# Topological aspects of the physics of polymers: The theory and its biophysical applications 

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The formation of polymer ring structures leads to new physical effects that arise from topological restrictions. These restrictions consist of the fact that the topological state of a ring polymer molecule cannot vary. Interest in this type of object has increased sharply since the proof that in most cases the molecules of DNA function in the cell in a closed circular form. This review presents the necessary information on the physical properties of circular DNAs. The mathematical basis of the theory of knots and linkages are briefly presented, together with the theory of ribbons. This apparatus is employed to obtain quantitative data on the behavior of closed polymer chains by Monte Carlo calculations on a computer. The results of the calculations are compared with the experimental data for circular DNAs. This comparison has made it possible to obtain valuable information on the properties of the double helix, such as its torsional rigidity. The biological role of the topological restrictions in DNA is discussed, as well as the significance of the recently discovered enzymes, topoisomerases, which alter the topological state of circular DNAs.

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## 1. INTRODUCTION

The study of long polymer molecules has posed a number of theoretical problems, which have long since become a part of the group of problems of statistical physics (see, e.g., Refs. 1, 2). The analogy between a polymer chain and a Brownian trajectory has proved very fruitful. In fact, the configurations adopted by the simplest model of a polymer chain, in which links remote along the chain can pass through one another without hindrance, are equivalent to the trajectories of a random walk. This model serves as the starting point of the statistical physics of polymers (see Refs. 3-7). A polymer chain differs from a Brownian trajectory in its corporeality, i.e., in the fact that self-overlap is forbidden in it. This leads to the well known effect of swelling of polymer coils, and an extensive literature has been devoted to its theoretical study (see the reviews of Refs. 6-8 and the studies cited therein).

Effects of a completely different type, also caused by the corporeality of the polymer chain, must arise in treating the statistical physics of ring polymers. In fact, the configurations that a ring polymer chain or a system of ring chains can adopt are limited solely to topologically equivalent states, i.e., to those that can be derived from one another by continuous deformation
without self-intersections. Topological restrictions of this type give rise to completely new problems in the theoretical analysis of the behavior of systems made of closed chains, which require the application of a special mathematical apparatus for their solution.

This review will analyze the approaches that. have been proposed to overcome the stated difficulties, and which allow one to solve a number of problems of statistical physics of polymer chains with account taken of the topological restrictions. The interest in problems of this type arises from a number of factors. First,


FIG. 1. Topological "cross-linking" in a polymer network. The dots denote ordinary chemical cross-links.

a)

b)

FIG. 2. Examples of topologically nontrivial structures made of circular polymer chains: a knot (a) and a linkage or catenane (b).
ever more attention has been attracted in recent years to the possible effect of entanglement of polymer chains on mechanical and other properties of polymer networks ${ }^{9-12}$ (Fig. 1). Second, the purely chemical problem of synthesizing polymer molecules having the shape of a knot or of polymer molecules linked together (catenanes) possesses a certain interest (Fig. 2). Third, and most importantly, it has turned out in recent years that ring molecules play a very great role in biology. Namely, it has turned out that DNA molecules practically always function in the cell in a closed circular form. In other words, the functioning DNA molecule amounts to two closed polymer chains, which in addition form with one another a linkage of very high order (Fig. 3). The existence of such structures poses a number of very interesting topological problems. Understanding of the physical, and ultimately the biological, properties of DNA molecules hinges on their solution. Considerable attention will be paid in this review to examining these problems.

## 2. CIRCULAR CLOSED DNAs

## a) The circular form of DNA and its biological role

The fundamental stimulus for the development of the theory to be presented in this review was the discovery of circular DNAs. We recall that DNA molecules, which contain all the information on the structure of living organisms, consist of two polymer chains attached to one another by weak, noncovalent interactions. These chains form a double helix in which $\gamma_{0} \approx 10$ monomer links (base pairs) occur per turn. Figure 4 depicts a packing model of a fragment of the DNA molecule. Actual DNAs contain from several thousand to billions of monomer links. Initially the main attention was focused on studying the properties of linear DNA molecules, since this is precisely the form of DNA that could be extracted from cells and virus particles. A number of


FIG. 3. Schematic diagram of closed circular DNA. Each of the two complementary chains of the double helix is closed. Consequently the chains turn out to be linked. A quantitative measure of the linkage is the quantity $L k$, which is the number of times that one of the chains passes through a surface stretched on the other chain (for the case drawn in the diagram, we have $L k=9$ ). In actual cases we have $L k \geq 10^{2}$.


FIG. 4. Space model of a region of the double helix of DNA. The dark circles, which are connected for the sake of perspicuity with helices, are the phosphate groups. The nitrogen bases, in whose sequence the genetic information is recorded, lie inside. There are 10 base pairs per turn of the helix.
reviews (see, e.g., Refs. 16-18) have been devoted to the physical studies of these DNAs.

In was unexpectedly found in 1963 that DNA exists in certain viruses in a closed circular (CC) form. In this new state the two single chains of which the DNA consists are each closed on themselves. CC DNA is illustrated schematically in Fig. 3. We see that the two complementary filaments in CC DNA proved to be linked. Here they form a high-order linkage (of the order of $N / \gamma_{0}$, where $N$ is the number of pairs in the DNA). Initially this discovery was not seen to be very significant, since this form of DNA was regarded as exotic. However, in the course of time, the CC form of DNA was discovered in an ever greater number of organisms. Currently it is generally acknowledged that precisely this form of DNA is typical of the simplest DNAs, and also of the cytoplasmic DNAs of animals. Also most virus DNAs pass through a stage of the CC form in the course of infection of cells. Such a widespread occurrence of this form of DNA in nature has elicited the interest in its structure and properties that has been manifested in recent years (see the review of Ref. 19).
The discovery of CC DNA has led to the formulation of fundamentally new problems, since it has turned out that many of the physical properties of the CC form differ radically from those of the linear form. The difference between the properties of these two forms of DNA is not at all due to the existence of end effects in the one case but not in the other. They involve all regions of the molecule and are caused entirely by the topological restrictions that arise in the CC form of DNA. The topological restrictions are the strict invariance of the order of linkage of the two complementary chains. Evidently these topological restrictions are immediately eliminated after even one of the chains has
been broken. Therefore the special properties of CC DNA vanish not only when it is converted to the linear form, but also when the nicked circular form is formed, i.e., the form in which one of the filaments has been broken, while the other remains closed into a ring.

## b) Superhelix formation and its effect on the properties of closed circular DNA

The existence of a topological invariant of CC DNA gives rise to a new physical parameter that characterizes this form and determines many of its properties. This parameter is introduced as follows. Let us denote by $L k$ the linking number of the two complementary chains. This quantity amounts to the algebraic number of intersections by one chain with a surface stretched over the second chain (see also Sec. 4). The supercoiling number of CC DNA denotes the quantity

$$
\begin{equation*}
\tau=L k-\frac{N}{\gamma_{0}} . \tag{1}
\end{equation*}
$$

Here $N$ is the number of base pairs in the DNA, and $\gamma_{0}$ is the number of base pairs per turn of the double helix in linear DNA under the given conditions. Often one employs the supercoiling density, which is defined as $\sigma$ $=10 \tau / N$. In all cases when $\tau \neq 0$, one says that the CC DNA is supercoiled. Evidently the double helix as a whole proves to be under stress in the supercoiled state. This stress can lead both to a change in the actual number of base pairs per turn of the double helix in CC DNA, and to a regular bend of the axis of the helix in space (Fig. 5). The latter possibility has given rise to the term "supercoiling", since structures of the type shown in Fig. 5 are often observed in CC DNA in the electron microscope.

Native DNA as extracted from cells proves to be not only circular and closed, but also supercoiled. Here the supercoiling always possesses the same sense-it is negative and amounts to $-\sigma=0.03-0.10$ (see Ref. 19). This means that CC DNA in the cell is somewhat untwisted as compared with the linear form of the double helix under the same conditions. For a long time, the


FIG. 5. Supercoiled CC DNA. This is how DNA looks when carefully extracted from the cell. The supercoiling is always negative.
problem has been discussed of whether negative supercoiling is necessary for the normal functioning of CC DNA in the cell, or whether it arises during extraction of the DNA. At present it has been firmly established that negative supercoiling is actually necessary for the normal functioning of CC DNA in the cell. Thus, the fundamental biological process that occurs in DNA, its replication (i.e., the formation of two daughter molecules), does not happen if the CC DNA is not negatively supercoiled. Moreover, it has turned out that a special enzyme, DNA-gyrase, occurs in cells, which creates negative supercoiling in CC DNA. This enzyme is only one of the members of an entire class of enzymes that can change the topology of DNA, and which have received the general name of topoisomerases (see also Sec. 6).

The stress that arises in negatively supercoiled CC DNA considerably facilitates the formation of unwound regions in these DNAs, i.e., regions in which the weak bonds between the two complementary chains forming the double helix have been broken. In fact, the filaments are not twisted about one another in the unwound regions (for more details, see Ref. 16). Therefore the formation of open regions relieves the stress in the remaining parts of the molecule. An opening of the fraction $\theta$ of pairs in DNA such that $\theta=-\left(\sigma \gamma_{0} / 10\right) \approx-\sigma$ completely relieves the stress. However, further opening of the DNA has the result that stress arises again.

Thus the double helix in the negatively supercoiled CC form of DNA is substantially weakened as compared with the linear form or the closed circular but not supercoiled form. Experimentally this is manifested in the fact that negatively supercoiled CC DNA is considerably more subject than the other forms to modification by many chemical agents, in particular mutagens and carcinogens. It also possesses an elevated affinity for a number of important proteins, e.g., such as RNApolymerase, which reads the genetic information from the DNA molecule.

Formation of uncoiled regions is not the only way to relieve the stress in CC DNA. The negative supercoiling can also be relieved by formation of cross-shaped structures in regions of the double helix in which the sequence of bases has a second-order symmetry axis (Fig. 6). Another way is to bind molecules (ligands) to the DNA that diminish the rotation angle between adjacent base pairs.

This effect is employed for measuring the values of $\tau$ (or of $\sigma$ ). The method is based on the fact that the DNA "straightens out" as the stress is relieved in negatively supercoiled CC DNA as larger numbers of ligand molecules are bound to it. That is, its mobility decreases. Then, when the number of bound molecules exceeds the quantity

$$
\begin{equation*}
\mu=\frac{360}{\varphi} \tau \tag{2}
\end{equation*}
$$

( $\varphi$ is the change in the angle between adjacent base pairs when ligand molecules are intercalated between them), the stresses in the DNA again begin to increase and the mobility will increase. Consequently the curve of the


FIG. 6. A cross-shaped structure that can arise in CC DNA. Formation of this type of structure requires negative supercoiling as well as a special sequence of nucleotides possessing a twofold symmetry axis.
dependence, e.g., of the sedimentation constant on the number of bound ligand molecules has a minimum (Fig. 7). Upon determining the value of $\mu$ at the minimum and substituting it into Eq. (2), one can find $\tau$ if one knows the value of $\varphi$. For the data shown in Fig. 7, where ethidium bromide has been used as the ligand, we find $\varphi=-26^{\circ}$.

In recent years, yet another method of determining the number of supercoiling turns has become widespread. It is based on employing the method of gel electrophoresis. The method has such a high resolving power that molecules that differ in $\tau$ by unity yield separate bands. Application of the method of gel electrophoresis to studying CC DNA has led to a series of remarkable experimental studies. The results of some of these studies will be presented and discussed in the light of the theory set forth in this review in Sec. 6.

The development of very sensitive and strictly quantitative methodologies of studying CC DNA has made it possible to realize the unique possibilities involved in the properties of this form of DNA. Consequently, CC DNA has become a powerful tool for studying the properties of the double helix of DNA in general. In this re-


FIG. 7. Dependence of the sedimentation constant of circular DNA from the tumorigenic virus SV 40 on the number of molecules per base pair of a ligand (ethidium bromide) bound to the polymer $(\nu=\mu / N)$. Curve 1 pertains to the closed circular form, and the curve 2 to the nicked circular form (from the study of Bauer and Vinograd ${ }^{44}$ ).
gard it is interesting to note that when doubts arose not so long ago as to whether DNA is a double helix, owing to the appearance of an alternative model, the latter could be definitely rejected, precisely on the basis of data on CC DNA (see Ref. 20).

The vigorous development of studies of the CC form of DNA has in a compelling manner posed especially sharply the need for constructing an adequate theory, i.e., a theory of ring polymer structures that takes into account the topological restrictions. Considerable progress has been attained in the past ten years in constructing this type of theory. We shall present these results in detail in Secs. 3-5, while in Sec. 6 we shall return to examining circular DNAs and shall show how the results of the theory enable one to interpret the experimental data on these new and extremely interesting objects of living nature.

## 3. THEORY OF KNOTS AND LINKAGES

## a) The statistical integral for closed chains

In calculating the statistical integral (partition function) for a closed chain or a system of closed chains, one must restrict the region of integration only to the region of phase space that corresponds to the topologically equivalent states of the system. Therefore one must know how to characterize the topological states of a system by starting with the configurations of the polymer chains contained in it. Such a characteristic, which depends only on the topology of the system, i.e., remains invariant upon any deformation of the chains that can be carried out without destroying their integrity, is called a topological invariant. Thus, in the case of a single closed chain, a topological invariant must primarily answer the question of whether the closed chain is knotted (Fig. 8). In the case of a system of two closed chains, the simplest question consists of whether they are linked or not, etc.

Let us clarify what we have said with the example of the statintegral for a single closed polymer molecule represented by a model of a freely-linked chain made of $n-1$ identical links of length $b$. The statistical integral of this chain has the form

$$
\begin{align*}
Z=\int \prod_{i=1}^{n} d \mathbf{r}_{i} \prod_{i=1}^{n-1} \delta\left(\left|\mathbf{r}_{i+1}-\mathbf{r}_{i}\right|\right. & -b) \delta\left(\mathbf{r}_{i}\right) \delta\left(\mathbf{r}_{n}\right) \\
& \times\left(4 \pi b^{2}\right)^{-n} e^{-\beta \Phi \delta} \delta\left(G_{0}-G\left(\mathbf{r}_{1}, \ldots, \mathbf{r}_{n}\right)\right) . \tag{3}
\end{align*}
$$

Here $r_{i}$ is the radius vector from the coordinate origin to the $i$ th vertex of the chain, and $\Phi$ is the total intra-


FIG. 8. Examples of topologically equivalent and nonequivalent polymer chains.
molecular potential. This expression coincides with the standard expression for the statsum of an open chain having the distance between the ends $r$ (see, e.g., Ref. 2, Chap. 7, Sec. 9) for $\mathbf{r}=0$, with a substantial exception consisting of the presence of the last $\delta$ function. In it $G_{0}$ is a certain value of a topological invariant of the closed chain, while $G$ symbolizes a function with the aid of which one can find the value of the topological invariant by starting with the spatial configuration of the chain. The appearance of this $\delta$-function expresses explicitly the fact that the statistical physics of closed chains can in no case be considered a simple limiting case of the statistical physics of linear chains for $r=0$. One might say that, just as the physics of linear polymer chains is governed by the linear memory (see, e.g., Ref. 8) arising from the fixed sequence of links, the physics of closed chains is further governed by the topological memory arising from the mutual impenetrability of the links with one another.

The simplest topological invariant that allows one to distinguish the states of two closed curves $C_{1}$ and $C_{2}$ is the following expression (the Gauss integral; see, e.g., Ref. 21, Chap. 3, Sec. 15):

$$
\begin{equation*}
G\left(C_{1}, C_{2}\right)=\frac{1}{4 \pi} \oint_{C_{1}} \oint_{C_{2}} \frac{\left(d \mathbf{r}_{1} \times d \mathbf{r}_{8}\right) \mathbf{r}_{12}}{r_{12}^{\mathbf{1}}} \tag{4}
\end{equation*}
$$

Here $\mathbf{r}_{1}$ and $\mathbf{r}_{2}$ are radius vectors that pass along the curves $C_{1}$ and $C_{2}$, respectively, and we have $\mathbf{r}_{12}=\mathbf{r}_{1}$ $-r_{2}$. By using the Stokes theorem, we can easily show that the integral in (4) equals the algebraic number of intersections by one of the curves with a surface stretched on the other curve. This directly implies that the Gauss integral is not a complete topological invari-ant-examples exist of linked curves for which $G=0$, just as for unlinked curves (see below, Fig. 10 and Table II). Thus, the Gauss integral is a weak topological invariant. The situation is even worse with knotsno integral invariant exists for them at all. The assumption that the integral in (4) for $C_{1}=C_{2}$ can betreated as the invariant of a knot ${ }^{10}$ has proved false (see Ref. 22; the true meaning of the Gauss integral taken over a single curve will be treated in detail below in Sec. 4).

Thus the calculation of the integral in (3) by analytic methods is impossible owing to the lack of an analytic expression for the function $G$ for knots. As for linkages, the employment for them of analytic methods for calculating the statintegral while using the function $G$ in the form (4) faces serious mathematical difficulties. ${ }^{10,23}$ Even more importantly, if one were able to overcome these difficulties, it is not evident how far one could trust the results obtained, owing to the defects of the Gauss integral as an invariant of linkages. Therefore another approach has been proposed, ${ }^{22,24}$ based on using numerical, machine methods. The attractiveness of this approach consists of the fact that it enables one to use algebraic invariants of knots and linkages. The construction of such invariants has been one of the remarkable advances of algebraic topology. Without taking up the mathematical details, which are contained in the monograph of Ref. 25 we shall take up below the fundamental ideas that have led to the construction of the algebraic invariants of knots and linkages.

## b) Classification of knots and linkages

Before we proceed to describe the algebraic invariants of knots and linkages, let us dwell briefly on the problem of what are knots and linkages in general and how are they classified. To do this, we must stipulate the reduction of any knot or linkage to a certain standard form. Naturally, in such a reduction we must take case not to allow self-intersection of chains. This type of deformation is called in topology an isotopic deformation. Two knots (or linkages) that can be converted into one another by isotopic deformation belong to the same isotopic type. The standard form of a knot (or linkage) has been adopted as being its representation that realizes the minimal number of crossings when projected on a plane. The simplest knot has three such crossings (see Fig. 2), and it is called the trefoil.

Knots are simple or compound. A knot is called compound if an unbounded surface exists that is intersected by the knot at only two points, and which divides it into two knots. Figure 9 shows the initial part of the table of simple knots. In particular, all knots are presented having a number of intersections less than nine when in the standard form. A knot and its mirror image are treated as belonging to the same type of knot, although they can belong to the same or to different isotopic types. Thus the trefoil and its mirror image belong to different isotopic types, while the figure-eight (the knot $4_{1}$ ) and its mirror image belong to a single isotopic type. The table of knots is actually the table of types of knots, since it presents only one representative of mirror pairs. With increasing number of intersections, the number of types of simple knots increases very rapidly. Thus, tables have been constructed at present of all the types of knots with a number of intersections


FIG. 9. Table of the simple knots having in the standard form a number of crossings less than nine.
less than ten, ${ }^{26}$ with ten, ${ }^{27,}{ }^{28}$ and a perhaps incomplete table of knots with eleven intersections (see Ref. 27). It turns out that 49 types of knots exist with nine intersections, 165 types of knots with ten intersections, and about 552 types of knots with eleven intersections (pictures of all these knots can be found in the cited studies).

Curiously, this distinctive activity of compiling complete tables of knots, which was begun (and very strongly advanced) more than one hundred years ago by Tait, Kirkman, and Little, has been continued after a long interuption by Conway ${ }^{27}$ and Perko. ${ }^{28}$ We note that such tables are very valuable for the problems treated in this review, since they allow one to test the effectiveness of invariants of knots.

In contrast to the table of knots, the table of linkages was first compiled quite recently. ${ }^{27}$ It contains 275 types of simple linkages with a number of intersections less than eleven, and is constructed by the same principle as the table of knots. The initial part of the table of types of linkages is shown in Fig. 10.

## c) Algebraic invariants of knots and linkages

The first and most essential step in constructing an invariant of a knot is to introduce the concept of the knot group. The knot group amounts to a special case of the fundamental group (see Ref. 21), the concept of which is the basis of many branches of algebraic topology.
Thus, let us treat a knot in three-dimensional space (Fig. 11). A knot is understood to be any closed curve, and in particular the topological equivalent of a circle (in this case it is called a trivial knot). Let us remove from the space being considered all the points belonging to the knot. The remaining set of points is called the complementary space of the knot. Let us choose an arbitrary point $p$ in this space. We shall call any trajec-


FIG. 10. Table of the simple linkages having in the standard form a number of crossings less than nine.


FIG. 11. On the concept of a knot group. The loops $a_{1}$ and $a_{2}$ are equivalent ( $a_{1} \approx a_{2}$ ) and we have $a_{4} \approx a_{1}^{-1}, a_{1} a_{2} \approx a_{3}$.
tory a loop with the basis point $p$ if it completely belongs to the complementary space, and begins and ends at the point $p$. Let us assign a direction of passage around the loop. Two loops are termed equivalent if they can be transformed into one another by continuous deformation in the complementary space of the knot. It is essential to stress that any self-intersections of the loops are allowed during their deformation, though it is strictly forbidden to go outside the complementary space (see Fig. 11). Thus all possible loops are subdivided into classes of equivalent loops. A remarkable fact is that the classes of equivalent loops form a group, which is called the knot group. Here the product $a b$ of the two loops $a$ and $b$ is taken to be trajectory that successively passes along the loop $a$ and then the loop $b$. The loop $a^{-1}$, or inverse of the loop $a$, passes through the same points of space, but in the reverse direction. The identity element of the group is the class of loops that can be retracted into the point $p$ (cf. loop $a_{0}$ in Fig. 11). We should stress that the knot group does not depend on the choice of the basis point $p$. Knots that belong to the same type (and a fortiori to the same isotopic type) correspond to isomorphic groups.

Let us clarify what we have said with the example of the trivial knot, i.e., a circle. Evidently, in this case each class of equivalent loops is unambiguously characterized by the number of turns that the loops contained in it make around the given circle. That is, the set of these classes is equivalent simply to the set of integers. Thus the group of the trivial knot is isomorphic with the group of integers. Moreover, a very important theorem has been proved in the theory of knots: no nontrivial knot has a group isomorphic with the group of integers. Hence we see that the knot group is a very powerful topological invariant. However, examples are known of knots of different types that have isomorphic groups (Fig. 12). Nevertheless, the knot group would be a very good invariant if a universal method existed for establishing the isomorphism of two knot groups. However,


FIG. 12. Example of knots of different types that have isomorphic groups.


FIG. 13. On the calculation of an Alexander polynomial. Here $x_{1}, x_{2}$, and $x_{3}$ are the generators, and 1,2 , and 3 are the crossing points in the projection of the knot.
no such method exists because the knot group always consists of an infinite number of elements. Therefore the next step is taken in the theory of knots-one seeks invariants of the knot group, which unavoidably prove to be weaker topological invariants, but for which the problem of equivalence is easily solved. The most convenient invariant of this type is the so-called Alexander polynomial. We shall present here the algorithm for calculating the Alexander polynomial in a final form convient for machine calculations. ${ }^{22}$

First we shall project the knot on a plane along an arbitrarily chosen axis, while drawing breaks at the crossing points in the part of the curve that lies below (Fig. 13). Now the projection of the knot amounts to the set of segments of curves, which are called the generators. Let us fix arbitrarily the direction of passage of the generators and number them, having selected arbitrarily the first generator. The crossing that separates the $k$ th and $(k+1)$ th generators will be called the $k$ th crossing. The crossings are of two types (Fig. 14). Thus each crossing is characterized by its number, by its type (I or II), and by the number of the generator passing over it. Now we can correlate the knot with a square Alexander matrix, in which the $k$ th row corresponds to the $k$ th crossing and which consists of $n$ elements ( $n$ is the total number of crossings in the projection of the knot). Here all the elements except $a_{k h}, a_{k, n+1}$ and $a_{k i}$ ( $i$ is the number of the overpassing generator) are zero. The nonzero elements of the $k$ th row are defined as follows:

1) when $i=k$ or $i=k+1$, independently of the type of crossing, we have:

$$
a_{k h}=-1, \quad a_{k h+1}=1
$$

2) when $i \neq k, i \neq k+1$, we have for a type I crossing:

$$
a_{k h}=1, \quad a_{k h+1}=-t, \quad a_{k i}=t-1
$$

and for a type II crossing:

$$
a_{k k}=-t, \quad a_{k k+1}=1, \quad a_{k t}=t-1
$$

These relationships hold for all $k=1, \ldots, n$ under the condition of the substitution $n+1 \rightarrow 1$.


FIG. 14. The two types of crossings.


FIG. 15. Nontrivial knots for which the Alexander polynomial $\Delta(t)=1$. The knot $11_{493}$ belongs to the same isotopic type as the knot shown in Fig. 6 of Ref. 22.

An invariant in the form of an Alexander polynomial also exists for linkages, but it is a function of two variables, rather than one (or more than two, depending on the number of chains being considered). The algorithm for calculating the Alexander polynomial $\Delta(s, t)$ for two chains is analogous to that for calculating the Alexander polynomial for a knot, but is somewhat more unwieldy. It can be found in Ref. 24. Table II gives the Alexander polynomials for the linkages shown in Fig. 10. For unlinked curves we have $\Delta(s, t)=0$.

There is a very simple relation between the Alexander polynomial and the Gauss integral of (4):

$$
\begin{equation*}
|G|=|\Delta(1.1)| . \tag{5}
\end{equation*}
$$

Hence we see that the Gauss integral is generally a far weaker invariant than the Alexander polynomial. An especially substantial defect of the Gauss integral as an invariant of a linkage is that it vanishes for many rather simple types of linkages and thus does not enable one to distinguish them from unlinked curves (see Table II). At the same time, among the 91 types of linkages with a number of crossings less than nine given in Ref. 27,

TABLE II. Alexander polynomials $\Delta(s, t)$ and values of the Gauss invariant in (4), $\Delta(1,1)$, for the linkages shown in Fig. 10.

| Type of linkage | $\Delta(s, t)$ | $\|\Delta(1.1)\|$ |
| :---: | :---: | :---: |
| 21 | 1 | 1 |
| 41 | $s+t$ | 2 |
| 51 | (s-1) $(t-1)$ | 0 |
| ${ }_{6} 1$ | $s^{2}+t^{2}+s t$ | 3 |
| 6 | $s t(s+t)-s t+s+t$ | 3 |
| 63 | $2 s t-(s+t)+2$ | 2 |
| 71 | $s^{2} t^{\mathbf{2}}-s t(s+t)+s t-(s+t)+1$ | 1 |
| 72 | $s t(s+t)-t^{2}-s^{2}-3 s t+s+t$ | 1 |
| 73 | $2(s-1)(t-1)$ | 0 |
| $7{ }_{4}$ | $(s-1)(t-1)\left(s^{2}+1\right)$ | 0 |
| 73 | $2 s^{3} t-t^{3}-s+2$ | 2 |
| $7{ }_{6}$ | $(s+1)^{2}(s-1)(t-1)$ | 0 |
| 7, | $5^{3}+t$ | 2 |
| 78 | $(s-1)(t-1)$ | 0 |
| 81 | $(s+t)\left(s^{2}+t^{2}\right)$ | 4 |
| 88 | $s t(s+t-1)(s t+1)+s+t$ | 4 |
| $8{ }_{8}$ | $2 s^{2} t^{2}-s t(s+t)+3 s t-(s+t)+2$ | 3 |
| 8 | $s^{4} t^{2}(s+t)-2 s^{2} t^{3}+2 s t(s+t)-2 s t+s+t$ | 4 |
| 8 | $s^{2} t^{2}-2 s t(s+t)+3 s t-2(s+t)+1$ | 3 |
| $8{ }_{8}$ | $2 s t-3(s+t)+2$ | 2 |
| $8_{7}^{7}$ | $s^{2} t^{2}-2 s t(s+t)+s^{3}+3 s t+t^{2}-2(s+t)+1$ | 1 |
| $8{ }_{8}$ | $s^{2} i^{2}-2 s t(s+t)+s^{2}+s t+t^{2}-2(s+t)+1$ | 3 |
| 8 | $s^{3}+2 s^{3} t-4 s^{2}-4 s t+s+2 t$ | 2 |
| 810 | $\left(s^{3}-1\right)(t-1)$ | 0 |
| 811 | $s^{3} t-2 s^{2}(s+t)+2 s(s+t)-2(s+t)+1$ | 2 |
| 812 | $\left(s^{3}-1\right)(t-1)$ (t) | 0 |
| $8_{13}$ | $\left(s^{2}+1\right)(s-1)(t-1)$ | $\square$ |
| $8{ }_{8}$ | $s^{3} t-4 s^{2} t+4 s^{2}+4 s t-4 s+1$ | 2 |
| 815 | $(s-1)$ $s^{3}-2 s(t-1)$ $(s+t)+t$ | 0 2 |
| 816 | $s^{3}-2 s(s+t)+t$ | 2 |

Note. We have $\Delta(s, t)=0$ for unlinked contours. The form of the Alexander polynomial is fixed apart from the replacement $s \rightarrow 1 / s$ and/or $t \rightarrow 1 / t$. Thus the polynomials $s^{2}+t^{2}+s t$ and $s^{2} t^{2}+s t+1$ are equivalent.
there is not one for which $\Delta(s, t)=0$, while we find $\Delta(s, t)=1$ only for the simplest linkage $2_{1}$. Thus the Alexander polynomial is a very good invariant of knots and linkages that is highly suitable for use in the statistical physics of closed polymer chains.

## 4. THEORY OF RIBBONS

Along with the application of purely topological concepts and methods to analyzing ring polymer structures that were discussed in Sec. 3, considerable attention has been paid during the past decade to employing a new mathematical apparatus that has arisen at the boundary of topology and differential geometry for treating a special class of closed polymer molecules, namely the circular DNA molecules that were discussed in Sec. 2. A broad range of external conditions exists in which we can treat the double helix of DNA as being a smooth ribbon whose edges are the sugar-phosphate skeletons of the two complementary strands (Fig. 4). Closed circular DNA will always correspond to a two-sided ribbon, since the chemical structure of the molecule rules out the possible formation of one-sided ribbons of the type of the Moebius strip. Thus each edge of the ribbon forms a separate closed curve, and these two curves can be unlinked or form linkages of various types. The types of linkages that can arise here comprise a subclass of the entire set of linkages. As we can easily convince ourselves, for this subclass of linkages the Gauss integral of (4) is a complete topological invariant. ${ }^{1 \prime}$ The value of this integral, which can be only an integer, is called the linking number of the edges of the ribbon, and is denoted by $L k$. Thus the quantity $L k$ is a natural topological invariant of a ribbon. A given value of $L k$ corresponds to a set of different geometrical forms of the ribbon. The problem consists in finding the differential-geometric characteristics of the ribbon that are associated with its topological characteristic, i.e., with $L k$. Evidently one such characteristic is the twist $T w$ of the ribbon, a concept well known in differential geometry ${ }^{29}$ [see also Eq. (10) below]. Situations exist in which $L k=T w$. In particular, this happens if the axis of the ribbon lies completely in a plane. On the other hand, we can at least partially realize a given value of $L k$ by fixing the axis of the ribbon over a certain interval in the form of a helical line, rather than by twisting the ribbon. Thus, in the general case we have $L k \neq T w$. This fact, unexpected at first glance, involves the fact that the quantity $T w$ is the sum of the small rotations of the vector perpendicular to the axis of the ribbon as measured in the local system of coordinates, which per se rotates as we move along the ribbon.

As an example to illustrate what we have said, let us examine a ribbon tightly applied to the surface of a cylinder, and whose axis describes a helix (Fig. 16). Let $R$ be the number of turns that the axis of the ribbon makes ( $R>0$ for a right-hand helix and $R<0$ for a left-

[^0]FIG. 16. A ribbon wound flat on the surface of a cylinder ( $R=-2$ )
hand helix), $p$ be the pitch of the helix, and $r$ the radius of the cylinder. In this case we have:

$$
\begin{align*}
L k & =R  \tag{6}\\
T w & =R \frac{p}{\sqrt{p^{2}+4 \pi^{2} r^{2}}} \tag{7}
\end{align*}
$$

Thus, in addition to the twist of the ribbon, at least one more differential-geometric characteristic of the ribbon must exist that is associated with its topology. This characteristic was found relatively recently in Refs. 30-33. It was shown that we always have

$$
\begin{equation*}
L k=T w+W r \tag{8}
\end{equation*}
$$

Here the quantity $W r$, which has been called the writhing number (from the English verb urithe) is determined solely by the arrangement in space of the axis of the ribbon $C$ and is expressed in the form of the Gauss integral (4) with $C_{1}=C_{2}=C$ :

$$
\begin{equation*}
W r=\frac{1}{4 \pi} \oint_{C} \oint_{C} \frac{\left(d \mathbf{r}_{1} \times d \mathbf{r}_{2}\right) \mathbf{r}_{12}}{r \underline{l}_{\mathbf{2}}} . \tag{9}
\end{equation*}
$$

Formula (8) was proved by White ${ }^{33}$ in 1969 . Since the cited studies, and in particular Ref. 33 are written for mathematicians and are difficult to understand for a reader not having special preparation, we shall give a simplified proof of White's theorem due to V.V. Anshelevich.

Let $\mathbf{r}(t), 0 \leqslant t \leqslant 1$ be a doubly differentiable closed curve without self-intersections, i.e., a curve for which $\mathbf{r}(l)=\mathbf{r}(0) ; \mathbf{r}^{\prime}(l)=\mathbf{r}^{\prime}(0) ; \mathbf{r}^{\prime \prime}(l)=\mathbf{r}^{\prime \prime}(0)$. Moreover, let $\left|\mathbf{r}^{\prime \prime}(t)\right|<$ const. That is, the curve has a bounded curvature. ${ }^{2)}$ As the parameter $t$, let us select simply the distance between the points $r(t)$ and $r(0)$ along the curve. Then $\left|\mathbf{r}^{\prime}(t)\right|=1$.

Further, let $\mathrm{a}(t)$ be a unit vector normal to the curve $r(t)$. Let us consider the two closed curves, $r(t)$ and $\mathbf{r}(t)+\varepsilon \mathbf{a}(t)$. We shall consider the parameter $\varepsilon$ to be so small that the type of linkage of the two curves does not change upon further decrease in $\varepsilon$. Such an $\varepsilon$ exists, since we have $\left|r^{\prime \prime}(t)\right| \leqslant$ const. Thus we have correlated the curve $\mathbf{r}(t)$ with a certain ribbon having the edges $\mathbf{r}(t)$ and $\mathbf{r}(t)+\varepsilon \mathbf{a}(t)$.

Let us calculate the limiting value of the Gauss integral of (4) for these two curves:

$$
\frac{1}{4 \pi} \int_{0}^{l} \mathrm{~d} s \int_{0}^{l} \mathrm{~d} t \frac{\left[(\mathbf{r}(t)-\mathbf{r}(s)-\mathrm{E} \mathbf{a}(s)) \times \mathbf{r}^{\prime}(t)\right]\left(\mathbf{r}^{\prime}(s)+\varepsilon \mathbf{a}^{\prime}(s)\right)}{|\mathbf{r}(t)-\mathbf{r}(s)-\mathrm{Ea}(s)|^{3}}
$$

[^1]as $\varepsilon \rightarrow 0$. By definition, this value is the quantity $L k$.
Let us subdivide the interval of integration with respect to $t$ into two parts: a small neighborhood of the point $s$, i.e., $s-\delta \leqslant t \leqslant s+\delta$, and the remaining part of the segment $[0,1]$, where the integrand has no singularities. In the $\delta$-neighborhood we can consider $\mathbf{r}(t)$ to be a segment of a straight line:
\[

$$
\begin{gathered}
\mathbf{r}(t)=\mathbf{r}(s)+\mathbf{r}^{\prime}(s)(t-s) \\
\mathbf{r}^{\prime}(t)=\mathbf{r}^{\prime}(s)
\end{gathered}
$$
\]

Then the integral with respect to $t$ in the $\delta$-neighborhood of the point $s$ will be:

$$
\begin{aligned}
&-\frac{1}{4 \pi} \int_{0}^{l} \mathrm{~d} s\left(\mathbf{a}(s) \times \mathbf{r}^{\prime}(s)\right) \mathbf{a}^{\prime}(s) \varepsilon^{2} \int_{-0}^{s+\delta} \frac{\mathbf{d} t}{\left[(t-s)^{2}+\varepsilon^{\prime}\right]^{3 / 2}} \\
&=\frac{1}{2 \pi} \int_{0}^{l}\left(\mathbf{r}^{\prime}(s) \times \mathbf{a}(s)\right) \mathbf{a}^{\prime}(s) \mathrm{d} s
\end{aligned}
$$

apart from terms small in comparison with the parameter $\varepsilon$.

The integral over the remaining part of the segment has no singularities, and to the same accuracy is equal to:

$$
\frac{1}{4 \pi} \int_{0}^{t} \int_{0}^{t} \frac{\left[(\mathbf{r}(t)-\mathbf{r}(s)) \times \mathbf{r}^{\prime}(t)\right] \mathbf{r}^{\prime}(s)}{|\mathbf{r}(\mathrm{s})-\mathbf{r}(t)|^{s}} \mathrm{~d} t \mathrm{~d} s
$$

Here we can extend the integration over the entire segment $[0,1]$, since the value of this integral in the region $s-\delta \leqslant t \leqslant s+\delta$ approaches zero as $\delta \rightarrow 0$.

## Finally we have:

$L k=\frac{1}{2 \pi} \int_{0}^{l}\left[\mathbf{r}^{\prime}(s) \times \mathbf{a}(s)\right] \mathbf{a}^{\prime}(s) \mathrm{d} s+\frac{1}{4 \pi} \int_{0}^{t} \int_{0}^{l} \frac{\left[(\mathbf{r}(t)-\mathbf{r}(s)) \times \mathbf{r}^{\prime}(t)\right] \mathrm{d} s \mathrm{~d} t}{|\mathbf{r}(t)-\mathbf{r}(s)|^{3}}$.
The first term in this expression is the total twist of the ribbon. ${ }^{29}$ In the special case in which we take $\mathrm{a}(t)$ to be the principal normal of the curve $r(t)$, this term will amount to what is called in differential geometry the twist of the curve. Formula (10) was derived specifically for this special case in the pioneering study of Calagareanu. ${ }^{30,31}$ White ${ }^{33}$ showed that this formula is valid for an arbitrary ribbon. The principal point is that the second term of Eq. (10) does not contain the vector a $(t)$. That is, this term depends only on the form of the axis of the ribbon. It amounts to a new differential-geometric concept of the writhing number of a curve. This term was introduced in 1971 by Fuller, ${ }^{34}$ who first pointed out the importance of the mathematical results presented above for the theoretical analysis of the properties of circular DNAs (see also Crick's article ${ }^{35}$ ).

Let us examine the fundamental properties of the writhing number. First of all, we must stress that the writhing number in (9) is generally a cont inuous function of the form of the curve, in contrast to the classical Gauss integral for two curves, which can adopt only integral values. The quantity Wr suffers discontinuities only when one region of the curve passes through another, and here the magnitude of the jump is always +2 . It is evident from Eq. (9) that the quantity $W r$ is zero for curves possessing a center or plane of symmetry. One can say that the writhing number serves as a measure of the right-left asymmetry of the curve, i.e.,
a measure of its chirality
Similarly to $W r$, the quantity $T w$ is also a continuous function, which depends both on the form of the curve fixed by the axis of the curve and on how the ribbon is "wound" on this curve. Thus, the right-hand side of Eq. (8) contains the sum of two continuous functions, while the left-hand side contains a topological invariant that can adopt only integral values. The stated properties of the quantities $W r$ and $T w$ are well illustrated by the example of a ribbon wound on a cylinder discussed above (see Fig. 16). Equations (6)-(8) imply that in this case we have

$$
\begin{equation*}
W r=R\left(1-\frac{p}{\sqrt{p^{2}+4 \pi^{2} r^{2}}}\right) . \tag{11}
\end{equation*}
$$

This same formula can also be derived directly by calculating the integral in (9). We note that Eq. (11) is general for all ribbons whose axes describe a helix having the pitch $p$, radius $r$, and number of turns $R$. This does not pertain to Eqs. (6) and (7), which hold only for the doubly special case of a ribbon tightly applied to the surface of a cylinder. The same configuration of the axis of a ribbon, i.e., the same value of Wr , can correspond to a set of ribbons having different values of $T w$ and correspondingly of $L k$.

## 5. CALCULATIONS BY THE MONTE CARLO METHOD

## a) Methods of calculation

We see from the material presented in Sec. 3 that very effective invariants of knots and linkages exist. However, these invariants have a nature such that they do not enable one to calculate statintegrals of the type of (3) by analytical methods. Therefore one naturally turns to numerical methods of calculating the statistical characteristics, i.e., to Monte Carlo methods. As applied to the problems treated here, this implies generating closed chains in a random fashion and classifying them according to their topological states. Further, one can study by such a machine experiment any of the statistical properties of chains existing in a certain topological state.

1) Generation of closed chains. A problem that arises in calculating the statistical properties of closed chains by the Monte Carlo method consists of the need to generate closed chains having an undeformed distribution function. The most direct method, albeit absolutely inapplicable from the practical standpoint, would be the random generation of chains having a nonfixed distance between the ends, with selection of those for which the ends fortuitously meet. The inefficiency of this method is obvious. Therefore the development of special algorithms that enable generation of only closed chains of a given length was required for the set of problems under study. Such algorithms could be constructed only for the simplest model of a polymer chain in which the directions of adjacent segments are uncorrelated. This model is very popular in polymer physics, since, when the chains are long enough, the properties of real polymer chains are practically indistinguishable from those predicted by
this simple model. The connection between the actual and the idealized polymer chain is established by selecting an appropriate value of the length of a segment (it is called the statistical or Kuhn segment, see, e.g., Refs. 3-5).

Instead of generating chains in which the probabilities of any direction for each link are the same and then choosing from them the closed chains, one can proceed in a different way. One can construct chains segment by segment so that the direction of each link is deter mined by the conditional probability calculated taking into account the fact that the chain must close at the last step of the construction. It is important to emphasize that this approach must yield the same statistical ensemble of closed chains as does the first, direct approach.
Let us calculate this conditional distribution function $P\left(n-i, r_{i}, 8\right)$, where $i$ is the number of links already constructed, $r_{i}$ is the radius vector from the origin of the chain to the end of the $i$ th link, and $\delta$ is the direction of the $(i+1)$ th link. Let $P\left(k, r_{k}\right)$ be the distribution function of the vector of the distance between the ends of an unclosed chain made of $k$ segments. In order to calculate the function $P\left(n-i, r_{i}, \delta\right)$, let us examine the remaining part of the closed chain, which consists of $n-i$ segments, and which joins the point with the radius vector $r_{i}$ with the origin of the chain. The sought distribution function is the ratio of the number of trajectories passing through the point $\mathbf{r}_{i}+8$ to the total number of trajectories reaching the point
$r_{i}$ :
$P\left(n-i, r_{i}, \delta\right)$
$=P\left(n-i-1, \mathrm{r}_{i}+8\right) P(1,8)\left(\int P\left(n-i-1, \mathrm{r}_{1}+8\right) P(1,8) d \delta\right)^{-1}$.
According to the Chapman-Kolmogorov equation, we have:

$$
\int P(1, \delta) P\left(n-i-1, \mathrm{r}_{i}+\delta\right) \mathrm{d} \delta=P\left(n-i, \mathrm{r}_{\mathrm{j}}\right) .
$$

Consequently we finally obtain:

$$
\begin{equation*}
P\left(n-i, \mathrm{r}_{l}, \delta\right)=\frac{P\left(n-i-1, \mathrm{r}_{l}+0\right) P(1,8)}{P\left(n-i, \mathrm{r}_{l}\right)} \tag{12}
\end{equation*}
$$

Equation (12) is universal in nature and it can be used both for lattice and non-lattice models. The problem consists only in having the expressions for $P\left(k, \mathrm{r}_{n}\right)$ turn out simple enough so that the obtained function $P\left(n-i, r_{i}, \delta\right)$ would be convenient for machine generation of closed chains. Two models have been proposed that satisfy this requirement.
A lattice model of a polymer chain has been treated ${ }^{22}$ that consists of segments of identical length, for the case of a body-centered lattice, for which the function $P\left(k, \mathbf{r}_{k}\right)$ has the form

$$
P\left(k, x_{1}, x_{2}, x_{3}\right)=\frac{1}{8^{k}} \prod_{j=1}^{3} C_{k}^{\left(k-x_{j}\right) / 2}
$$

In this case Eq. (12) yields the expression

$$
\begin{equation*}
P(m, \mathbf{r}, \delta)=\prod_{i=1}^{3} \frac{m-\delta_{j} x_{j}}{2 m}, \text { where } \delta_{j}= \pm 1 \tag{13}
\end{equation*}
$$

The lattice model has the defect that cases are unavoidable in it in which the end of a segment falls at
an already occupied lattice node. In order to avoid such events, a simple method was employed in Ref. 22 that consists of having the whole lattice shifted by a small random vector at every such event, and constructing the chain thenceforth on the new, shifted lattice.

A convenient distribution function for the lattice-free model was proposed in Ref. 36. It treats a freely linked chain consisting of segments of unequal length. Specifically, it was proposed that the projection of the length $\delta_{j}$ of the segments on an arbitrary axis has a normal distribution:

$$
P\left(1, \delta_{j}\right)=\frac{1}{\sqrt{2 \pi} l} e^{-\delta_{j}^{j} / 2 l} .
$$

In this case the function $P\left(k, \mathrm{r}_{k}\right)$ will have the following form:

$$
\begin{equation*}
p\left(k, \mathbf{r}_{k}\right)=\left(\frac{1}{2 \pi \pi^{2} k}\right)^{3 / 2} e^{-r k^{2} / 2 \mu^{2}} . \tag{14}
\end{equation*}
$$

The formula (14) coincides with the well-known distribution function for the distance between the ends for a freely-linked chain made up of $k$ identical segments of length $l$ in the case $k \gg 1$. The convenience of the model being treated consists of the fact that in it the expression (14) is rigorous for any $k$ (including $k=1$ ). Upon substituting (14) into (12), we finally get:

$$
\begin{equation*}
p(m, \mathrm{r}, \delta)=\left(\frac{1}{2 \pi \pi^{2}} \frac{m}{m-1}\right)^{9 / 2} e^{-(\mathrm{r}+m \delta)^{1 / 2} 2^{\bullet} m(m-1)} . \tag{15}
\end{equation*}
$$

There also exist other, formally less rigorous algorithms, yet not inferior in practice to that presented here, which enable one to treat a lattice-free model of closed chains made up of segments of differing lengths (see, e.g., Ref. 37).
2) Calculation of the Alexander polynomials. In performing the machine calculations, one conveniently does not compare with one another the Alexander poly nomials $\Delta(t)$, but the numerical values of these polynomials for certain previously selected values of $t$. Here again the tables of knots come to one's aid, or more exactly, the corresponding tables of Alexander polynomials. Analysis of these tables shows that the Alexander polynomials are as a rule sign-alternating. Consequently the value $\Delta(-1)$ corresponds to the sum of the absolute values of the coefficients of the polynomials, which is larger than 3 for all polynomials but $\Delta(t)=1$ and $\Delta(t)=t^{2}-t+1$. Thus, in order to distinguish the trivial knot and the trefoil from one another and from the rest of the knots, it suffices in practice to calculate only $\Delta(-1)$, as was first proposed in Ref. 22. If we add as well the value of $\Delta(-2)$, we can distinguish all the polynomials of knots with a number of crossings less than 10.

Before we calculate the Alexander polynomial on the basis of the Alexander matrix, it is very convenient to reduce the order of the matrix according to the following rules: ${ }^{36}$
a) If the $k$ th row contains only two nonzero elements $a_{k k}=-1$ and $a_{k k+1}=1$, then we should add the column $k$ to the column $k+1$. Then we delete the $k$ th column and the $k$ th row and renumber the rows and column afresh.


FIG. 17.
b) If in two adjacent rows of the matrix having the numbers $k$ and $k+1$ elements having the value $t-1$ lie in a single column and elements equal to $t-1$ are lacking in the $(k+1)$ th column, then we should add the $k$ th column to the $(k+2)$ th, and then delete the $k$ th and ( $k+1$ )th rows and columns and renumber all the rows and columns afresh.

These two operations performed on the Alexander matrix correspond to removing the crossings shown in Fig. 17a and b, respectively. They are identical to the first two operations on the table of crossings of a knot proposed by Le Bret. ${ }^{37}$

In order to distinguish unlinked curves from the simplest linkage $2_{1}$ and these two situations from all more complex linkages, it suffices to restrict the treatment to calculating the value of the Alexander polýnomial $\Delta(-1,-1)$ (see Table II).

## b) Results of the calculations

1) Probability of knot formation. The first problem that arises in analyzing ring polymer chains consists of the following. Let a ring molecule be formed by fortuitous closure of a linear molecule consisting of $n$ segments. What is the probability of forming a knotted chain, i.e., a nontrivial knot? This problem has been clearly formulated by Delbrück ${ }^{38}$ and first solved ${ }^{22,39}$ by the methods presented in Sec. 3 and in the last subsection. Analogous calculations have recently been performed in Refs. 36 and 37. All the published data on the relationship between the probability of knot formation and the number $n$ of segments in the chain, as well as the results of our recent calculations, are collected together in Fig. 18.
We see that the results obtained by various authors. agree very well with one another. This is not surprising, since, in spite of a certain difference in the polymer models employed, to which certain differences in the results are due, the presented data in all cases


FIG. 18. Dependence of the probability $P$ of formation of a nontrivial knot during random closure of a polymer chain on the number of segments in it. 1-data of Refs. 22, 39; 2data of Ref. 36; 3-data of Ref. 37; 4-recent data of the present authors.
fit the model of an infinitely thin polymer chain.
We see from Fig. 18 that the probability of knot formation has an evident tendency to approach unity as $n \rightarrow \infty$, though as yet it has been possible to perform the calculations only up to $n$ values such that $P$ barely exceeds 0.5 . The extension of this relationship into the region of large $n$ is hindered by the memory size and speed of the computer. Of course, there is a limitation in principle, which involves the fact that certain nontrivial knots have the same Alexander polynomial as an unknotted chain (trivial knot) does. How ever, such knots constitute such an infinitesimal fraction of all the knots (see Sec. 3), that this restriction becomes substantial only at $n$ values for which the probability of knot formation already has become unity.
It has been shown ${ }^{22,37,39}$ that the probability of knot formation declines sharply with increasing ratio of the thickness $d$ of a segment to its length $b$. Le Bret ${ }^{37}$ has generalized the results of the calculations of the probability $P$ of knot formation in the form of the following interpolation formula:

$$
\begin{align*}
P=\left(-1.97 \cdot 10^{-2}+2.76 \cdot 10^{-3} n+\right. & \left.5.60 \cdot 10^{-8} n^{2}-2.37 \cdot 10^{-8} n^{3}\right) \\
& \times \exp (-11.6 d / b), \quad 6 \leqslant n \leqslant 150 . \tag{16}
\end{align*}
$$

The probability of knot formation for chains having an excluded volume can become so small that doubts can arise whether the probability of knot formation always approaches unity as $n \rightarrow \infty$. At one time Hammersley ${ }^{40}$ proposed that the fraction of unknotted closed chains becomes exponentially small with increasing $n$, even for chains having an excluded volume. As Frisch and Klempner ${ }^{41}$ have shown, this fact stems from the work of Kesten ${ }^{42}$ and from one of the theorems of knot theory. As we see from Eq. (16), however, the probability of knot formation at fixed $n$ declines exponentially with increasing ratio of the thickness to the length of a segment.

The sharp decline in the probability of knot formation with increasing thickness of the chain, i.e., increasing excluded-volume effect, suggests that this probability will increase when attraction between remote segments of the chain is introduced, i.e., in the case of a negative excluded volume. To test this hypothesis, we have performed calculations of the probability $P$ of knot formation as a function of the value of the swelling parameter $\alpha$ of the polymer chain in the region $\alpha<1$.

The quantity $\alpha$ was defined as usual (see, e.g., Refs. 5,8 , i.e., $\alpha^{2}=\left\langle S^{2}\right\rangle /\left\langle S^{2}\right\rangle_{0}$, where $\left\langle S^{2}\right\rangle$ is the rms radius of inertia of the chain under study, and $\left\langle S^{2}\right\rangle_{0}$ is the same for the $\theta$-point, i.e., for an infinitely thin chain without interaction between the segments. A model potential of the form $U=-U_{0} \exp \left(-u r^{2}\right)$ was used, where $r$ was taken to be the distance between any pairs of vertices of the freely-linked chain. The calculations were performed for two fixed values of $x(0.2$ and 0.5$)$. Each time the quantity $\alpha$ was varied by changing the parameter $U_{0}$. The results do not depend on the parameter $x$. Moreover, a potential of a quite different type led to exactly the same results. Thus the probability of knot formation can be considered to be a


FIG. 19. Dependence of the probability of knot formation on the swelling parameter $\alpha$ of the polymer in the region $\alpha<1$, i.e., in the presence of attraction between the segments. The calculations were performed for a chain consisting of $30 \mathrm{seg}-$ ments.
single-valued function of the swelling parameter $\alpha$, without depending on the specific form of the interaction potential between the segments. Figure 19 demonstrates the very sharp dependence of the probability of knot formation on $\alpha$. It was not possible to proceed into the region of smaller values of $\alpha$ since the calculations require too much machine time.

Data are also given in Refs. 22 and 39 on the probability of formation of knots of different types for the model of an infinitely thin interaction-free chain. The results of calculations of the probability of knot formation have been discussed in connection with various problems in Refs. 22 and 39. Extremely interesting experimental data have appeared since then, and we shall discuss them below in Sec. 6.
2) Topological interactions of polymer chains. Just as the first problem of the statistical mechanics of knots is the problem treated above of the probability of knot formation upon random closure of a solitary chain, the first problem associated with the behavior of two chains is the probability of formation of the linked (or unlinked) state during the random closure of the two chains at a given distance ( $R$ ) between their centers of mass. The results of this type of calculations for the model of an infinitely thin chain (i.e., at the $\theta$-point), which were performed in Refs. 24 and 39, are shown in Fig. 20. It turned out that the results of


FIG. 20. Dependence of the probability of formation of the unlinked state of two polymer chains on the distance $R$ between their centers of mass. The different curves correspond to different values of the number $n$ of segments in each chain (both chains are assumed to consist of the same number of segments); 1-20, 2-40,3-60, and 4-80. Data from Ref. 24.
the calculations satisfy very well the interpolation formula

$$
\begin{equation*}
P_{0}=1-A_{0} \exp \left(-\alpha_{0} R^{3}\right) . \tag{17}
\end{equation*}
$$

Here $A_{0}$ and $\alpha_{0}$ depend on the number of segments in the chains (see Refs. 24, 39).

Equation (17) shows that the number of states of a system made of two unlinked rings is a sharply varying function of the distance between them. This means that a gas (or solution) of unlinked, infinitely thin ring polymer chains will not be ideal. Repulsion between the chains of an entropic type arises in it. The second virial coefficient for a gas (or solution) of such chains will have the following simple form (see Refs. 24, 39):

$$
\begin{equation*}
B=\frac{2}{3} \pi \frac{A_{0}}{a_{0}} . \tag{18}
\end{equation*}
$$

Analysis of the results of machine calculations shows that the values of $B$ obtained are very close to that corresponding to spherical, mutually impenetrable particles having a radius equal to the rms radius of gyration of a closed polymer chain. Thus, ideal closed chains must experience a mutual repulsion completely caused by toplogical restrictions.
On the other hand, the quantity $B$ enables one to calculate the concentration of linked molecules (catenanes) $c_{k}$ that will be obtained upon random closure of linear polymers having the concentration $c$ :

$$
\begin{equation*}
c_{h}=c^{2} B . \tag{19}
\end{equation*}
$$

3) Writhing-number distribution of closed chains. In addition to the purely topological characteristics, a differential-geometric quantity that was discussed in Sec. 4 plays a very important role in the description of circular DNAs, the writhing number Wr. The problem of calculating the writhing-number distribution of closed chains was first proposed by Benham ${ }^{43}$ and solved in Ref. 44. Here the writhing-number distribution of chains belonging to a given topological type was treated.

Figure 21 shows the results of the calculations of the variance of the writhing number for unknotted chains (trivial knots), as taken from Ref. 44. Exactly the same results have been obtained by Le Bret. ${ }^{37}$ The calculations were performed by the Monte Carlo method for lattice-free models of a freely linked, infinitely thin polymer chain. In this case the double integral of (9) can be converted into a double summation over the


FIG. 21. Dependence of the variance of the writhing number on the number of segments in a closed, unknotted polymer chain.
segments, since this integral for two arbitrarily oriented rectilinear segments can be calculated analytically. Le Bret ${ }^{37}$ employed a different, but completely equivalent definition of the writhing number.

The results of the calculations showed that the quantity $W r$ for unknotted closed chains (trivial knots) has a normal distribution with a maximum at zero and a variance that depends almost linearly on the number of segments in the chain (see Fig. 21). For the trefoil one obtains a bimodal distribution that is the sum of two normal distributions, each of which corresponds to the two mirror-isomeric forms of the trefoil (see Ref. 37). The centers of these distributions lie at the points $W r \approx \pm 3$. For the knot $4_{1}$, the center of the distribution lies at zero, as is natural, since this knot is isotopically equivalent to its mirror reflection. $\mathrm{Bi}-$ modal distributions are again obtained for the knots $5_{1}$ and $5_{2}$, and here the maxima are separated respectively by the amounts 126 and 9.4. ${ }^{37}$
It is important to emphasize that, even with a relatively small number of segments in the chain, for which the fraction of knotted chains obtained by random closure is still very small, one must not calculate the value of $\left\langle(W r\rangle^{2}\right\rangle$ without treating the knotted chains. ${ }^{44}$ This involves the fact that the knots lacking mirror symmetry give a disproportionately large contribution to the variance of the writhing number, owing to their bimodal distribution.

## 6. APPLICATIONS OF THE THEORY TO CIRCULAR DNAs

The topological problems in polymers began to be discussed in connection with attempts at chemical synthesis of knots and catenanes. ${ }^{13}$ Then the importance of topology in polymer networks was recognized. ${ }^{9-12}$ These two lines of study have been discussed in detail in the review by Frisch and Klempner. ${ }^{41}$ The experimental situation in these fields has changed but little since the publication of that review. Thus, in spite of persistent, many-years of work on the chemical synthesis of knots, this has not yet succeeded. However, during these years vigorous development has occurred in the applications of topology to a completely new field: the field of circular DNAS. This problem was touched upon very cursorily in the review of Ref. 41 solely in connection with the discovery of linked DNA rings (catenanes). Currently it is precisely in the field of study of closed circular (CC) DNAs that topological ideas and methods are finding the most widespread application.

The application of topological ideas to studying the properties of CC DNA was started by Fuller ${ }^{34}$ in 1971, when he applied the results of ribbon theory to analyzing the properties of these molecules. According to this theory (see Sec. 4), besides the topological characteristic of a ribbon-its linking number $L k$, also two differential-geometric characteristics play an important role, the twist $T w$ of the ribbon, and its writhing number $W r$. Here all three characteristics are interrelated by the condition (8).

We recall that CC DNA is generally not characterized
by the total quantity $L k$, but by the number of excess turns (the number of supercoild $\tau$ ), as defined by using Eq. (1). We stress that the quantity $N / \gamma_{0}$ in Eq. (1) is rigorously fixed under given external conditions. However, upon changing the external conditions (temperature, composition of the solvent, etc.), the quantity $\gamma_{0}$ varies. Therefore the number $\tau$ of supercoils, in contrast to $L k$, is a topological invariant of DNA only under fixed external conditions.

The true value of the twist $T w$ of the ribbon, that is the axial twist of CC DNA, can generally be anything whatever, depending on the value of $W r$, since the sole condition for a ribbon is the condition (8). Thus we can require that $T w=N / \gamma_{0}$. Then, according to (8), we have $W r=\tau$. Conversely, one can make $W r=0$ so that all the supercoiling is realized in the form of an axial twist. In this case we have $T w=L k=\tau+\left(N / \gamma_{0}\right)$, and the mean number of base pairs per turn of the double helix will be

$$
\gamma=\frac{N}{T w}=\frac{1}{\left(1 / \gamma_{0}\right)+(\tau / N)} .
$$

The real situation, i.e., what fraction of the supercoils is realized on the average in the form of axial twist, and what fraction in the form of writhing, i.e., bending of the axis of the double helix, is determined by the relationship between the coefficients of rigidity of the double helix for twist and for axial bending. They are what determine the amplitude of the thermal fluctuations of the quantities $W r$ and $T w$ about their equilibrium values.

Experimentally one can measure directly only the quantity $\tau$. The currently existing methods are based on the fact that each value of $\tau$ corresponds to its own mean value of the writhing number, and consequently, to its own value of the mobility of the CC DNA (see Sec. 2). This section will present the results of the studies on circular DNA in which the theory presented in the earlier sections has been employed in an essential manner. We must remember in comparing theory with experiment that DNA amounts to a very rigid polymer, for which the ratio of the thickness of a segment to its length is of the orde: of $10^{-2}$. Moreover, in the usually studied region of ionic conditions and DNA lengths, one can neglect the interaction between segments remotely spaced along the chain in DNA (volume effects). Therefore the results of Sec. 5 that pertain to the model of an infinitely thin chain without interaction between the segments are applicable to DNA.

## a) Distribution of closed circular DNAs with respect to topoisomers

Thus far we have been discussing conformational rearrangements of CC DNA in which $L k$ is strictly invariant. That is, both strands of DNA remain continuous. Very valuable information on the energy and conformation characteristics of CC DNA has arisen from experiments in which the value of $L k$ could vary, and the equilibrium distribution of the CC molecules with respect to $L k$ was studied. The most convenient way to vary $L k$ is to employ special enzymes, which


FIG. 22. Equilibrium distribution of CC DNA with respect to the topoisomers. CC DNA of SV 40 virus was treated with a type I topoisomerase and the obtained preparation was subjected to gel electrophoresis. The abscissa is the coordinate $x$ along the gel; the ordinate is the corresponding amount of DNA ( $y$ is in arbitrary units). Adjacent peaks correspond to topoisomers that differ by unity in the value of $\tau$.
have been called topoisomerases.
The studies under discussion ${ }^{45,46}$ employed type I topoisomerases, which alter the topological state of CC DNA by breaking and recombining only one of the strands of the double helix. These enzymes relax the distribution of the molecules with respect to $L k$ to its equilibrium form. The very sensitive gel-electrophoresis method was used for analyzing the distribution of the CC DNA molecules with respect to $L k$ in these studies. This method can separate molecules of CC DNA that differ by unity in $L k$. An example of the distribution of material obtained after gel electrophoresis is shown in Fig. 22. Naturally, the maximum of the equilibrium distribution always corresponds to $\tau=0$. We note that, although the quantity $\tau$ can only adopt discrete values that differ by no less than unity, it is not required to be an integer. Therefore, as a rule, molecules having $\tau=0$ do not appear in a preparation. It is also evident that molecules having values of $\tau$ close in absolute magnitude must have close-lying mobilities and lie close together in the gel. A distribution of the type shown in Fig. 22, in which the molecules having positive and negative values of $\tau$ are separated, are obtained when the electrophoresis is performed under conditions differing from those in which the reaction with the topoisomerase is conducted. The change in conditions means that we must substitute some other value $\gamma_{0}^{\prime}$ in place of $\gamma_{0}$ in Eq. (1) without changing $L k$. This means that the entire distribution is shifted by the amount $\delta \tau=N\left[\left(1 / \gamma_{0}\right)-(1 /\right.$ $\left.\left.\gamma_{0}^{\prime}\right)\right]$. Then the molecules that possessed the value $\tau$ in the original distribution will possess the values $\tau^{\prime}$ $=\tau+\delta \tau$ in the new distribution. If $\delta \tau$ is large enough, all of the topoisomers are well separated.

Experiments have shown that the obtained distribution is always normal. The variance of this normal distribution $\left\langle\tau^{2}\right\rangle=\left\langle(\Delta L k)^{2}\right\rangle$ as measured for different DNAs is shown in Fig. 23. For numbers of pairs $N$ $z 3000$, the results of the experiments satisfy the following phenomenological formula:

$$
\begin{equation*}
P(\tau) \propto \exp \left(-1000 \frac{\tau^{2}}{N}\right) . \tag{20}
\end{equation*}
$$

We see from Fig. 23 that the results obtained by different authors, who have used completely different


FIG. 23. Dependence of the variance $\left\langle(\Delta L k)^{2}\right\rangle$ of the distribution with respect to linking number of the DNA strands on the number of segments in the DNA chain. The length of a segment was taken as 115 nm , i.e., 338 paris. 1-data of Ref. 45, 2-data of Refs. 46, 47.
enzyme systems, fit a single curve. This very fact convinces one that the distributions of molecules with respect to $L k$ obtained in Refs. 45-47 are actually equilibrium distributions. Each of these studies has given other highly convincing proofs of the equilibrium nature of the observed distributions.

These experiments have played a very important role in studying the physical properties of CC DNA. They have made it possible to find the relationship of the free energy of CC DNA to the number of supercoiling turns:

$$
\begin{equation*}
F=1000 R T \frac{\tau^{2}}{N} . \tag{21}
\end{equation*}
$$

Moreover, these data, together with the theoretical results presented in Sec. 5, have made it possible for the first time to obtain a reliable estimate of the torsional rigidity of the double helix and to find what fraction of the supercoiling $\tau$ is realized in the form of a change in the axial twist $\Delta T w$, and what fraction in the form of the writhing number $W r$. Under conditions allowing multiple breaks and reconnections of one of the strands of DNA, i.e., in the presence of topoisomerases, $\Delta T w$ and $W r$ are independent random quantities, while the resultant quantity $\Delta L k$ equals their sum [cf. Eq. (8)]:

$$
\begin{equation*}
\Delta L k=\Delta T u+W r . \tag{22}
\end{equation*}
$$

Evidently the mean values are $\langle\Delta T w\rangle=\langle W r\rangle=\langle\Delta L k\rangle$ $=0$. However, the quantities $\left\langle(\Delta T w)^{2}\right\rangle$ and $\left\langle(W r\rangle^{2}\right\rangle$ differ from zero. Then, owing to the independence of the random quantities $\Delta T w$ and $W r$, we have

$$
\begin{equation*}
\left\langle(\Delta L k)^{2}\right\rangle=\left\langle(\Delta T w)^{2}\right\rangle+\left\langle(W r)^{2}\right\rangle . \tag{23}
\end{equation*}
$$

The quantity $\left\langle(\Delta L k)^{2}\right\rangle$ is known from experiment (see Fig. 23), and the quantity $\left\langle(W r)^{2}\right\rangle$ is obtained by calculation (see Fig. 21). Therefore Eq. (23) makes it possible to find $\left\langle(\Delta T w)^{2}\right\rangle$ as a function of the length of the DNA.

The results are shown in Fig. 24. On the other hand, the quantity $\left\langle(\Delta T w)^{2}\right\rangle$ is unambiguously related to the value of the torsional rigidity of the double helix. In


FIG. 24. Dependence of the variance $\left\langle(T w)^{2}\right\rangle$ of the axial twist of DNA on the number of segments in the molecule. ${ }^{44}$ The points were obtained by subtracting the calculated values of $\left\langle(W r)^{2}\right\rangle$ shown in Fig. 21 from the experimental values of $\left\langle(\Delta L k)^{2}\right\rangle$ shown in Fig. 23.
fact, let a deviation of the angle between adjacent base pairs of DNA by the amount $\Delta \phi$ increase the free energy by the amount

$$
\begin{equation*}
f=\frac{1}{2} g(\Delta \psi)^{2} \tag{24}
\end{equation*}
$$

Here $g$ is the torsional rigidity.
When the fluctuations of the adjacent angles $\Delta \phi$ are independent, we have

$$
\begin{equation*}
\left\langle(\Delta T \psi)^{2}\right\rangle=N\left\langle(\Delta \varphi)^{2}\right\rangle=N \frac{R T}{g} . \tag{25}
\end{equation*}
$$

In full agreement with Eq. (25), the value of $\left\langle(\Delta T w)^{2}\right\rangle$ that was found proved to be strictly proportional to $N$ (see Fig. 24). The slope of the straight line made it possible to determine $g$, which proved to be

$$
\begin{equation*}
g=0.036 R T \tag{26}
\end{equation*}
$$

This value of the torsional rigidity of DNA corresponds to an rms amplitude of thermal fluctuations in the value of the angle $\phi$ between adjacent base pairs of $5^{\circ}$. Thus the analysis of the experimental data on circular DNAs employing the results presented in the theoretical part of this review made it possible to estimate one of the fundamental characteristics of the double helix. We should note that other attempts have also been undertaken to estimate the torsional rigidity of DNA on the basis of completely different experimental data (see Refs. 48-51). However, all these at tempts led to substantially less definite estimates. Only recently has a rather accurate estimate been obtained of the torsional rigidity of DNA from data on the kinetics of depolarization of the fluorescence of a dye bound to DNA. ${ }^{52}$ These data confirmed the results given above from Ref. 44.

The presented results depend in an essential manner on knowledge of another very important characteristic of the double helix: its flexional rigidity. The flexional rigidity of DNA is commonly characterized by the value of the persistence length $a$, or, as is equiva lent, by the magnitude of the statistical segment $b=2 a$. Studies during the past decade have yielded a reliable estimate $b=115 \pm 10 \mathrm{~nm}$ (see Refs. 53-56). It has been shown very recently that this value remains constant over a broad range of variation of external condi tions ${ }^{57,58}$ (ionic composition of the solvent, etc.). It was assumed in Ref. 44, whose results have been
presented above, that $b=115 \mathrm{~nm}$ independently of the ionic conditions under which the DNA was treated with topoisomerases. On the other hand, Le Bret ${ }^{37}$ could not obtain a concrete estimate of the torsional rigidity, although he also obtained correct results on the variance of the writhing number (see Sec. 5). This is because he relied on the data of Harrington, ${ }^{59}$ which indicated a very strong dependence of the persistence length of DNA on ionic conditions. The complete inconsistency of Harrington's conclusions has been clearly demonstrated in Refs. 57 and 58.

We see directly from comparing Figs. 23 and 21 that the value of $\left\langle W r^{2}\right\rangle$ amounts to almost exactly half of the value of $\left\langle(\Delta L k)^{2}\right\rangle$ for all lengths of circular DNAs. This directly leads to the conclusion that in CC DNAs half of the supercoils $\tau$ must be realized in the form of the writhing number, and half in the form of a change in the axial twist.

## b) Knots and catenanes

Above we have been treating the properties of CC DNA that are determined by its two-strandedness. In this case the topological characteristic is the quantity $L k$. In this section we shall take up the problems in whose analysis we can treat the double helix of DNA as a unitary polymer chain. The different topological states in which closed polymer chains can exist, i.e., knots and linkages, have been described in detail in the theoretical part of this review.

As mathematical objects, knots and linkages have been studied already for more than a hundred years. The problem of the possible existence of such topological states in molecules has been raised relatively recently (see the review of Ref. 41). It has acquired special interest since the discovery of circular DNAs. Shortly after the discovery of single circular molecules of DNA, catenanes were found ${ }^{60,61}$ in certain cells, i.e., linkages, and even entire networks of linked circular DNAS (see the review of Ref. 62). Catenanes are of ten obtained upon replication of DNA in vitro (see Ref. 63 and the references cited therein), and also upon closure into a ring of linear DNAs having "sticky ends" at sufficient concentration. ${ }^{64}$ The problem has arisen of the mechanism of replication of catenanes and networks. In fact, it is very difficult to imagine how this type of structures can duplicate itself in cell division.

The calculations of the probability of knot formation upon closing a polymer chain, the results of which are given in Sec. 5, have posed the problem of the possible existence of knotted DNAs. Thus, according to these results, the equilibrium fraction of knotted DNAs must be appreciable for circular DNAs containing more than $10^{4}$ base pairs ( $\mathbf{3 0}$ segments). In most cases DNA molecules have even a greater length, and the hypothesis has been advanced of the existence in the cell of special mechanisms that prevent the formation of knotted DNAs. ${ }^{39}$ In fact, in the replication of a knotted chain the daughter filaments cannot separate. That is, the replication of knotted DNAs involves serious problems.

Although the theory has predicted the possible forma-
tion of knotted molecules, at least in vitro, for a long time they were not observed. This involved the fact that, for the short DNAs ( $N<10^{4}$ ) for which one can expect an appreciable difference in physical properties (e.g., mobility) between knotted and unknotted molecules, the probability of knot formation is very small. On the other hand, for long molecules ( $N>10^{4}$ ), for which this probability is considerable, the expected changes in the physical properties are very small. Knotted molecules were first detected in preparations of single-stranded circular DNAs after they had been treated under certain special conditions with a type I topoisomerase. ${ }^{65}$ This was the first case of a discovery of knotted polymer chains.
However, the problem of discovering knots in nor mal, two-stranded DNAs continued to be very intriguing. Knots in such DNAs have been obtained very recently. ${ }^{66}$ It turned out that there is a special subclass of topoisomerases, i.e., enzymes that can alter $L k$ in CC DNAs, called type II topoisomerases, and are capable of untying and tying knots in CC DNAs. Moreover, these enzymes catalyze the formation of catenanes from pairs or from a larger number of molecules of CC DNA. ${ }^{67,68}$ Here entire networks are formed, similarly to those observed in vivo in kinetoplasts. ${ }^{62}$ In contrast to type I topoisomerases, type II topoisomerases break, and then directly rejoin both strands of DNA molecules. It has been shown that the enzyme "draws" a segment of the same or of another molecule lying nearby through the "gap" that is formed in the intermediate state between the ends that arise through breakage (Fig. 25). This operation with an individual CC DNA corresponds to a change in the writhing number by $\pm 2$ (see Sec. 4). However, it evidently does not alter $T w$. Consequently, we have $\Delta L k= \pm 2$. That is, type II topoisomerases can change the value of $L k$ only by an even number. In fact, experiment shows that type II topoisomerases, in contrast to type I, always alter $L k$ only by an even number. ${ }^{66,69}$ Thus type II topoisomerases catalyze the process of mutual penetration of segments of the double helix through one another. Consequently, these topoisomerases must lead to establishment of complete topological equilibrium, i.e., to a distribution of the


b)

c)

FIG. 25. Three "topological reactions" catalyzed by a type II topoisomerase. a) change in the number of supercoiling turns ( $\Delta \tau= \pm 2$ ); b) untying and tying of knots; c) unlinking and formation of catenanes.
molecules over the topological states that would correspond to freely penetrable strands.

As we have noted above, these molecules need not be very long for a reliable proof of the detection of knotted molecules, but then the fraction of knots, as calculated, must be small. Liu and his associates ${ }^{66}$ have been able to overcome this contradiction by using topoisomerase II in very large concentrations in which it substantially changed the macromolecular properties of the DNA itself. Moreover, they did not add ATP to the enzyme, which is necessary for its normal operation. Precisely under these extreme conditions, they found even in short DNAs having $N=4.5 \times 10^{3}$ a considerable fraction of knotted molecules. They were able to detect them initially from the appearance of new bands in the gel electrophoretogram that corresponded to a greater mobility. Study of the properties of these fractions by various methods including electron microscopy has made it possible to show unequi vocally that they correspond to knots of various types. If now one adds topoisomerase II in the normal amount and ATP to a purified preparation of knotted molecules, rapid untying of the knots takes place. That is, the system rapidly relaxes to the equilibrium state for pure DNA molecules, in which, as calculated, there should be practically no knots for the given length. As regards the reasons why the enzyme in high concentration sharply shifts the equilibrium toward knot formation, in the light of the results presented in Sec. 5, the most likely explanation consists of the idea that the protein in high concentration decreases the dimensions of the polymer coil of DNA by changing the character of the interaction of segments remote along the chain. As we see from Fig. 19, even a small change in the dimensions of the polymer coil sharply increases the equilibrium fraction of knots. Another explanation ${ }^{66}$ of the effect of the protein owing to a change in the dimension of a segment seems less plausible. Knotted molecules of DNA (and also catenanes) have been obtained also by a highly refined method by employing special cell extracts that cause recombination, and preparation by gene engineering of special "chimeral" DNAs. ${ }^{70}$

Thus it has been experimentally shown possible to form knots in vitro. Moreover, a class of enzymes has been found that can tie and untie knots in the cell. Now, after the discovery of type II topoisomerases, the existence and replication of knotted DNAs do not seem so improbable. In fact, in principle these topoisomerases eliminate all the topological problems that can arise here. Hence it becomes quite possible to assume the existence of knotted DNAs in vivo. They have not yet been discovered, but the publication of Refs. 66 and 70 permits us to expect that the problem will be elucidated in the very near future of the existence of knotted DNA molecules in the living cell.

## 7. CONCLUSION

The presented material shows that the theory of circular polymer structures possesses a specific, well developed apparatus that rests on the results of algebraic topology and modern differential geometry. The
special interest in this theory arises from the fact that it has found application in recent years in studying the properties of circular DNA molecules, the most popular objects of modern molecular biology. The DNA molecule has proved a highly favorable object for applying the theory also because its properties can be described by simple models for which exact results can be obtained owing to the very great rigidity of the DNA chain. These results can be quantitatively correlated with experiment.

We should emphasize that the problems discussed in this review, which involve the properties of closed circular DNAs, pertain to the case of small values of the supercoiling. The most reliable results have been obtained in this region. At the same time, the study of arbitrary values of the density of supercoils of CC DNA is of substantial interest for application. A number of problems arises here that have as yet been studied insufficiently. Thus, there have been a number of attempts to answer the question of the spatial configuration of the chain of CC DNA in the supercoiled state. ${ }^{71-73}$ However, the authors of these studies restricted the treatment of the purely mechanical problem, treating DNA as an elastic rod of infinitely small thickness and without taking thermal motion into account. There are serious grounds for suspecting that both the finite thickness of the chain (see Ref. 34) and the statistical properties of the chain substantially af fect the configuration of CC DNA.

Another, even more important problem bearing on CC DNA having a sufficiently large negative supercoiling involves the fact that the stresses that arise in such a DNA can lead to substantial conformational rearrangements, as was mentioned in Sec. 2. Analysis of this type of conformational changes is closely associated with the theory of the helix-coil transition DNA (see the review in Ref. 16). A number of attempts have been made in recent years to construct a theory of the conformational changes in CC DNA with topological restrictions taken into account. ${ }^{74-77}$ One of the most interesting conclusions of the theory was the prediction of a very sharp increase in the probability of formation of cross-shaped structures of the type shown in Fig. 6 as the supercoiling increases. ${ }^{75}$ Quite recently the formation of structures of this type in supercoiled DNAs has been convincingly demonstrated in the elegant experiments of Lilley ${ }^{78}$ and of Panayotatos and Wells. ${ }^{79}$

The recent discoveries that were discussed in Sec. 6 have considerably expanded the experimental possibilities of directed variation of the topological state of DNA molecules. Undoubtedly this will lead in the next few years to the appearance of new, interesting data on the physical properties of CC DNA in the knotted and linked states.

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[^0]:    ${ }^{1)}$ For simplicity we assume in this section that the ribbon as a whole forms a trivial knot. However, this does not restrict the generality of the results.

[^1]:    ${ }^{2)}$ All the results remain valid also for piecewise smooth curves.

[^2]:    ${ }^{1}$ L. D. Landau and E. M. Lifshits, Statisticheskaya fizika (Statistical Physics), Gostekhizdat, M., L., 1951 (Engl. Transl., Addison-Wesley, Reading, Mass., 1st edn., 1958; 2nd edn., 1969).
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