs. (59), (64), and (65), and the width of the melting range by Eq. (1). The quantity $|\partial\vartheta/\partial T|$ was calculated by the recurrent formulas[39]

$$\left|\frac{\partial\vartheta}{\partial T}\right| = \left|\frac{\partial\vartheta}{\partial \ln s}\right| \frac{U_{AT}}{T^2}. \qquad (76)$$

where

$$\frac{\partial\vartheta}{\partial \ln s} = \frac{1}{N}\sum_{i=1}^{N} S_i, \qquad (77)$$

$$S_i = Q_i(1-Q_i) + \frac{b_i}{G_i G_{i-1}} \times [Q_{i-1}(1-Q_{i-1}) + S_{i-1}]. \qquad (78)$$

The calculation made it possible to get the melting curves and the mean length per helical region in the polymer for various values of the parameters listed above. Figures 15, 16, and 17 give appropriate examples. The curves in these diagrams have been drawn for values of the parameters close to the real values:

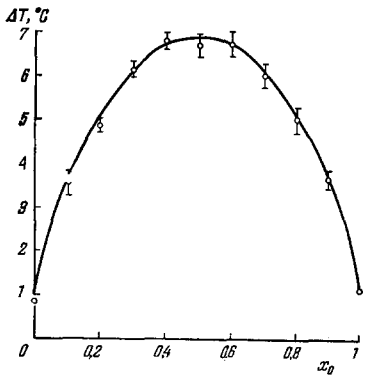


FIG. 17. Relation of the width of the melting range of a one-stranded heteropolymer having a random sequence to the GC content. [39] The sizes of the root-mean-square deviations are indicated.

$T_{AT} = 340^\circ K$, $T_{GC} = 380^\circ K$, $U_{AT} = 7$ kcal/mole ($3500^\circ K$) $\sigma = 5 \times 10^{-5}$. The number N of links in the calculated chain in different series was taken from 2×10^4 to 10^6. The scatter in the theoretical points shown in Fig. 17 (root-mean square deviation) corresponds to the fact that the chains of identical composition that are generated in the calculation differ in sequence. The marked interval contains 68% of all the cases. This scatter approaches zero only as $N \to \infty$. The nucleotide compositions of the chains generated in the calculation for the taken values of N differ insignificantly, and they cannot give rise to such a scatter in values of ΔT (at $N = 10^5$, it can give a scatter in ΔT values in the region of $x_0 = 0.5$ of no more than $10^{-4\,\circ}C$). In this sense, we should not distinguish between the a priori and a posteriori compositions of the chains, and the remark made on this point in[41] is not important in this case. However, as we see, the difference in nucleotide sequence of different DNA molecules with a given composition can lead to differences in ΔT of the order of 0.2–0.3°C (when $N = 10^5$; this quantity declines like $1/\sqrt{N}$ with increasing N). In order, when needed, to calculate the width of the melting range for different values of the parameters, it is convenient to use the following interpolation formula, which satisfies the results of the machine calculation:[47]

$$\Delta T \approx 2.16 \frac{T_0^2}{U_{AT}} \left(\frac{T_{GC} - T_{AT}}{T_{AT} T_0} U_{AT}\right)^{1.6} \frac{x_0(1-x_0)}{\lg \frac{1}{\sigma}} + 4\sqrt{\sigma}\frac{T_{AT}^2}{U_{AT}}. \qquad (79)$$

According to the machine calculations, the melting temperature T_0 satisfies Eq. (37). Equation (79) holds over a wide range of values of the parameters, and has an accuracy no worse than 10%. This accuracy is maintained over the following ranges of variation of values: the parameter x_0 from ≈ 0.15 to ≈ 0.85; $T_{GC} - T_{AT}$, at least from 20 to 100°; σ from 10^{-3} to zero; for U_{AT}, practically any positive value can be used, Naturally, not all the permissible values of the parameters have real meanings, and the concrete choice of parameters is determined by the experimental data.

It is interesting to compare Eq. (79) with the formulas derived above in the approximate analysis. In this case, when the heterogeneity is the determining factor (the first term in Eq. (79) considerably exceeds the second term), we see that Eq. (79) gives the same

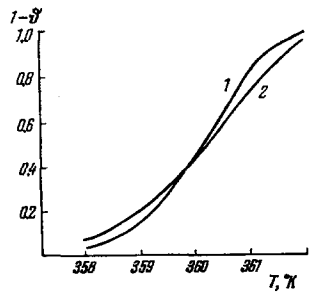


FIG. 18. Melting curves of a two-stranded heteropolymer having a random sequence. [45] $1-\sigma = 5 \times 10^{-5}$; $2-\sigma = 5 \times 10^{-4}$.

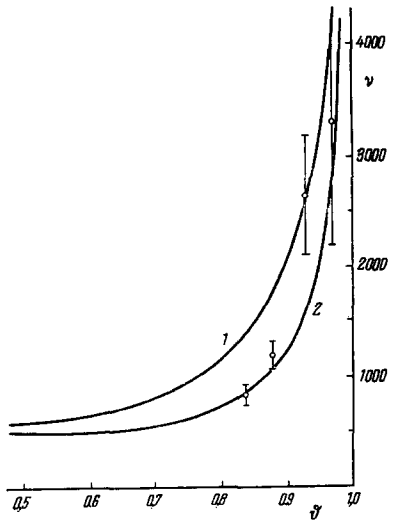


FIG. 19. Relation of the mean length per helical region to the helicity. The curves are the theoretical ones calculated for a two-stranded heteropolymer having a random sequence: [45] $1-\sigma = 5 \times 10^{-5}$, U_{AT} = 7 kcal/mole; $2-\sigma = 5 \times 10^{-5}$, U_{AT} = 9 kcal/mole. The points are the experimental values for T2 phage DNA. [5]

dependence on x_0 and on σ as the above-derived formulas do, but the exponent of $T_{GC} - T_{AT}$ is 1.6, rather than 2. Thus, the approximate theory not only reflects correctly the fundamental qualitative features of the melting of a heteropolymer, but it also gives a quantitatively correct result for the relation of ΔT to σ in the region of small σ. In the region of actual values of σ, the second term in Eq. (79) contributes about 1° to ΔT. We note, besides, that Eq. (79) remains valid if we go from a heteropolymer to a homopolymer (cf. Eq. (8)).

In discussing the melting of closed ring DNA, we shall compare below the experimental data with the outlined results of calculation for the case in which melting occurs without formation of loops. However, we should give for comparison with experiment the results of calculation for melting with loop formation.

The melting curves of DNA with account taken of loop formation were calculated by the above-described iteration procedure, with the same values of the parameters as in the calculations whose results are shown in Figs. 15–17. Figure 18 shows these curves. The width of the melting range for these curves was found to be: $\Delta T = 2.7°$ for $\sigma = 5 \times 10^{-5}$, and $\Delta T = 3.2°$ for $\sigma = 5 \times 10^{-4}$. When loops were not accounted for, values of $\Delta T = 7-8°$ were obtained (by Eq. (79)) instead of these values. This narrowing of the melting range agrees with Eq. (52).

If we take the value 9 kcal/mole for U_{AT}, which is apparently closer to the truth than 7 kcal/mole, then ΔT increases somewhat, and attains values $\approx 3°$ for $\sigma = 5 \times 10^{-5}$.

Thus, the results of calculating the width of the melting range of heteropolynucleotides having a random sequence, with account taken of loop formation, give the value $\Delta T = 2.8-3.2°$ in the region of actual values of the parameters.

Figure 19 gives the calculated curves for the relation of the mean length of a helical region to the helicity for two extreme values of U_{AT}.

The obtained results agree with those of Crothers' calculations,[48] which were performed by a different method. However, in Crothers' study, the calculations were performed for rather small chain lengths N(5000), and he assumed to simplify the calculations that melting can only occur in regions of several tens of links (from 30 to 50). In this respect, Eichinger and and Fixman[49] have seriously criticized the results of[48]. In turn, Fixman and his associates[49,50] have proposed a method of calculating the helix-coil transition in heteropolymers that is highly artificial and has no firm justification. The only justification is the fact that their calculations for single-stranded heteropolymers agree well in a certain range of parameter values with the results of the exact theory (see[50]). However, the results for a two-stranded heteropolymer disagree sharply with those given above for this case, and give a width of the melting range of about 0.2°. The elementary theory presented above clear shows that taking account of loops in a heteropolymer cannot give such enormous effects. Hence, we think that the results of[49] are in error.

We shall proceed to compare the theory with experiment. Unfortunately, the only experimentally measurable characteristic until recently has been the melting curve. The shape of the melting curve agrees with experiment, almost independently of the calculated model, and it cannot serve as a criterion for the correctness of the theory. Hence, it makes sense only to compare the numerical value of the width of the melting range. At present, sufficiently complete experimental data exist for two conditions: standard ionic conditions (SSC), and for a 7.2 M $NaClO_4$ solution. In the former case, $T_{GC} - T_{AT} = 41°$, and in the latter case, 56°. The rest of the parameters vary relatively slightly. The table shows the comparison of theory with experiment. The data for T2 phage DNA are from[4], and the rest of the data from[12]. The DNA's of T2 and T7 pha phages are linear, and the DNA of polyoma II is annular, but with a break in one of the chains, so that its chains can freely unwind. Hence it should obey the same theory as linear DNA. Polyoma I DNA is annular and closed, and it should obey the theory for single-stranded polymers.

The theory that predicts the width of the melting range contains a single "purely theoretical" parameter σ. All the other parameters are determined directly from independent experiments (calorimetry and determination of T_m). We note that, while ΔT for a homopolynucleotide depends strongly on σ (by a power law), this dependence is considerably weaker

Comparison of theory with experiment

DNA	ΔT_{exp}	ΔT_{theor}	DNA	ΔT_{exp}	ΔT_{theor}
SSC (0.15 *M* NaCl), $T_{GC}-T_{AT}=41°$			7.2 *M* $NaClO_4$, $T_{GC}-T_{AT}=56°$		
T2	3.2°	2.8—3.2°	T2	5°	4.8—5.2°
T7	2.5—3°		T7	5.5°	
Polyoma II	2.5—3°		Polyoma II	8°	
Polyoma I	—	≈8°	Polyoma I	19°	≈14°

(logarithmic) for a heteropolynucleotide. Hence, it is reasonable to select σ by comparing theory with experiment for homopolynucleotides. As we pointed out above, this gives a value of 10^{-4}—10^{-5}. Naturally, some uncertainty remains here, which we discussed above in the section dealing with melting of homopolymers. However, owing to the logarithmic dependence on σ of the melting parameters of a heteropolymer, this uncertainty can hardly affect the result appreciably.

We should deem the agreement that we get between the experimental and calculated values of ΔT to be quite satisfactory. In 7.2 M $NaClO_4$ solution, the experimental values for polyoma DNA proved to be substantially larger than the theoretical values. Here there is an internal contradiction in the experimental data, since T7 phage and polyoma II DNA have identical values of ΔT under SSC conditions, but different in 7.2 M $NaClO_4$ solution. In line with this, we shouldn't yet grant any serious meaning to a certain discrepancy between theory and experiment.

It is of great interest to compare the theory with other experimentally found characteristics of the helix-coil transition in DNA. Some experimental data obtained in[4,5] are plotted (as the points) in Fig. 19. As we see, the results of calculations for various possible values of the parameters (U_{AT} from 7 to 9 kcal/mole) bracket the experimental points. Since $\bar{\nu}$ depends somewhat more strongly on σ than ΔT does (see, e.g., Eqs. (48) and (74)), comparison of the experimental values of $\bar{\nu}$ with the theoretical permits one in principle to make an additional refinement in the choice of σ. The results of this comparison favor a choice of σ of about 5×10^{-5}.

The obtained agreement of theory with experiment indicates that some DNA's, in particular the experimentally well-studied T2 phage DNA have sequences of base pairs which behave, when subdivided into regions of hundreds of links, just like the random sequence that was adopted in the theoretical calculations. However, this type of DNA, which has a narrow melting interval (about 3°) is the exception, rather than the rule. Even among the virusses, there are some whose DNA consists of long regions that strongly differ in their mean GC content. These DNA's melt in stepwise fashion: each step corresponds to melting of an individual region, or block. DNA from higher organisms has very broad melting curves with $\Delta T = 10°$, though smooth enough. This has been shown recently to be explained by the block character of the base-pair distribution in the DNA of higher organisms.[51,52] In a first approximation, we can consider the DNA of higher organisms to be a sequence of blocks of mean dimension about 10^4—10^5 pairs. Within each block, the sequence of base pairs is close to random with a fixed value of x_0, but the value of x_0 differs for different blocks. The DNA of bacteria has an analogous structure, but the blocks in them differ less in their mean GC content, and hence ΔT is smaller for them.*

3. Relation of the Width of the Melting Range of DNA to the Chain Length

The results discussed thus far have referred to melting of long molecules in which end effects play no role. Decrease in the chain length lowers the melting point and broadens the melting range, both for homopolymers and heteropolymers. This effect is manifested especially clearly when the chain length becomes considerably shorter than the mean length of a helical region that is formed in the course of melting of a polymer. Then we can assume that melting of such fragments occurs as a whole, without formation of intermediate states. Melting of a short fragment is facilitated by the fact that it has a surface energy equal to the energy F_S of two boundaries in a polymer, so that the condition of equilibrium has the form (cf. Eq. (35)):

$$\nu\Delta F = -F_s, \tag{80}$$

Here ΔF is the difference in free energy per link between the helical and coil-like states of a long polymer. Since $\Delta F = -U + T\Delta S$, and $U/\Delta S = T_0$ is the melting point of the long polymer, then the melting point of a fragment of length ν is

$$T_m = T_0 - \frac{F_s}{\Delta S}\frac{1}{\nu}. \tag{81}$$

Eq. (81) is valid for either a homopolymer of a heteropolymer. The melting curve of a solution of fragments of length ν will be described by the mass-action law (Boltzmann distribution):

$$\frac{N_2}{N_1} = e^{-\frac{F_2-F_1}{T}} = e^{-\frac{\nu\Delta F+F_s}{T}},$$

whence

$$\vartheta = \frac{1}{1+e^{\frac{\nu\Delta F+F_s}{T}}}. \tag{82}$$

Thus, the melting point in (81) corresponds to the point at which $\vartheta = 1/2$, while the width of the melting range has the form

$$\Delta T = \frac{4}{\nu}\frac{T_0}{\Delta S} = \frac{4}{\nu}\frac{T_0^2}{U}. \tag{83}$$

Evidently, this formula holds only for a homopolymer or for a preparation consisting of molecules of a heteropolymer that have strictly identical sequences (see Note 7).

*The presented results make expecially evident the inadequacy of the attempts made in [41] to explain the different values of ΔT for DNA from various sources by arbitrary adjustment of the parameter σ (which had to be varied over several orders of magnitude) within the framework of calculating the helix-coil transition for a heteropolymer with a random sequence without account taken of loops.

In the fragmentation of a long heteropolymer molecule having a random sequence, the different fragments have concentrations of GC pairs that differ from the mean x_0 by the amount Δx. The latter quantity has the Gaussian distribution (37′). The relation between the melting point T of a fragment and Δx is given by the formula (cf. Eq. (36)):

$$T = T_0 + (T_{GC} - T_{AT})\,\Delta x - \frac{F_s}{\Delta S}\frac{1}{\nu}. \tag{84}$$

Hence, the melting curve will have the shape of an error curve:

$$\vartheta = \frac{1}{\sqrt{2\pi}\,\sigma_\nu}\int_{\Delta x(T)}^{\infty} e^{-\frac{t^2}{2\sigma_\nu^2}}\,dt, \tag{85}$$

where $\Delta x(T)$ is given by Eq. (84). By using the definition in (1), we obtain from Eq. (85):

$$(\Delta T)^2 = 2\pi\,(T_{GC} - T_{AT})^2\,x_0\,(1 - x_0)\,\frac{1}{\nu}. \tag{86}$$

These are the elementary asymptotic (for small ν) formulas relating the melting point and the width of the melting range to the chain length. These formulas prove to be very useful for rough estimates of the most varied types (see, e.g.[53]). However, they cannot pretend to describe experiment quantitatively, especially among heteropolymers.

The first attempt to devise a quantitative theory relating the melting curves of fragmented DNA to the chain length and to compare it with experiment was made in[54]. They showed in this study that quantitative comparison of the melting point with experiment is hindered by the fact that this characteristic is sensitive to whether the strands separate completely upon melting. In other respects, this study is not convincing, since the numerical calculations carried out in it referred only to the limiting case of short lengths, and were compared with experiment for great lengths (>400 links), for which the width of the melting range of the fragmented DNA differed little from that of DNA of high molecular weight. We should also note that the experiments performed in[54] have too low an accuracy to permit quantitative comparison with theory.

The melting curves of fragmented molecules can be calculated by the general formulas (59), (61), and (62). Breaking a chain made of N links into pieces containing n_0 links each is carried out in the theory by imposing the condition (it is assumed that $N = n_0 k_0$):

$$\zeta_i = \begin{cases} 1 & \text{for } i \neq kn_0, \\ 1/\sigma & \text{for } i = kn_0, \end{cases} \quad k = 1, 2, \ldots, (k_0 - 1). \tag{87}$$

This condition implies loss of interaction between the links numbered kn_0 and $kn_0 + 1$. Experimentally, such a loss of interaction can be effected both by actual fragmentation of the DNA, or by formation in some way of locally denatured regions in it. Figure 20 shows the melting curves for different values of n_0, as calculated by Eqs. (59), (61), (62), and (87). We see that a regular shift to the left and broadening occur with decreasing n_0. Since the calculations were performed under the assumption that the strands do not separate completely upon melting, we should not expect good agreement with experiment of the melting-point shift calculated in this way. This involves the fact that, as was noted above, the melting-point shift depends on whether the strands

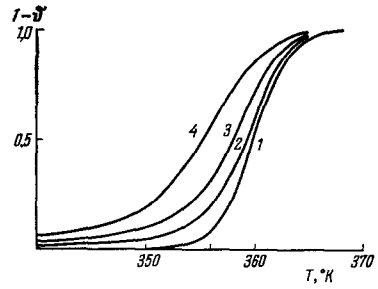


FIG. 20. Theoretical melting curves of a heteropolymer having a random sequence, when "fragmented" to different values of n_0: [55] 1–$n_0 = 3 \times 10^5$; 2–$n_0 = 200$; 3–$n_0 = 100$; 4–$n_0 = 50$.

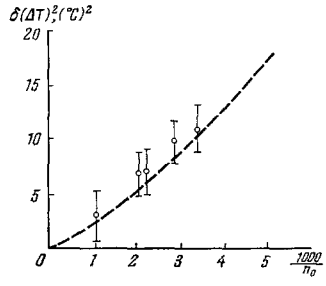


FIG. 21. Theoretical (dotted line) and experimental (circles) relations of $\delta(\Delta T)^2$ to $1/n_0$.

separate completely. Conversely, the variation in the width of the melting range should not depend on these factors. Moreover, calculations show[55] that the quantity

$$\delta\,(\Delta T)^2 \equiv (\Delta T)^2 - (\Delta_0 T)^2, \tag{88}$$

where $\Delta_0 T$ is the asymptotic value of the width of the melting range, is practically independent as $n_0 \to \infty$ of the value of the cooperativity factor σ adopted in the calculation. Since shifting from the model of a single-stranded heteropolymer to a model of a two-stranded heteropolymer is equivalent in theory to changing the effective value of σ, the calculated values of $\delta(\Delta T)^2$ should not depend on the model. Finally, the calculations showed[55] that the size of $\delta(\Delta T)^2$ is practically independent of the nature of the length distribution of the fragments. The following interpolation formula has been derived from the results of machine calculations:[47]

$$\frac{100}{n_0} \approx (0.87y + 0.20)\,\frac{y}{y + 0.074}, \tag{89}$$

Here y is a quantity that is related to $\delta(\Delta T)^2$:

$$\delta\,(\Delta T)^2 \approx 0.103\left(\frac{T_0^2}{U_{AT}}\right)^2\left(\frac{T_{GC} - T_{AT}}{T_0 T_{AT}}\,U_{AT}\right)^{1.4} x_0\,(1 - x_0)\,y. \tag{90}$$

One can compare this formula with experiment. The melting curves of DNA that had been fragmented to different molecular weights were obtained in[13] (see Fig. 9). The shape of the curves in Fig. 9 agrees qualitatively with what the theory predicts. Actually, the rise in the left-hand branch of the curves with decreasing molecular weight exceeds the expected amount. Apparently, this involves inexact cross-rupture of the molecules upon fragmentation, which gives rise to single-stranded "tails" at the ends of the fragments. Figure 21 shows the relation of $\delta(\Delta T)^2$ to $1/n_0$. The value of n_0 was determined by sedimentation and by electron microscopy.

The circles in Fig. 21 are the experimental points, and the dotted line has been drawn according to Eq. (89), with values of the parameters corresponding to standard ionic conditions. We see that the theory agrees with experiment. Thus, measurement of the width of the melting range can be used to determine the chain length of fragmented DNA. Furthermore, since the effect of broadening of the melting curve involves loss of interaction between links along the chain, rather than specifically the rupture of the sugar-phosphate backbone, measurement of the width of the melting range can serve as a universal method for determining the concentration of defects in the secondary structure of DNA. Such a method was proposed in[13], and its potentialities were demonstrated with the example of DNA that had been subjected to ultraviolet irradiation, which brings about in DNA defects in the secondary structure without breaking the sugar-phosphate backbone.

V. CONCLUSION

We can see from all that has been said that the phenomenon of the helix-coil transition in DNA is among the physical phenomena that are currently amenable to detailed theoretical and experimental analysis. The studies that have been conducted show that in natural DNA's we are dealing with an actual one-dimensional aperiodic crystal. Synthetic homopolynucleotides are periodic crystals. What are the forthcoming problems in the theoretical and experimental study of the helix-coil transition in DNA? First of all, we must note the lack of information on the properties of synthetic homopolymers. This involves primarily the poor accessibility of preparations of homopolynucleotides of high molecular weight. Performance of systematic, careful studies of homopolynucleotides would substantially speed progress in this field. One must also make careful measurements of melting curves of actual DNA's under different ionic conditions in order to get quantitative data on the relation of ΔT to the conditions in the medium. This would make it possible to compare theory with experiment in further detail.

With regard to theory, the main difficulty involves the problem of knots in the melted region of the polymer. On this point, the theory of the helix-coil transition proves to face difficulties that are analogous in many ways to those encountered in other fields of polymer physics (see, e.g.,[56-58]).

We have restricted our treatment in this article to the thermodynamics of the helix-coil transition in DNA. The kinetics of this process has been studied intensively in recent years (see[59-61]). A number of interesting experimental results have already been obtained, but there is as yet no adequate theory.

Another interesting approach that has developed in recent years is to study the kinetics of unwinding of DNA when acted on by chemical agents whose reactions with the bases prevent the latter from forming complementary pairs. In particular, formaldehyde is such an agent. The kinetics of unwinding of DNA treated with formaldehyde has been already studied in considerable detail. On the basis of this study, a highly sensitive method of detecting locally denatured regions in DNA has been developed (see[62,27,63]). This method in itself, as well as in combination with some methods based on thermodynamic properties of DNA, makes it possible to get results that can be of direct biological interest.[53,63,64]

The purpose of this article has been to present the existing theoretical concepts of the thermodynamic properties of DNA as a one-dimensional aperiodic crystal. Study of the helix-coil transition in DNA is interesting in itself, since we are dealing with the unusual physical phenomenon. However, the importance of an all-sided study of the thermodynamic properties of DNA, and especially of its complexes with proteins, has recently become more and more evident in understanding how the fundamental stages of functioning of DNA occur in the cell. Perhaps the most interesting study along this line is that of Alberts and Frey.[65] In this study they isolated the protein of gene 32 of T4 bacteriophase. The presence of the latter in large amounts is necessary for normal occurrence of replication (duplication of DNA) and genetic recombination. The studies performed in[65] showed that this protein has a much higher binding constant with one-stranded than with two-stranded DNA. Hence, presence of this protein in the solution should substantially facilitate separation of the strands of DNA. The unwinding action of the protein was shown directly in its interaction with the synthetic two-stranded polynucleotide poly-(dAT : dAT). A number of interesting properties of this protein were also studied. Thus, special proteins exist in the cell that break down (or weaken very greatly) the helical structure of DNA, and this breakdown is necessary for the functioning of the cell.

Studies along this line are just beginning. However, it is already clear that they will lead to a considerably deeper understanding of how DNA performs its functions in the cell.

NOTES

1. Watson and Crick established the structure of the DNA molecule from x-ray crystal-structure data and stereochemical considerations in 1953. The correctness of the Watson-Crick model was subsequently proved rigorously by Wilkins and his associates on the basis of much more complete x-ray data. The establishment of this structure has been acknowledged to be the greatest achievement of the 20th Century in the field of biology (J. Watson[66] has vividly described the history of the discovery of the structure of DNA in his book). This discovery founded a new science: molecular biology.

The so-called B-form of DNA has the parameters cited in the text. DNA can change its structure when the external conditions are changed greatly. Thus, for example, when a DNA preparation is dried, it transforms to the A-form, in which the DNA contains 11 base pairs per turn to the helix, and the normal to the plane of the base pairs is inclined by 20° to the axis of the helix. At higher humidity, DNA occurs in the B-form. Therefore, people assume that DNA has the same structure in dilute solution. The correctness of this assumption is confirmed by a number of indirect

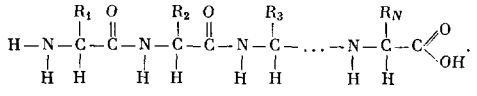
data (flow birefringence, small-angle x-ray scattering, optical dichroism, etc.). Very convincing data of this type have been obtained recently (see[67]). They showed that the circular dichroism spectrum of DNA in solution coincides with that of DNA films at high relative humidities at which DNA occurs in the B-form according to x-ray data. A marked change in the circular dichroism spectrum is observed with decrease in humidity as the DNA transforms to the A-form.

Information has recently been accumulating that small changes in the structure of DNA can occur (e.g., the angle between neighboring base pairs) upon changing the properties of the solution (the temperature or the salt concentration).

2. In oder to study the properties of DNA molecules, they are isolated from cells or viruses by purification from all other components (mainly proteins). The methods commonly used to isolate DNA are rather severe. The velocity gradients that arise in the isolation process, as well as certain biochemical factors, break the DNA molecules into fragments containing no more than several tens of thousands, or at the most, hundreds of thousands of links. The true length of the molecules in a living cell or in a virus particle differs greatly for different living organisms. Thus, the length of the DNA molecule for different viruses can vary from several thousand links to hundreds of thousands of links. The DNA molecules of bacteria already contain several million links. Here it has been established that the entire set of genetic information of a bacterium is contained in the sequence of one gigantic DNA molecule whose contour length is approximately a millimeter. In more complex, multicellular organisms, the genetic information is not written down continuously, but is contained in several individual DNA molecules. The total contour length of all the DNA molecules that bear the total, unitary bulk of information (this complete set of genetic information is called the genome) is equal, for example, to about 1.8 meters for man. That is, it contains several billion links.

Besides the increase in bulk of genetic information, multicellular organisms differ from bacteria and viruses in that the DNA in multicellular organisms is very firmly complexed with protein. The existence of this complex is manifested especially distinctly in the stage of cell division during which the DNA and protein form special particles that can be seen in the optical microscope, or chromosomes. Very little is yet known about the structure of these nucleoprotein complexes.

3. The genetic information written in the form of a sequence of nucleotides in a DNA molecule is realized as follows (we give below a highly schematic and somewhat exaggereated description of the processes; for more details, see, e.g., the excellent book by J. Watson[68]). One one of the strands of the DNA molecule (which is called the significant strand), an RNA molecule complementary to this strand is synthesized with the aid of the special enzyme RNA polymerase. This RNA is called informational RNA (or mRNA from the English word messenger). This process is called transcription. However, not the whole DNA molecule is copied, but only a small part of it called the gene. The gene contains the information for the chemical structure of one protein molecule. The mRNA synthesized on the gene forms a complex with special particles called ribosomes, which synthesize protein according to the pattern brought by the mRNA. The point is that protein molecules, which perform practically all the functions of the living organism, are linear polymer chains that are packed quite capriciously in space (all enzymes are proteins; moreover, all the structural components of the cell and the living organism consist of proteins to a considerable degree). The monomeric links of a protein chain are amino-acid residues, so that a protein chain (a part of it is also called a polypeptide chain) has the form



The amino-acid residues differ from one another in the radical R. Twenty types of amino-acid residues in all are found in proteins. The simplest of them is glycine, where simply hydrogen occurs as R. Then follows alanine, which has the methyl group CH_3 as R, etc.

The ribosomes perform the translation from the polynucleotide language to the poly-amino acid language. The dictionary that the ribosome uses in translation is called the genetic code. The genetic code has already been deciphered (see, e.g., Watson's book[68]). Some special, very small RNA molecules called transport RNA (tRNA) play an essential role in this process. They contain only about a hundred nucleotides, and they transport the amino-acid residues from the solution to the polypeptide chain.

4. One establishes the macromolecular structure of one-stranded and two-stranded DNA, or as one often says, the morphology of the DNA molecule in solution, by using the ordinary methods of polymer solution physics (see the monograph[69]). Thus, the statistical segment of single-stranded DNA has been determined from viscosity data in[4] for T2 phage DNA, and in[70] for the homopolymer poly-U, and they got practically identical results. There is as yet no complete certainty about the size of the statistical segment of double-helical DNA, since light scattering and hydrodynamic methods give divergent results (see[71]). The hydrodynamic data give a size of the statistical segment of about 300 base pairs. The methods of polymer solution physics do not permit one to choose between two models of flexibility of a polymer chain: zigzag, in which the molecule is a chain of freely-jointed, completely rigid rods; and worm-like (persistent), in which the molecule behaves like a rubber hose that has a certain very small flexibility at every point. This problem was settled in favor of the worm-like model when it was shown[62] that locally denatured regions that could serve as the joints for a zigzag model are absent in DNA for distances of at least several thousand base pairs.

5. A well-studied example, both experimentally and theoretically, of a single-stranded homopolymer that undergoes a helix-coil transition with changing conditions in the medium is a polypeptide (see Note 3). A polypeptide molecule can form a so-called α-helix, in which the chain is twisted into a helix. It is reinforced by hydrogen bonds formed along the axis of the helix

between the N-H and C-O groups of amino-acid residues separated by three links along the chain. When the external conditions are changed (the temperature, the pH, or the composition of the solvent), the α-helix can break down and transform into an ordinary flexible polymer chain. Zimm and Bragg first applied the Ising model to describe the helix-coil transition in a polypeptide in 1958. Subsequent experimental and theoretical studies have shown that the results of calculations performed within the framework of the Ising model agree quantitatively with experiment. These problems have been presented in detail in the monograph.[72]

6. As we have mentioned, each state of a freely-jointed "immaterial" chain (i.e., without interaction between remote portions along the chain) is equivalent to the trajectory of a Brownian particle. Here the number ν of segments of the chain plays the role of the time. Hence, the probability P(h) that the ends of a chain of ν segments will be separated by a distance from h to h + dh is (see e.g.,[73]):

$$P(\mathrm{h})\,d\mathrm{h}=\left(\frac{3}{2\pi\nu l^2}\right)^{3/2} e^{-\frac{3h^2}{2\nu l^2}}\,d\mathrm{h}.$$

The probability that both ends will occupy the small volume δV will evidently be $P(0)\delta V$, which leads to Eq. (9) of the main text.

7. The results given in the main text were obtained essentially by applying the assumption (upon which the entire treatment of the helix-coil transition in DNA is based) that the strands do not separate completely upon melting. This is just why we could use the first-order reaction equation (82) to describe the helix-coil equilibrium. There are a number of experimental situations in which such a treatment is justified, even for short regions. That is, the strands actually do not separate. For example, in[53], Eqs. (81) and (83) were used to estimate the length of low-melting regions continued in T2 phage DNA that melt at a temperature below the onset of melting of the main part of the molecule. Here it was assumed that they are sequences consisting almost exclusively of AT pairs. Evidently, what we should consider in this case is precisely a first-order reaction.

However, there are other situations that one encounters in studying homogeneous two-stranded oligonucleotides (i.e., very short polynucleotides) in which equilibrium between two-stranded and one-stranded oligonucleotides can be established within the time of experiment. In this case, one must consider a second-order reaction. This has been done by Applequist and Damle.[74] Following their work, we shall treat the equilibrium between single and double-helical chains of the complementary oligonucleotides A and B. As before, we shall assume that the process follows an "all-or-none" principle:

$$\mathrm{A}+\mathrm{B}\rightleftharpoons\mathrm{AB}.$$

The equation for equilibrium has the form

$$\frac{[\mathrm{AB}]}{[\mathrm{A}]\,[\mathrm{B}]}=K.$$

We shall introduce the total concentration C of one-stranded oligonucleotides of one type:

$$[\mathrm{A}]+[\mathrm{AB}]=C.$$

Upon assuming that [A] = [B], we get the quadratic equation

$$K\,[\mathrm{A}]^2+[\mathrm{A}]-C=0,$$

Hence, we obtain the following formula for the fraction of melted links $1-\vartheta=[\mathrm{A}]/C$:

$$1-\vartheta=\frac{1}{2KC}\,[(1+4KC)^{1/2}-1].$$

This formula describes the helix-coil equilibrium in short oligonucleotides. It implies that the degree of conversion from the helical to the coil-like state depends both on the temperature (via the equilibrium constant K) and on the concentration C of the oligonucleotide. Evidently, the melting point at which half of the links are in the coil-like state is defined by the condition

$$K_m C=2.$$

Evidently, the equilibrium constant K will be (cf. (82)):

$$K=\frac{2}{C_0}\,e^{-\frac{\nu\Delta F+F_s}{T}},$$

where C_0 is a constant having the dimensions of concentration that does not depend on ν and T.

From the condition $K_m C = 2$, we find for T_m the equation

$$\frac{1}{T_m}-\frac{1}{T_0}=\frac{1}{\nu}\left[\frac{F_s}{U}\,\frac{1}{T_m}-\frac{1}{U}\ln\frac{C}{C_0}\right],$$

where T_0 is the melting point of a long homopolynucleotide (as $\nu\to\infty$). This formula differs substantially from (81) in that it contains the concentration C of the oligonucleotide in the solution.

If we differentiate the expression for $1-\vartheta$ with respect to T, we get the following formula for the width of the melting range:

$$\Delta T=\frac{6}{\nu}\,\frac{T_m^2}{U}.$$

This formula is fully analogous to Eq. (83), and differs from it only in the numerical coefficient.

A number of experimental studies have recently appeared on the melting of very short two-stranded homopolynucleotides. They have shown that the theory of Applequist and Damle presented above is applicable to those systems.

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