The Mathematics of Supercoiled DNA:

An Essay in Geometric Biology

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To my parents,

and to "Chumley"

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Preface

In this essay, we study the basic spirals of life. Not the pleasant spirals on the shells of snails or the heads of sunflowers; no, we study the very secret of life, the molecular spiral known as the DNA double helix. Under certain circumstances, the double helix can itself be coiled into a higher-order spiral: a "superhelix". The remainder of this essay deals with the mathematical description and biological importance of these beautiful higher-order structures.

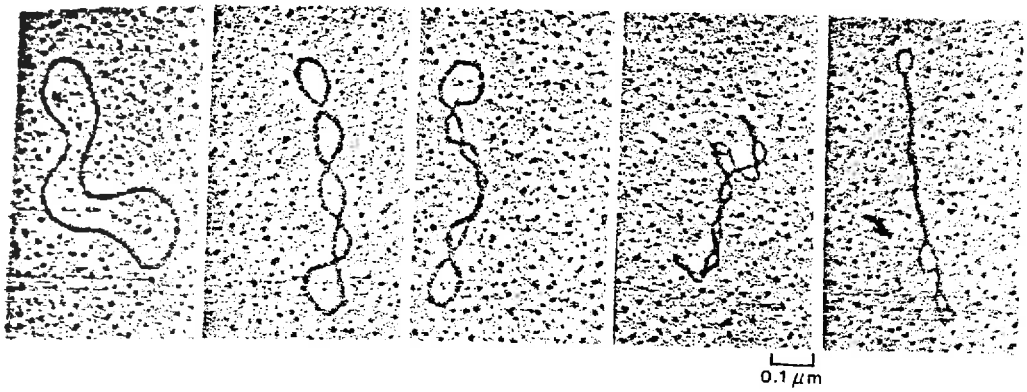
I thank Dr. Abe Worcel for sharing his biochemical wisdom, his enthusiasm, and his unwavering faith in symmetry. Thanks to Ms. Maureen Kirkham for her cheerfulness and careful typing. Finally, thanks to Professor Fred Almgren, Jr., my adviser, for his good-natured skepticism which was always tempered by the warmth of his encouragement.

Steven Strogatz
April 13, 1980

Electron micrographs of supercoiled DNA -

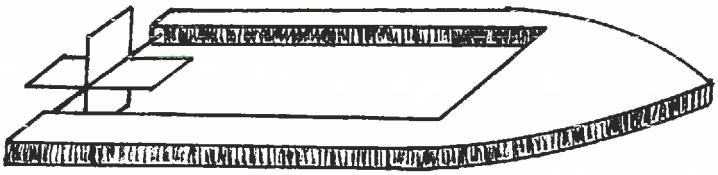
from relaxed to tightly wound.

(from Kornberg 1980)



Introduction

A child is winding up the paddle-wheel of his little toy boat. The paddle-wheel is attached by a rubber band to two arms of the boat.



As he winds the paddle round and round, little coils appear in the straight segments of rubber band. Nothing very interesting happens for a while. Suddenly, an additional turn of the paddle causes the straight rubber band to writhe and bunch up. At this critical point, the rubber band has writhed in order to relieve some of its local twisting. We say that the rubber band has supercoiled.

The domestic scene provides other examples of supercoiling, as anyone who has tried to untangle a telephone cord or garden hose can attest. But supercoiling is far more than a quotidian nuisance. In a very real sense, life as we know it depends on supercoiling.

The blueprint of life is DNA. This long and twisted molecule generates diversity among organisms but at the same time ensures that elephants give birth to baby elephants and not to baby salamanders. Moreover, the ongoing processes of life are commanded by these chemical threads. To store so much information the DNA has to be very long - in fact, a single human being contains a length of DNA equal to fifty round trips to the sun! Even in the most primitive organisms, the packaging of DNA is an engineering problem of astonishing proportions. Nature brilliantly solved the problem faced by these little bugs - their DNA is bunched up and compacted by supercoiling.

If the packaging problem hadn't been solved for bacteria and other primitive organisms, higher forms of life would have been impossible and we wouldn't be here.

What causes supercoiling of the DNA? A first step towards an answer comes from the following observation. The DNA of primitive organisms usually consists of two interlocked circles. More precisely, the strands of the DNA wind around one another in double helical fashion, as usual - except that here each strand is itself a closed loop. A closed circular DNA is always found to be underwound compared to a linear DNA of the same length. The stressed molecule then compensates for the underwinding by assuming a supercoiled structure. What causes underwinding? Furious work is being done on that question - the answer appears to involve an enzyme called gyrase.

Supercoiling has many more ramifications besides packaging; DNA replication, transcription, energetics, chemical reactivity, hydrodynamic behavior and enzymology are also affected. Research in any one of these areas absolutely requires a clear and precise scheme for the description of supercoiling. Mathematics enters here. Unfortunately the first scheme developed by the biochemists suffered from vagueness. Curiously, at about the time that circular DNA was first being characterized by Vinograd and his colleagues, mathematicians were studying very similar problems in the context of differential geometry.

We discuss, in the following pages, a scheme for the description of supercoiling. We have integrated the work of many mathematicians and biologists, whose perspectives and approaches were often widely divergent. It is especially hoped that this essay will aid and encourage those mathematicians interested in the geometry of life.

Mathematical Description of Supercoiling

How can we describe the conformation of a supercoiled DNA molecule? We hope to capture its salient features and express them in terms of a few vivid parameters. From one point of view, the important feature of supercoiled DNA, is,tautologically, its degree of supercoiling. In other words, we seek some measure of the tortuousity of the duplex axis.

A second point of view emphasizes the two polynucleotide strands which together form the duplex. Supercoiling is, after all, unique to those DNAs whose duplex consists of intertwined closed loops. So now we concentrate less on the axis, and instead focus our attention on the relative spatial arrangement of the strands. They both twist about the duplex axis, and in so doing, become very highly linked with one another.

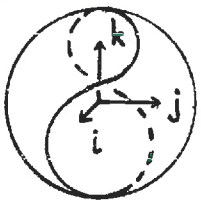
It was probably heuristic arguments like these which led Vinograd and other biochemists to define the conformational parameters, α, β, and τ. Roughly speaking α measures linking, β measures twisting, and τ measures supercoling. Usual definitions are: "the topological winding number α is the number of revolutions made by one strand about the duplex axis when the axis is constrained to lie in a plane; the duplex winding number β is the number of revolutions made by one strand about the duplex axis in the unconstrained molecule: the superhelix winding number τ is the number of revolutions made by the duplex about the superhelix axis." (Vinograd et. al. 1966) A proposed relationship among the parameters is $\alpha = \beta + \tau$, based on the observation that in closed duplex DNA, introduction of a duplex turn is offset by concomitant winding of the superhelix. (Before we continue, let us clarify our use of an expression which may seem offensive. For brevity, we write "the biochemists claim..." when we really mean "in the biochemical literature, it is often claimed that..."

We certainly do not mean to generalize about all biochemists. It is hoped that the reader will tolerate this and similar abuses of language. Also, in what follows, we abbreviate "closed duplex DNA" by cd DNA. Return now to the α, β, τ scheme.)

This descriptive scheme served the biochemists well enough for a few years. Purported proofs of the equation $\alpha + \beta = \tau$ were offered (Glaubiger & Hearst 1967) and accepted by the biological audience. However one should be very skeptical of any proofs which depend on definitions as loose as those given above. Upon close examination, the definitions retreat from intelligibility and enshroud themselves in a fog of confusion. For example, consider the duplex winding number β. The number of revolutions made by a strand about a planar duplex axis is an intuitively clear notion but if the duplex axis is itself helical, or perhaps some wildly writhing space curve, how is one to compute β? In this case, the concept of duplex turns becomes problematical. Yet, for some reason this natural question never arose, at least not in public. The biologists' complacence may perhaps be understood if we recall that the holiest of the holy, the Watson-Crick model of DNA, asserts a duplex twisting rate of 1 revolution per 10 base pairs. This rate, well-defined and invariable for linear DNA, may have been subconsciously assumed by the early workers to be equally well-defined for supercoiled DNA. But this is historical speculation on our part. The key point here is that the suggested definition of β is vague in all but the simplest cases.

Now consider the superhelix winding number τ (frequently called "the number of superhelical, or tertiary, turns"). When the duplex axis assumes the form of a regular (super)helix, one can merely infer a value of τ from

inspection. Once again the proposed definition becomes problematical if the duplex axis is some general space curve. For example, let the axis follow the curve below, which lies on a sphere. (Fuller 1971)

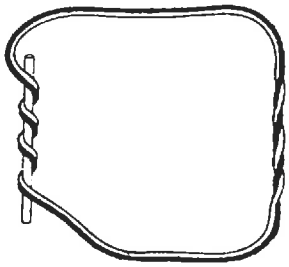


$\tau_i = -1$

$\tau_j = 0$

$\tau_k = +1$

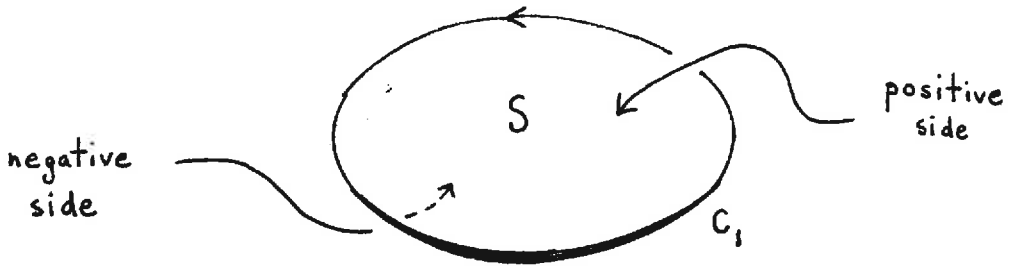
Here we see that τ depends on direction, an unsavory feature conveniently avoided by a regular superhelix because of its rotational symmetry. As a second example illustrating the ambiguity of this descriptive scheme, consider the diagram below, taken from Vinograd et. al. 1968. (Also see Bauer 1978)



It was originally proposed that for the left half of the duplex above, $\beta = 0$ and $\tau = -3$. As Bauer points out, the strands do twist about the duplex axis, and $\beta \neq 0$, unless the superhelical pitch is zero.

We turn now to the problem of constructing precise definitions for the parameters which describe linking, twisting, and supercoiling. It happens that the biochemists' "topological winding number" α is familiar to topologists and geometers, who call it the "linking number". This quantity was first studied rigorously by Gauss, in connection with his work on electromagnetism, and since then has played an important role in knot theory. To see the reason for the terminology, we consider two closed curves C_1 and C_2 (which represent the two DNA strands.) We choose an orientation, or sense of

direction, along each one, and then imagine constructing a surface S having one of the curves, say C_1 as a boundary. In the diagram below, we've chosen C_1 to be a circle for visual simplicity - this choice involves no loss of mathematical generality, as is shown formally in Pohl 1968. The surface S is then the circular region bounded by C_1. Our orientation for C_1 defines a positive side for S ; it is that side of S which "lies on our left as we walk along C_1", to use the standard explanation (Pohl and Roberts 1978, which we're following)



Now to calculate the linking number of C_1 with C_2, we count the number of times C_2 passes through S from the negative side, and subtract the number of times C_2 passes through S from the positive side. The resulting integer is the <u>linking number</u> of C_1 with C_2, and is denoted by Lk. Examples are shown below.

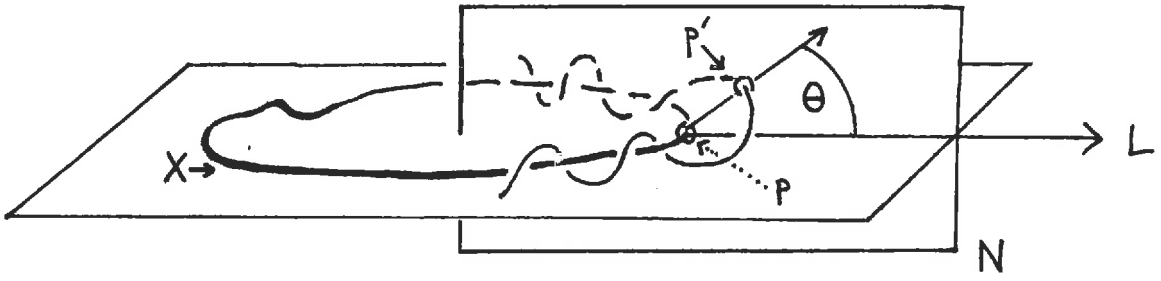
Topologists express the key property of Lk by saying it is an "invariant of isotopy". In laymen's terms, the linking number is an integer which remains constant throughout all deformations during which C_1 and C_2 do not pass through each other. Biologically speaking, a closed DNA molecule can writhe, twist, or undergo all sorts of violent battering, but its

characteristic Lk will stay the same, as long as the strands remain unbroken. Thus each intact cd DNA has a permanent and distinctive topological signature.

Later we will discuss Lk in greater detail. It is the conceptually simplest of the parameters necessary to describe cd DNA, and was rediscovered in an essentially correct form by the biochemists. So let us now undertake to lift the descriptive fog which envelops twisting and supercoiling.

A hint about how to attack duplex twisting comes from analyzing the simple case in which the duplex axis is planar. To this end, imagine a long DNA duplex with free ends and a linear axis. We put the molecule down on a plane, grab hold of the free ends, and while constraining the molecule to be planar we bend the duplex axis and butt the ends together so that the axis is now a simple closed curve and the strands form a closed duplex. (Some twisting may be necessary to align the strands before closing the duplex, but this is irrelevant as long as we keep the axis planar.)

Let the duplex axis be called X, and assume X is smooth, with an everywhere well-defined, non-zero tangent vector t. (We will be precise a bit later - excessive rigor would only obscure the main idea at the point). At a point p on X , we consider the plane through p which is perpendicular to t. This plane will be referred to as the normal plane N (at p). It intersects both of the neighboring strands; focus on only one of the strands, and let p' be its point of intersection with N. (See below). Let L be the line defined by the intersection of N at the plane of X .



In the diagram, we have shown only one of the strands, for clarity.

Let θ be the angle between pp' and L. As p travels along X, θ will sweep out $\frac{\Delta\theta}{2\pi}$ revolutions. In other words, the number of duplex turns is given by $\beta = \frac{\Delta\theta}{2\pi}$. Note that since the strands are closed , β is an integer. In fact, β = Lk in this special case of planar X . If X is parametrized, say by arc length s , we have a more precise formula for β:

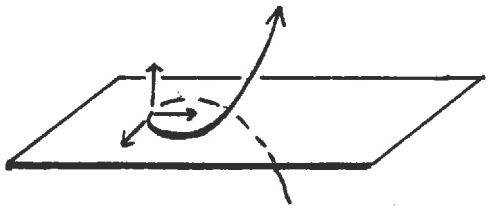
$$\beta = \frac{1}{2\pi}\int_0^{\ell} \frac{d\theta}{ds}\, ds \qquad [1]$$

We prefer [1] to $\beta = \frac{\Delta\theta}{2\pi}$ because there is possible ambiguity associated with the angular measurement $\Delta\theta$. More importantly, we'll now see that [1] generalizes naturally when X is a non-planar closed space curve.

The non-planarity of X introduces one primary difficulty with respect to our first approach. The twisting angle θ was there easily defined because the place of X served as a canonical reference. Having observed the crucial importance of this reference plane, one expects that the appropriate generalization of β will involve a <u>local</u> reference plane. Such an object will now be defined, first mathematically and then physically. The reader may select either description, according to his taste.

Let X be a C^{∞} map, X: $[a,b] \longrightarrow \mathbb{R}^3$ and $x'(t) \neq 0$ for all $t \in [a,b]$. Then X may be reparametrized by arc length s, whence X'(s) is the unit tangent vector. We further assume that all derivatives of X (including the zeroth) agree at s = 0 and s = ℓ, so that X is a smooth closed curve. Next we demand that X be simple, i.e. $X(s_1) = X(s_2)$ iff $s_1 = s_2$, with the allowed exception $X(0) = X(\ell)$. Such an X is a suitable duplex axis. if we demand finally that X have non-vanishing curvature at all points. Because of our assumptions, the Frenet trihedron is defined all along X , and in particular the osculating plane is well-defined. This plane is the desired canonical reference. (For an alternative approach to these matters which involves no assumptions on curvature, see Fuller 1971.)

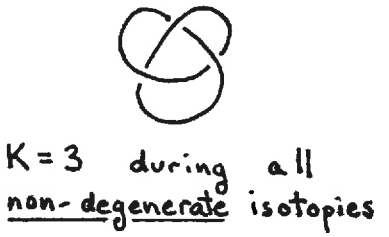
Here the above discussion is translated into physical language. Consider a particle moving in space with unit speed along a smoothly curved path X. Then the acceleration vector is always normal to the velocity vector, assuming that the radius of curvature is everywhere finite and non-zero. (Note especially that the path contains no straight line segments) Then the plane spanned by the tangent and acceleration vectors is called the osculating plane; to use sloppy but vivid language, it is the plane in which X resides locally. (See diagram)

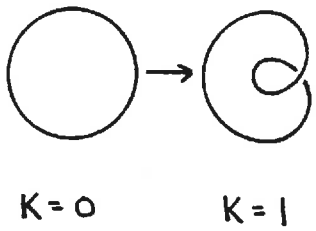


Now that the osculating plane has been defined, we can define an angle θ exactly analogous to the earlier one. We merely refer θ to the osculating plane, rather than to some fixed plane. As before, we can then consider $\beta^* = \frac{1}{2\pi}\int^{\ell} \frac{d\theta}{ds}\, ds$ to be a measure of duplex twisting. Note that β^* is a refined version of β. It is the number of revolutions made by a strand about the tangent to X, as counted by an observer sitting on the Frenet trihedron while the trihedron makes one complete cycle of X. As Calugareanu puts it β^* gives the "nombre de tours relatif" of the strand about the axis. (Calugareanu 1961) As is clear from its definition β^* is an integer. However, unlike the planar case, here it need not equal the linking number of the strands. The difference $Lk-\beta^*$ is called K by Calugareanu, and SL, i.e. self-linking number, by Pohl (Pohl 1968). It too is an integer, and the amazing theorem, due to Calugareanu, is that K depends only on X, and not on the spatial disposition of the surrounding strands. (Note that the strands determine both Lk and β^*, and thus might

be expected to determine their difference.) So in some sense K measures the non-planarity of X and therefore could be a candidate for the parameter of supercoiling. Have we found in β^* and K the descriptive parameters sought originally?

Unfortunately not, for two reasons. First of all, neither K nor β^* is sufficiently sensitive to slight conformational changes in a cd DNA. As noted earlier, both parameters are integer-valued and must necessarily remain constant or else change discontinuously during molecular deformations. Examples of both types of behavior are shown below (Calugareanu 1961). (See Historical Comments for Calugareanu's restrictive definition of isotopy.)





Ideally, our descriptive parameters would vary continuusly during continuous molecular deformations, so that tiny structural changes could be monitored.

A second problem is that β^* really doesn't measure the quantity of interest. It suffers from a rather serious weakness - β^* measures duplex twisting in relation to the Frenet trihedron rather than in relation to some fixed external observer. An example will clarify this point. Suppose we consider the path traced out by the endpoint of the principal normal vector (= acceleration vector) as the Frenet trihedron travels along a tortuous axis X. It is not difficult to imagine that the traced path, which we may call a strand, is twisted about X when viewed from the vantage point of an outsider. Yet since in this example $\theta \equiv 0$, then $\frac{d\theta}{ds} = 0$ as well, as so $\beta^* = 0$. How do we reconcile this result with our intuitive sense of twist?

Clearly the notion of "relative turns" encompassed by β^* must be supplemented with a parameter which, roughly speaking, measures the self-twisting of X. In other words, we'd like to characterize the total rotation of the Frenet trihedron about the tangent vector. But now our problems are solved! From classical differential geometry it is known that τ, the torsion of X, may be interpreted as the desired angular rate of rotation, assuming X is parametrized by arc length s. Then the total number of turns of the Frenet trihedron about the tangent vector is given by $\frac{1}{2\pi}\int_0^\ell \tau \, ds$.

We have found the proper method of describing duplex twisting (with respect to external observers) expressed by the following decomposition:

Duplex turns w.r.t. outsiders	=	Duplex turns w.r.t. Frenet trihedron (β^*)	+	Total # of turns of Frenet trihedron w.r.t. outsiders

$$Tw = \frac{1}{2\pi}\int_0^\ell \frac{d\theta}{ds}\, ds + \frac{1}{2\pi}\int_0^\ell \tau \, ds \qquad [2]$$

The sum of terms on the right is called the twist, and is denoted Tw. Unlike β^*, this quantity need not be an integer, and it does vary continuously as the molecule is deformed. This parameter quantifies the biochemists' β; Tw is both precisely defined and readily computable. Note that for a planar duplex axis, Tw reduces to β^* because $\tau=0$. But because $\beta^* = Lk$ in this case, we have Tw = Lk too. Linking and twisting coincide in the planar case.

At this point, we hasten to emphasize the differences between linking and twisting. First, Tw is a geometric property; deformation of the duplex will generally alter the twist. Lk, however, is a topological property and

cannot be changed by deforming the duplex (excluding those deformations which rupture the strands). Second, Tw is an additive property in the sense that we may calculate it for all local sections of the duplex, and then sum over all sections to find Tw for the whole structure. The linking number is a global property and can be found only by consideration of the entire molecule.(Fuller 1978) None of these crucial distinctions is manifested when X is a plane curve.

However, if we allow X to be a closed space curve, not necessarily planar, then in general $Lk \neq Tw$. Calugareanu's theorem is that the difference Lk-Tw depends only on the duplex axis, and not on the arrangement of the strands. Fuller has called this difference the "writhing number" of X, denoted Wr (Fuller 1971). Thus we have

$$Lk = Tw + Wr \qquad [3]$$

Before we relate Wr to the parameter K , defined earlier in a similar fashion, let us discuss qualitatively the main features of the writhing number.

Most importantly, Wr is a sensitive measure of supercoiling. It varies continuously during continuous deformations of the duplex axis, and can thus reflect slight conformational changes. Unlike Lk, it is not a topological invariant, but a geometric property of the duplex axis. (Recall that both Lk and Tw are properties of the strands themselves). We can best get an understanding of Wr by seeing a few examples, which should also clarify Lk and Tw. After the examples, we'll consider all three parameters in some mathematical detail. Finally, we present a "dictionary" which relates and deciphers previous descriptive schemes of supercoiling.

Examples of Wr, Tw, and Lk

Before beginning we refer the reader to the excellent paper by Crick (Crick 1976) wherein these concepts are thoroughly elucidated. He introduces a twisted ribbon model of DNA: the edges of the ribbon represent the DNA strands, and the ribbon's axis represents the duplex axis. Naturally we are primarily interested in closed ribbons.

First consider a deformable ribbon twisted around a cylinder, always lying flat on the surface and following a helical path with pitch angle α. For small pitch angles the ribbon has small twist, but large writhing. More specifically, if the ribbon makes N turns before the ends are joined by a twist-free strip, we can show (neglecting end effects) that

$$Lk = N \qquad Tw = N \sin \alpha \qquad Wr = N (1-\sin \alpha) \qquad [4]$$

Imagine stretching the helical ribbon along the cylinder while keeping the number of turns fixed. Then the twist becomes large, though the linking number remains constant. (Such an effect makes the difference between Tw and Lk transparent.) Note that pulling out a coiled telephone cord amounts fo the same thing; the highly writhing form, with low twist, is converted to a twisted form which writhes very little. (Bauer, Crick, White unpublished)

A more subtle example, illustrating the utility of the fact that Wr depends only on the duplex axis, is splendidly discussed by Crick (Crick 1976).

He asks us to consider the linking number of the structure (A) shown below.

A B

To calculate Lk for (A), Crick considers first the simple structure (B), in which one arm of the ribbon is on the cylindrical surface, while the other arm goes down along the cylindrical axis. Crick argues,

"Imagine the bottom of the cylinder to be stationary and the top to be rotated so that we generate a structure with two left-handed turns of flat ribbon on the outside and a twisted branch of the ribbon up the axis also having two left-handed turns. We now calculate the writhing number. The outside section of the ribbon contributes - 2 sin α to the twist while the contribution of the central section is -2. Thus $Tw = -2(1 + \sin \alpha)$. Since $Lk = 0$, we have $Wr = +2(1 + \sin \alpha)$ for this structure. However the axis of the ribbon follows exactly the same configuration as the structure we are interested in".

Let us review the strategy employed by Crick. To find the linking number of (A), he has twisted (B) into a structure having the same duplex axis, and therefore the same writhing number as (A). To compute this writhing number, he uses (i) the additivity of Tw when he sums over the inside and outside branches of twisted (B) , (ii) the constancy of Lk under smooth deformations - since Lk = 0 for (B), Lk = 0 for twisted (B) and (iii) the formula Lk = Wr + Tw. Now back to Crick, who was discussing the relation of (A) to twisted (B) when we interrupted.

"Thus, for this structure too, Wr = +2(1 + sin α). However, for (A) the value of Tw is clearly -2 sin α because the central part of the ribbon is untwisted. Thus, since Lk = Wr + Tw we obtain:

$$Lk = 2(1 + \sin \alpha) - 2 \sin \alpha = +2.$$

This calculation illustrates in a neat way one of the uses of the writhing number, Wr. It is sometimes easier to calculate Lk and Tw for a structure having the same axis as one in which we are interested. Then if for our structure of interest, the calculation of Tw is easy, we can immediately obtain Lk from it, or vice versa".

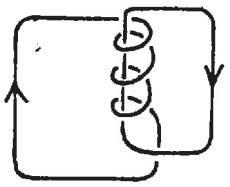
Further properties of Wr, Tw, and Lk.

Aside from their role in biochemistry, the parameters Wr and Tw are relatively unknown, especially among pure mathematicians. In elasticity theory, a quantity related to Tw is used in connection with the torques on a twisted rod. (See e.g. Love) The writhing number has been very little studied, and only a scanty bit of knowledge exists about its relation to more traditional parameters, such as curvature. In this section we will mention most of the important mathematical results in this area, along with references for further study.

Both the linking number and the writhing number may be calculated directly (though laboriously) by means of a certain integral, often called the "Gauss looping integral" in honor of its discoverer. Given two disjoint closed oriented curves, c , c' , the Gauss looping integral is written G(c,c');

$$G(c,c') = \frac{1}{4\pi} \int_c \int_{c'} \frac{1}{r^3} \begin{vmatrix} x'-x & dx & dx' \\ y'-y & dy & dy' \\ z'-z & dz & dz' \end{vmatrix} \qquad [5]$$

where (x,y,z) is on c , (x',y',z') is on c', and $r^2 = (x-x')^2 + (y-y')^2 + (z-z')^2$. For a proof that $G(c,c') = Lk$, see Edwards 1968. Gauss studied this quantity during his investigations of electromagnetism. The solenoid in the diagram illustrates the physical interpretation of $G(c,c')$ as "the work done on a unit magnetic pole in carrying it around one curve through the magnetic field produced by a unit electric current in the other" (Fuller 1962)



If we recall the original topological definition of Lk and supplement it with the equivalent equation [5], the following results become clear:

(a) Lk is independent of the parametrization of c and c'.

(b) $G(c,c') = G(c',c)$

(c) Lk is invariant under translations rotations, and dilations of the surrounding space

(d) The sign of Lk changes if the space is acted on by an orientation-reversing transformation, e.g. reflection in a plane.

If we consider a singular version of [5], with $c = c'$, the resulting integral is not zero in general - in fact it is the writhing number Wr! (Calugareanu 1959, 1961) In this case we interpret the integral as being performed over all distinct pairs of points on c. (Vranceanu 1972) It was in this integral form that Wr was first studied by Calugareanu in his quest for isotopic invariants of knots. Note that Edwards, in his work on statistical mechanics of knotted polymers, errs grieviously in assuming

that the isotopic invariance of [5] is inherited by the self-integral version of [5], i.e. the writhing number (Edwards 1968). Here, then, is the integral formula for Wr:

$$Wr = \frac{1}{4\pi} \int_c \frac{1}{r^3} \begin{vmatrix} x-x' & dx & dx' \\ y-y' & dy & dy' \\ z-z' & dz & dz' \end{vmatrix} \qquad [6]$$

where (x,y,z) and (x,'y',z') are distinct points on c, and r is the distance between them. We observe that Wr has the following properties:

(a) it is independent of the orientation of c.

(b) it is invariant under rigid motions or dilations of the space containing C.

(c) its sign is changed by reflection in a plane or reflection in a sphere, as long as the curve avoids the center of the sphere. Hence Wr = 0 for planar and spherical closed curves.

More direct geometric interpretations of Wr and Lk in terms of spherical area are possible. We will discuss only one such result, due to Fuller. (For a very sophisticated treatment of this subject, see White 1969, who generalized Calugareanu's results to higher dimensions; also Vranceanu 1972) In the following discussion, we will be using freely the arguments and illustrations given by Fuller 1978.

Suppose a simple closed space curve c is parametrized by some parameter t. "Then the unit tangents T(t) trace out, if their starting points are translated to the origin, a closed curve on the unit sphere. Let A be the solid angle in steradians (area on the unit sphere) enclosed by this curve. Then

$$1 + Wr \equiv \frac{1}{2\pi} A \pmod 2" \qquad [7]$$

Fuller points out that [7] is "only good modulo 2 because the solid angle enclosed by T(t) is determined only up to integer multiples of 4π (the area of the unit sphere)". He goes on to show how we can use [7] in conjunction with continuity arguments to obtain the right value of Wr in ambiguous cases.

To prove [7], invoke the Gauss-Bonnet theorem. (G.B.) For a closed curve on S^2 with a unit tangent to the sphere defined at each point of the curve:

Total rotation of tangent vector on sphere + area enclosed by curve $\equiv 0 \pmod 2$

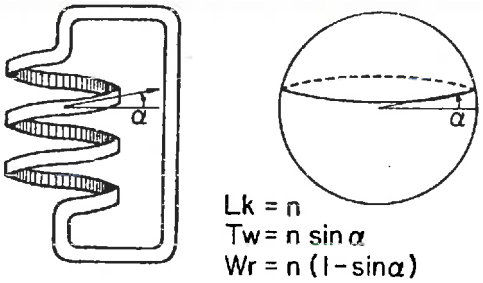
Now the unit tangents T(t) to our original curve c trace out a closed curve on S^2. Suppose we define a ribbon about c, given by $c + \varepsilon U(t)$, where U(t) is a unit vector which points from the axis c toward an edge of the ribbon. Then since U(t) is perpendicular to T(t), it is tangent to the unit sphere. Moreover, the total rotation of U(t) is just (2π)Tw, because Tw was defined in [2] to be the total rotation of such a vector.

Hence G.B. implies

$$Tw + \frac{1}{2\pi} A \equiv 0 \pmod 1 \qquad [8]$$

Since $Tw + Wr = Lk \equiv 0 \pmod 1$, we have $Wr \equiv -\frac{1}{2\pi} A \pmod 1$. To prove that this expression is correct modulo 2, Fuller argues that [7] is correct for a circle "and since it is correct modulo 1 it remains correct modulo 2 if the circle is deformed without passing through itself." If the deformed circle does pass through itself, Fuller has shown that Wr jumps by ± 2, which has no effect on [7]. Indeed, in all self-passages, both Lk and Wr jump by 2.

As an illustration of Fuller's theorem, consider the structure shown below (photocopied from his article)



Lk = n
Tw = n sin α
Wr = n (1 − sin α)
α = pitch angle of helix
n = number of turns

The ribbon above is partly wrapped n times around a cylinder, such that the ribbon axis is a helix of pitch α. The other part of the ribbon lies in a tangent plane to the cylinder. Then the "curve of unit tangents T(t) is a great circle enclosing an area 2π , plus n turns around a circle 'cap', enclosing an area $2\pi n(1 - \sin\alpha)$". (Fuller 1978) So the total area is $2\pi(1+Wr)$, since $n(1 - \sin\alpha) = Wr$ from [4]. The formula $1+Wr \equiv \frac{1}{2\pi} A \pmod 2$ is verified for this example.

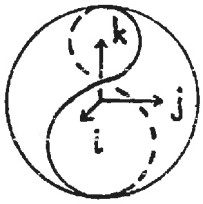
Perhaps at this juncture we should explain how equations like [4] are obtained. We use the fact that if a ribbon is generated by the end-point of the principal normal vector, then the twist reduces to the integral torsion $\frac{1}{2\pi}\int \tau \, ds$. We are free to choose such a simplifying ribbon because the writhing number is independent of any such choice. Since Lk = N by inspection in the case of a simple helix, and Tw may be computed using the relation $\tau = r^{-1} \cos\alpha \sin\alpha$, it is a matter of subtraction to find the writhing number. Note that the helical radius r never survives the calculations since all the parameters of supercoiling are dilation-invariant.

Another approach to the calculation of Lk and Wr involves counting cross-overs on planar projections. We project the duplex, in the case of Lk, or the axis, in the case of Wr. The algorithm for computing Lk of a ribbon consists of first marking the edges distinctively (say one red, and the other black) and then assigning orientations to each edge. We now inspect the configuration from some distant point - if this point is at infinity the view will be a projection, though this is not essential. Focus now on those points where the red edge is in front of black (of course, we could have made the other choice, but we must not choose <u>both</u> or else we'll later have to divide by two.) Each cross-over of red on top of black may be assigned a value of +1 or -1, where the conventions below are followed:

Then Lk is the algebraic sum of all these assignments (Crick 1976). It is not at all obvious that this sum is independent of our original choice of the plane on which the ribbon is projected. But because both edges are closed curves, the sum is in fact invariant and equal to Lk. For a proof see Pohl 1968.

To find Wr, we choose a direction in space and a plane perpendicular to that direction, and project the curve onto that plane. This time we assign values of +1 or -1 to <u>all</u> cross-overs; the value assigned follows the convention described earlier. The sum of these assignments is called the <u>directional writhing number</u>. Then the writhing number of the curve is the average over all directions of the directional writhing number (Fuller 1971, Benham 1978).

Note that these concepts triumphantly solve the directional problems associated with the biochemists' "superhelix winding number". A while back, we considered the spherical curve below (Fuller 1971)



At that time we observed the number of tertiary turns could be taken to equal 0 if the curve is viewed along j, and not equal to zero if we look down i-axis. Using the language just developed, the directional writhing numbers along the i,j,k directions are -1,0, and +1 respectively. It just so happens that the average over these three directions equals the average over <u>all</u> directions; we know Wr = 0 from [6] and property (c) of the writhing number.

The mathematics of writhing is virtually uncharted territory. In these few pages we have listed almost all that is known about the writhing number. Answers to the following questions are conspicuous by their absence:

(a) What families of closed curves have the same writhing number?

(b) Alternatively, what sorts of transformations preserve Wr?

(c) How is Wr related to the local curvature, total curvature, radius of gyration, etc?

A question of outstanding practical importance is related to (c):

(d) Given a curve of specified length and writhing number, what is the minimum value of the total squared curvature?

Question (d) arises during investigations of elastic properties of supercoiled DNA. The classical theory of elasticity posits a linear relation between the energy of bending and the total squared curvature. Since Lk of the molecule is fixed at the instant of duplex closure, this topological constraint imposes a coupling between Wr and Tw, and thus between the possible bending and torsional energies. An answer to (d) would elucidate the dependence of the latter coupling on the former. Presumably the molecule adopts a conformation which minimizes the total elastic energy, subject to the topological constraint. Any complete analysis of this energy-minimization problem requires the solution to (d). Later in this paper we will discuss the energetics of supercoiled DNA, though our approach will be semiempirical. For the moment, we leave mathematics and turn to lexicography.

Mathematics of Supercoiling - A Dictionary

Many different symbols and names for the parameters of supercoiling appear in the literature. So much confusion plagues the subject that we think it necessary to present a cross-referential dictionary. The terms are listed in order of decreasing importance. Our format is as follows:

A. Here you will find numbers corresponding to the pages in this essay where the term is discussed or defined. Thus A. is an index.

B. Cross-references and comparisons. The symbols listed refer to completely equivalent mathematical objects, unless otherwise indicated. Only the most important articles are cited; thus also literature. The references have been coded numerically according to the scheme below:

1. Fuller 1971
2. Fuller 1978
3. Fuller 1962
4. Crick 1976
5. Calugareanu 1959
6. Calugareanu 1961
7. Vinograd et. al. 1968
8. Bauer 1978
9. Pohl 1968
10. Pohl & Roberts 1978
11. White 1969
12. Edwards 1968
13. Vranceanu 1972

C. Here we make additional comments, reminders, or clarifications.

(1) Linking number

A. 3, 5-7, 9, 11-16, 20

B. Lk - 2

Lk $(X,X + \varepsilon U)$ - 1

Lk (C,C') -3

L - 4, 10

α - 7, 8

$G(C,C^*)$ - 6

I - 5

$L(f_1,f_2)$ - 9

$L(f,f_e)$ - 11

n - 12

C. In the biochemical literature, one often encounters "topological winding number", or "linkage number", denoted by α .

It is a topological invariant defined for a ribbon, or a pair of curves.

Bauer 1978 errs by called SL a linking number for a single closed curve; see (7).

(2) Writhing number

A. 12 - 22

B. Wr - 1, 2, 8

W - 4, 10

$J/4\pi$ - 6

J - 5

$\frac{1}{4\pi}\int_{C\times C} dS$ - 9

I - 13

$\frac{1}{4\pi}\int_{M\times M} dO_2$ - 11

C. Avoid confusion with "superhelical", or "tertiary turns". For example, in the case of a right-handed toroidal superhelix with N "superhelical turns" and pitch angle α, Wr = N(1-sin α). Thus Wr = N only if the superhelix has vanishingly small pitch.

In the biochemical literature, the symbol τ is not synonymous with Wr - it is the object we called N .

The writhing number is not a topological invariant. It is a real number associated with a closed space curve; frequently the curve is an "axis" of a "ribbon", in which case it is permissible to speak of the writhing number of the ribbon.

(3) Twist

A. 3-5, 7-15, 18, 19

B. Tw - 2, 8

Tw(X,U) - 1

T - 4, 10

$\frac{1}{2\pi}\int_C (\tau+\theta')ds$ - 6

β - 7

$\frac{1}{2\pi}\int_M \pi_{23}$ - 11

C. In the biochemical literature, the more usual name is "duplex winding number", or the "number of duplex turns". Fuller 1971 calls it the "total twist number".

The twist is defined for a ribbon, not for a duplex axis. Note that Calugareanu's definition assumes that the duplex axis admits a Frenet frame everywhere; White and Fuller drop this assumption and define the twist by means of an arbitrary unit normal vector field based on the axis.

The twist is not a topological invariant; however, it is a conformal invariant.

Even in its relaxed form, a cd DNA has a non-zero twist, due to Watson-Crick helical winding. The symbol ΔTw measures the deviation in twist as compared to the relaxed form, and $\Delta Tw = Tw - Tw_o$, where Tw_o is the twist of the relaxed (B configuration) molecule.

(4) Lk = Wr + Tw

A. 12-15, 18-19

B. Lk = Wr + Tw - 2

$Lk(X,X) + \varepsilon U) = Wr(X) + Tw(X,U) - 1$

$L = W + T - 4, 10$

$G(\mathcal{C}, C^*) = \frac{1}{4\pi} J + \frac{1}{2\pi} \int_C (\tau+\theta')ds - 6$

$L(f,f_e) = \frac{1}{4\pi} \int_{M\times M} dO_2 + \frac{1}{2\pi} \int_M \pi_{23} - 11$

C. One must be careful when comparing this to the earlier $\alpha = \tau + \beta$. If τ means "superhelical turns" (as it usually does), then the equation is false. If τ is defined as the difference of α and β, then everything is tautologically correct.

The formula $Lk = Wr + Tw$ was discovered by Calugareanu in 1960 - he proved it under restrictive assumptions on the smoothness and curvature of the axis curve. White offered the first general proof of the formula (no curvature assumptions) in 1969. Fuller did not publish a proof; he was the first to connect molecular biology and differential geometry through the above formula.

It is a formula about ribbons, and should not be confused with $K = Wr + \frac{1}{2\pi}\int_C \tau \, ds$.

(5) Superhelical turns

A. 3-5, 13, 19, 21

B. N - 1, 4

n - 2

τ - 7, 8 (see note below)

C. This term originated because regular toroidal or interwound superhelices were assumed to be the tertiary structures of supercoiled cd DNA. In these cases, a natural analogy exists between the turns of the strands about the duplex axis, and the turns of the duplex axis about the superhelix axis. When the duplex axis is a more general space curve, the term has no meaning. In particular, it is not the writhing number.

Synonyms are "tertiary turns" and "superhelix winding number".

(Note: we use the symbol τ to indicate torsion.)

(6) Angular twist rate

A. 11

B. w_1 - 1, 2

$\tau + \theta'$ - 6

π_{23} - 11

C. In Fuller 1971 and in texts on elasticity theory, this parameter is called the "twist". It is a property of a ribbon, whereas torsion is a property of the ribbon's axis.

(7) Calugareanu's invariant (self-linking number)

A. 9-10, 12

B. K - 5, 6, 13

SL - 8, 9

C. This quantity is an invariant of non-degenerate isotopy of a knot - see "Historical Comments" for a definition of non-degenerate isotopy. The invariant generated interest among Pohl and White, who developed much of the mathematics of higher-dimensional supercoiling. It attracted much more attention than $Lk = Wr + Tw$, which has since proved to be the significant result in Calugareanu's work.

The linking number of the ribbon $(C, C + \varepsilon n)$ where n is the principal normal vector to C, equals Calugareanu's invariant

$$K = Wr + \frac{1}{2\pi} \int_C \tau \, ds$$

Pohl calls the invariant "the self-linking number" of C.

Applications of the Mathematics of Supercoiling:

I. The Torsional Stiffness of DNA

The packaging of DNA staggers the imagination. A human being of length 2 m contains a length of DNA equal to 2.5×10^{13} m, i.e. 50 round trips to the sun! Even at the opposite end of the evolutionary spectrum, the bacterium E. Coli houses DNA one thousand times longer than itself. Such compactifications appear even more mind-boggling when we recall that the DNA must be readily retrievable and available for replication; moreover the squashing must not rip or break the DNA.

Consider the plight of E. Coli. Scaling up to human dimensions, we may pose the problem thus: take a piece of string 100 m long, and ball it up so you can hold it in both hands. In the thousand fold reduction just accomplished, have you tangled any of the string? Nature forbids such tangling of DNA - cell division and growth demand the ready separability of the genetic material. Packaging without tangling is as difficult as it is crucial.

As we mentioned at the beginning of this essay, the Earth's most primitive organisms solved the packaging problem by supercoiling their DNA. In procaryotic cells, as exemplified by E. Coli, an enzyme called DNA gyrase maintains the double helix in an underwound state (Champoux 1978). It is believed that gyrase makes a single-strand scission ("nick") in the DNA, and then promotes the unwinding of the helix. This energy-requiring process is driven by the hydrolysis of ATP, a high-energy phosphate ester which serves as the principal energy-storage compound of the cell (Watson 1976).

Upon cessation of DNA unwinding, the gyrase reseals the nick, thus creating a closed duplex DNA (cd DNA) which is underwound with respect to a linear DNA of the same length.

In its torsionally relaxed conformation, DNA has 10.4 base pairs per helical turn (Wang 1979). Hydrogen bonding and base-stacking interactions stabilize the structure at this twist rate. Thus, the unwinding action of gyrase induces torsional stresses in cd DNA. Unlike linear DNA, cd DNA cannot relieve these stresses by merely rewinding. Duplex twisting is here coupled to bending of the axis, by the basic equation $Lk = Tw + Wr$. In other words, if the underwound cd DNA tries to twist up, it can do so only at the expense of increased supercoiling. But supercoiling also introduces elastic stresses - in this case, bending stresses. So the DNA settles down in an energetic compromise, with the conformational free energy partitioned between twisting and writhing. The position of this compromise depends on the ratio of DNA's torsional and bending stiffnesses, called C and A, respectively. Before discussing this point further, we make some definitions.

A cd DNA is said to be relaxed if it has $Wr = 0$ when the twist rate is 10.4 base pairs per turn. The linking number of relaxed cd DNA is written Lk_o. Then we say the same molecule is underwound if it has $Lk < Lk_o$, and we write $\Delta Lk = Lk - Lk_o < 0$. We define Tw_o, Tw and ΔTw analogously; our basic equation becomes

$$\Delta Lk = \Delta Tw + Wr \qquad [9]$$

Now to return to the elastic parameters of cd DNA. Suppose DNA is far easier to twist than to bend, i.e. $C << A$. Then nearly all of ΔLk will be partitioned to ΔTw, and almost none to Wr. In other words, the action of gyrase will be manifested as helical unwinding, rather than as

supercoiling. Were this actually the case, the phenomenon of supercoiling would not be observed - after all, it arises only to relieve excessive torsional stresses (as in the paddle-boat example). And without supercoiling, our great-granddaddies in the primeval soup could not have solved the packaging problem.

So this anthropocentric argument leads us to expect that the torsional stiffness of DNA is not much less than its bending stiffness. The aim of the next section is to develop an elastic model which will enable prediction of the value of C in terms of known quantities. Our results imply that indeed C is not much less than A - in fact, they are found to be roughly equal.

Earlier researchers computed C by studying the torsional Brownian motion of linear DNA. Barkley and Zimm 1979 obtained a value which could not be reconciled with the torsional stiffness inferred from supercoiling data; we show that the discrepancy is due to a geometric oversimplification made by the authors, and that our more detailed considerations demonstrate the compatibility of the two sets of data.

To re-emphasize the main point of this and the following section, packaging of procaryotic DNA is achieved by supercoiling. Supercoiling is a manifestation of the torsional rigidity of DNA; the molecule prefers to writhe rather than accept the full brunt of helical untwisting caused by gyrase.

Life depends on the stiffness of the double helix. Just how stiff it is will be determined now.

An Elastic Model of Closed Duplex DNA

We mentioned earlier that a cd DNA underwound by an amount ΔLk undergoes both bending and torsional stresses. Gyrase has done work on these molecules by unwinding them - this work is then stored by the DNA in the form of increased conformational free energy. Such physical considerations suggest that topoisomers, i.e. chemically identical molecules which differ in their linking number, should differ in energy as well. The free energy difference between a relaxed molecule and the same molecule underwound by an amount ΔLk is referred to as the free energy of supercoiling.

Depew and Wang 1975 devised an especially elegant method for measuring this quantity. By treating a population of cd DNAs with an enzyme which makes single-strand scissions in the duplex (thus relieving the topological constraint of constant Lk), they brought the molecules to the nicked, relaxed form. Due to thermal fluctuations, both Tw and Wr of a nicked molecule vary about some mean values Tw^* and Wr^*. So at any time, some of the molecules are overwound, and some are underwound, assuming that the system has settled down to thermodynamic equilibrium. In the authors' words,

> "It immediately follows that if a homogeneous population of nicked circular DNA molecules is sealed by ligase, the resulting covalently closed molecules are not homogeneous with respect to (ΔLk), because the value of (ΔLk) for any product molecule is determined by the sum of (ΔTw) and (Wr) at the time of closure".

To measure the experimental distribution of topoisomers, Depew and Wang used Keller's gel electrophoresis method of resolving species differing in linking number (Keller and Wendel 1975). The basis of the method is that molecules with high $|\Delta Lk|$ migrate faster because they tend to be more compact. The observed concentration distribution over linking number is approximately Gaussian. Because of the Boltzmann distribution law of thermodynamics, the width of this distribution reflects how large the free energy of supercoiling is compared to the thermal energy kT (Depew and Wang 1975; Baver, Crick, and White, unpublished results). The results may be summarized by the equation

$$\Delta G = \frac{BkT}{n} (\Delta Lk)^2 \qquad [10]$$

where ΔG is the free energy of supercoiling, n is the number of base pairs in the DNA, and B is a pure number which depends slightly on the particular species of DNA. The values of B computed by Depew and Wang form the <u>empirical</u> basis of our subsequent work on the free energy of supercoiling. We'll soon write a <u>theoretical</u> expression for ΔG in terms of DNA conformational parameters (Wr, Tw, superhelical radius, etc.) and the elastic constants A and C. By then using some available data along with the experimental value of ΔG, we may deduce a value for C, the DNA torsional stiffness.

The model we are about to consider derives from the work of Camerini-Otero and Felsenfeld, to which reference should be made (Camerini-Otero and Felsenfeld 1978). Like those authors, we choose the DNA from the cancer-causing monkey virus SV40 as an exemplar, because it is the most extensively characterized of the known cd DNAs (Bauer 1978)

We now decompose the free energy of supercoiling ΔG into contributions from a few sources. The terms to be considered are the bending energy G_B ; the twisting energy G_T ; the configurational entropic free energy G_E , due to the orderliness inherent in the compact supercoils ; and the repulsive free energy G_R , due to excluded-volume effects and electrostatic repulsions caused by the self-proximity of the structure as compared to the relaxed form.

In what follows, we neglect the latter two contributions - thus we assume that, to a first approximation ,

$$\Delta G = G_B + G_T \qquad [11]$$

First consider G_B. In the usual first-order theory of elasticity, the bending energy is related to the total squared curvature (Landau and Lifshitz 1959):

$$G_B = \frac{1}{2} A \int_0^L K^2 \, ds \qquad [12]$$

Here K is the curvature of the duplex axis, s is the arc length parameter, L is the DNA contour length, and A is the bending stiffness (often called the "coefficient of flexural rigidity") of DNA.

To express the twisting free energy, we need a torsional analogue of the curvature. Such a quantity was discussed earlier, in connection with the twist - it is the angular twist rate, denoted here by w. Physically, w is the angular speed at which the strands rotate about the duplex axis. A more precise definition is as follows: the axis and one of its neighboring strands may be identified with, respectively, a differentiable space curve X and a curve generated by some smooth unit normal vector field defined along the axis X . Let T be the tangent

vector to the axis and N the vector field defined in the normal plane to T, and let $V = T \times N$. Then the frame (T,N,V) rotates in space with some angular velocity Ω as it moves along X with unit speed. Then $\Omega \cdot T$ is the desired angular twist rate w. (Note that when we defined $w = \dot{\tau} + \frac{d\theta}{ds}$ in equation [2], we assumed the existence of the Frenet frame - the definition given here allows us to weaken our earlier assumptions) (J.H. White, personal communication). The twist Tw is related by w by:

$$Tw = \frac{1}{2\pi} \int_0^L w \, ds \qquad [13]$$

For the relaxed molecule, we denote the angular twist rate by w_o. Then the torsional free energy is related to the deviation from w_o by

$$G_T = \frac{1}{2} C \int_0^L (\Delta w)^2 \, ds \qquad [14]$$

where $\Delta w = w - w_o$, and C is the torsional stiffness.

Combining [10], [11], [1], and [14], we obtain

$$\Delta G = \frac{BkT}{n} (\Delta Lk)^2 = \frac{1}{2} \int_0^L (AK^2 + C(\Delta w)^2) \, ds \qquad [15]$$

This equation relates the free energy of supercoiling to the purely geometric quantities K and Δw which govern the duplex conformation. Structural data for native SV40 DNA isolated from virions, in conjunction with [15], enable us to estimate C - thus our approach should be categorized as "semi-empirical".

We begin with the contribution from bending. The light-scattering experiments of Campbell and Eason 1975 indicate an asymmetric Y-shaped structure for native SV40 DNA in solution. We believe that these results from the light-scattering experiments are more pertinent than those obtained from X-ray diffraction or electron-microscopic studies. The

three-dimensional conformation in solution is what interests us, and is deducible from light-scattering; whereas X-ray diffraction requires the crystalline solid state of DNA, and the preparations used in electron microscopic studies subject the molecule to strong spreading forces which can easily distort the DNA (Campbell and Eason 1975).

The light-scattering data indicate that SV40 DNA consists of three interwound superhelical branches, having the same radii but lengths in the ratio 3:2:1. Hence if we neglect end effects, and assume a constant pitch angle for the superhelical winding, [12] may be rewritten

$$G_B = \frac{1}{2} AK^2 L \qquad [12a]$$

For an interwound superhelix of radius r, pitch angle α, $K = r^{-1} \cos^2 \alpha$ (Fuller 1971). Thus

$$G_B = \frac{AL}{2r^2} \cos^4 \alpha \qquad [12b]$$

If the superhelix has N turns ($\frac{N}{2}$ revolutions up, and $\frac{N}{2}$ down), then $\cos \alpha = \frac{2\pi rN}{L}$. Let the i^{th} superhelical branch have length L_i and N_i turns, where $i = 1,2,3$. If the bending energies of the branches may be treated independently, then [12b] becomes:

$$G_B = 8\pi^4 r^2 A \sum \frac{N_i^4}{L_i^3} \qquad [12c]$$

Assuming further that the superhelical turns are evenly distributed with respect to length, i.e. $\frac{N_i}{L_i} = \frac{N}{L}$, we find

$$G_B = \frac{8\pi^4 r^2 AN^4}{L^3} = \frac{2\pi^2}{L} AN^2 \cos^2 \alpha \qquad [12d]$$

Note that the explicit dependence of G_B on branching has disappeared.

For a right-handed interwound superhelix of N turns, pitch angle α, the writhing number $Wr = -N \sin\alpha$ (Fuller 1971 - note that the helix described by equation [4] is toroidal, not interwound.) Because the SV40 DNA is branched, one might expect its writhing number to differ from that of the corresponding non-branched superhelix. However, since the branches lie in the same plane (Campbell and Eason 1975) and thus do not generate a complex, e.g. pyramidal, structure, we expect that the approximation $Wr = -N \sin \alpha$ is reasonably close. From [12d], we have

$$G_B = \frac{2\pi^2}{L} A \cot^2\alpha \quad (Wr)^2 \qquad [12e]$$

The calculations leading from [12] to [12e] transform a general expression for G_B into one which incorporates the tertiary structural parameters α and Wr of the SV40 DNA. Hence direct observation can infuse our model with predictive power, and enable us to evaluate G_B.

We seek to transform [14] in an analogous way. To compute the torsional energy integral in [14], consider a representative molecule underwound by an amount ΔLk. Since we have fixed Wr by assuming a branched interwound superhelical structure, equation [9] implies that we have specified ΔTw. Then [13] yields a constraint on Δw:

$$\Delta Lk - Wr = \text{constant} = \frac{1}{2\pi} \int_0^{\ell} (\Delta w)\, ds \qquad [16]$$

Now we assume that SV40 DNA minimizes its twisting free energy G_T, subject to the side condition imposed by [16]. By variational methods, one can show that G_T is minimized when Δw is everywhere constant and equal to

$$\Delta w = \frac{2\pi}{L} (\Delta Lk - Wr) \qquad [17]$$

One can understand intuitively why a constant Δw minimizes G_T in [14]. Equation [16] essentially specifies the average value of Δw along the duplex; call that average value Δw^*. Now for all the times Δw exceeds Δw^*, there must be other times when Δw is less than Δw^* by counterbalancing amounts. However in G_T, which is of the form $\int(\Delta w)^2\, ds$, the surplus Δw will <u>not</u> be adequately counterbalanced by the deficient Δw, because they are both being squared. In other words, the twist energy integral is greater if Δw wobbles about Δw^* than if $\Delta w \equiv \Delta w^*$. It all boils down to the old rule: the mean square of something which deviates around an average is always greater than the square of the mean. (Feynmann 1964, Vol. II, Chapter 19 has an enlightening discussion of this point.) The upshot is that [14] may be written

$$G_T = \frac{2\pi^2 C}{L} (\Delta Lk - Wr)^2 \qquad [18]$$

Two further theoretical obstacles must be removed before we can estimate C. First, how is ΔLk, which is the quantity measured in gel electrophoresis experiments, partitioned between ΔTw and Wr? Is the deficit in linking number manifested as decreased duplex winding, or superhelical turns, or both? After preliminary results (Benham 1978, 1979) it appears the question has been answered definitively by the recent work of Vologodskii et. al. 1979. These workers utilized the computer simulation scheme known as the Monte-Carlo method, along with the available biochemical data, to deduce that about half of ΔLk is realized as "axial twisting", and the other half is realized as supercoiling.

Their procedure was as follows: from experimental data, they inferred a value for $Var(\Delta Lk^2)$, where the molecules being considered were the thermally fluctuating <u>nicked</u> DNAs of Depew and Wang 1975. Now the value of $Var(Wr^2)$ is fully determined by the bending stiffness of the

helix axis, which is a known quantity. Hence if $Var(Wr^2)$ could be theoretically calculated, then a value for $Var(\Delta Tw^2)$ would be implied - because in a nicked molecule the bending and twisting fluctuations are independent, so that $Var(\Delta Lk^2) = Var(Wr^2) + Var(\Delta Tw^2)$. Finally, the value of $Var(\Delta Tw^2)$ is connected with the value of the torsional stiffness in a simple way.

So their first step was to calculate $Var(Wr^2)$, given the bending stiffness A for the double helix. To do so, they developed an algorithm to generate "closed chains in space with a correct probability distribution". Another algorithm employed the method of Gauss integrals as well as directional writhing numbers (refer to "Further properties of Wr, Tw, and Lk") in order to calculate the writhing number of an arbitrary closed curve. Moreover, the authors point out that "when one randomly generates a closed chain it may prove to be knotted with some definite probability. To discard such knotted chains in the course of the Monte-Carlo calculations a reliable computer algorithm is needed to recognize them". Naturally they developed such an algorithm in their earlier work (Frank-Kamenetskii et. al. 1975) wherein knots are differentiated by their Alexander polynomials. Note that their assumption of the unknottedness of DNA extracted from cells is made on purely biological grounds.

Their results are

$$\begin{aligned} Wr &= .54\ \Delta Lk \\ \Delta Tw &= .46\ \Delta Lk \end{aligned} \qquad [19]$$

as inferred from graphs shown in their paper. We take this result to be the correct partitioning of ΔLk between ΔTw and Wr.

Our second obstacle is: what is the pitch angle of the SV40 DNA superhelix? Until definitive measurements are made, we must rely on the theoretical work of Camerini-Otero and Felsenfeld 1978. Because of the similarity between our assumptions and theirs, we cannot escape their result that the value of the pitch angle is restricted to the range 45° - 59°. (They attacked the energy-minimization problem with Lagrange multipliers, with a constraint on the minimum superhelical radius.) For the purposes of using [12e], we remark here that

$$.35 \leq \cot^2\alpha \leq 1 \qquad [20]$$

Having overcome the two obstacles, we can now write the basic formula which allows us to evaluate C. Using [10], [12e], [18] and [19], we find

$$\Delta G = \frac{BkT}{n} (\Delta Lk)^2 \qquad [20a]$$

$$= \frac{2\pi^2}{L} A\ (.54\ \Delta Lk)^2 \cot^2\alpha + \frac{2\pi^2}{L} C\ (.46\ \Delta Lk)^2$$

$$\text{Hence } \frac{(\Delta G)n}{(\Delta Lk)^2} = BkT \qquad [20b]$$

$$= 2\pi^2 \frac{n}{L} [A(.54)^2 \cot^2\alpha + (.46)^2 C]$$

Let $d = L/n$; it is just the separation between adjacent base pairs, known to be $d = 3.4 \times 10^{-8}$ cm. Therefore

$$\frac{(BkT)d}{2\pi^2} = (.54)^2 A \cot^2\alpha + (.46)^2 C \qquad [20c]$$

Since we have already delimited the possible values of α, we can evaluate C merely by evaluating A and B. Before doing so, let us pause to admire a nice feature of [20c]. The equation implies that C is independent of both the DNA length and its degree of supercoiling - these properties suggest a certain universality about C. (A,B, and α are thought to possess

similar generality; see Depew and Wang 1975, Camerini-Otero and Felsefeld 1978). So our model applies to all cd DNAs with branched interwound structures, and is not restricted to SV40 DNA.

The bending stiffness A is related to the DNA persistence length P by the formula $A = PkT$, where T is the absolute temperature and k is Boltzmann's constant (Barkley and Zimm 1979). The persistence length "measures the distance the DNA persists along a given direction before changing its course; to a good approximation, we may envision a long DNA molecule as a random-flights chain with Kuhn segments of twice the persistence length" (Barkley and Zimm 1979). Measurements place the value of the persistence length P at $T = 293^{\circ}K$ to be 660 ± 60 A° (Jolly and Eisenberg 1976), which implies a value of the bending stiffness: $A = 2.67 \times 10^{-19}$ erg-cm, with an uncertainty of about 10%.

The free energy proportionality constant B is 1100 ± 100 for most DNAs, at temperatures between $0^{\circ}C$ and $26^{\circ}C$ (Depew and Wang 1975). We should note that Depew and Wang originally evaluated B for values of ΔLk between 0 and 15, by fitting [10] to the data. We are soon going to extrapolate to our branched interwound structure, for which ΔLk is between -25 and -30. Such an extrapolation is questionable, and possibly not valid; yet throughout this analysis we must make do with the little experimental information available, and so we make calculational leaps, although reluctantly. Table 1 summarizes the data.

Table 1. DNA Parameters

Symbol	Interpretation	Value	Reference
P	persistence length	660 ± 60 A°	(1)
A	bending stiffness	2.67×10^{-19} erg-cm	(2)
B	Free energy proportionality constant for ΔG in [10]	1100 ± 100	(3)
α	superhelical pitch angle	52 ± 7°	(4)
d	base pair separation	3.4 A°	(5)

Key to references in Table 1

1. Jolly and Eisenberg 1976
2. Barkley and Zimm 1979
3. Depew and Wang 1975
4. Camerini-Otero and Felsenfeld 1978
5. Watson 1976

Finally we can calculate C. The relation $A = PkT$ yields

$$C = (.46^{-2})\left[\frac{Bd}{2\pi^2} - (.54)^2 P \cot^2\alpha\right] \quad kT \qquad [21a]$$

The typical values $T = 293^{\circ}K$, $\alpha = 52^{\circ}$, $B = 1100$, and $P = 6.6 \times 10^{-6}$cm imply

$$C = 1.41 \times 10^{-19} \text{ erg-cm} \qquad [21b]$$

At this juncture, something unfortunate happens. We would like to estimate the upper and lower bounds on C which are compatible with Table 1. Though the upper limit is plausible:

$$C \leq 2.79 \times 10^{-19} \text{ erg-cm} \qquad [21c]$$

The lower limit is, sadly, negative! (Of course, C must be non-negative by its definition.) Two explanations spring to mind: (i) This absurd result is symptomatic of fundamental problems in our elastic model or (ii) the "absurdity" is common to all equations like [21a], wherein one computes the difference between two very uncertain numbers which are equal, within their uncertainty. The first term in brackets is $\pm 10\%$ and the second is $\pm 60\%$ (mainly due to $\cot^2\alpha$); it just happens that the upper limit of the second overlaps the lower limit of the first. Experimenters could rectify matters by reliably measuring α - we are aware of but one attempt (Brady et. al. 1976) and controversy enshrouds its interpretation (Subirana and Puigjaner 1977; Campbell 1977). For now, we unwillingly depend on the theoretical estimates of Camerini-Otero and Felsenfeld 1978. Nevertheless our crude estimate [21b] agrees with those of previous authors; of course, the agreement may be fortuitous. A reliable, precise estimate of α will help us to judge the model proposed here.

Discussion of the Elastic Model and the Torsional Stiffness

Our most important finding is that the bending and torsional stiffnesses of DNA are of the same order of magnitude. Table 1 and equation [21b] permit the estimate

$$\frac{A}{C} \approx 1.8 \qquad [22]$$

This result supports our earlier speculation that C cannot be much less than A. Recall that anthropocentric argument: we owe our existence ultimately to those primitive organisms who managed to compact their DNA by unwinding and thus supercoiling it. Were DNA not torsionally rigid, such a resource would have been unavailable - a flabbier molecule would have docilely resigned itself to helical untwisting rather than writhing in protest.

One clarification is in order. All along, we have considered that type of supercoiling which arises solely in reaction to helical unwinding. Such supercoiling occurs in procaryotic organisms, and it represents Nature's most primitive solution to the packaging problem. In higher organisms, i.e. eucaryotes, an especially stunning improvement evolved: supercoiling may be generated by associations of proteins or other structural elements of the cell with DNA (Champoux 1978). Usually the DNA is wrapped on spools of protein. Such higher-order organization can be formed in linear or circular DNA and is not dependent on the strands being intact. Packaging capabilities increased by several orders of magnitude as a result of this evolutionary breakthrough. We will later discuss this subject at length; for now we return to the basic form of supercoiling adopted by circular procaryotic DNA.

Our elastic model enables us, for the first time, to calculate C straightforwardly, from the supercoiling data alone. In the past, this

important parameter has been difficult to estimate because the torsional stiffness of the double helix manifests itself only indirectly. The free ends of linear DNA allow relief of torsional stresses; thus the static geometry of such molecules tells us nothing about C. However, the torsional stiffness stamps its signature on cd DNA, where the topology couples bending and twisting. Guided by this principle, Vologodskii et. al. 1979 undertook their computer simulation studies in order to evaluate C. They report a temperature-dependent value equivalent to

$$C = (5.54 \pm 1.11) \times 10^{-22}\ T \quad \text{erg-cm} \qquad [23a]$$

$$\text{At } 293^{\circ}K = T \quad C = (1.62 \pm .32) \times 10^{-19} \quad \text{erg-cm} \qquad [23b]$$

Hence our results [21a,b], though obtained by radically different methods, overlap with theirs within the uncertainty.

At this point, we venture into the realm of subjectivity. We feel that our results extend and elucidate the earlier computer simulation findings. The simple physical model presented here is more perspicuous; one has a better "feel" for <u>why</u> the results are true. Unlike a computer's mysterious internal computations, our formulae are easily inspected and comprehended.

The torsional stiffness varies linearly with temperature according to [21a]. This result has been derived here subject to the assumptions that (i) the temperatures considered are near those for which the Depew-Wang data apply, i.e. 0°-26°c and (ii) the DNA persistence length is essentially constant over that range. Note that Vologodskii et. al. 1979 also obtained this result. But intuitively we'd expect some sort of inverse relationship between C and T; as T increases, the double helix should soften, not stiffen. This quandary disturbs us. A probable explanation involves the assumed relation between the persistence length

P and the bending stiffness A: A = PkT. Both A and P are likely to be decreasing functions of T - as the DNA heats up, it bends more easily and its effective straight segments shorten. Indeed, one study suggests that P varies inversely with T (Gray and Hearst 1968) over the range 5°-50°C; but therefore A is independent of T over that range. So even with this "correction", C is an increasing affine function of T, a physically unrealistic result. Unfortunately the authors offer only five data points as evidence. Taking their results at face value, we find that in the temperature range of interest to us, our assumption of constant P incurs an error of no more than 5%.

What, then, is the status of our equation [21a]? It is not a general law; it merely summarizes the approximate values of C predicted by the elastic model. As we consider temperatures close to 20°C, its precision increases. Only when experimentation determines the correct temperature variation of A and P can we hope to replace [21a] with a more satisfactory functional form.

The main sources of error in our results are (i) a reported uncertainty of 10% in the value of B for SV40 DNA. Note that B is called NK in Depew and Wang 1975. Also, our extrapolation of [10] to ΔLk between -25 and -30 is not strictly justified, since Depew and Wang derived their values of B for positively supercoiled molecules with ΔLk between 0 and 15 (as estimated from the photograph in their paper). We have already apologized for this unavoidable extrapolation. (ii) The superhelical pitch angle α hasn't been measured in the laboratory. We chose a value whose plausibility depends on the energy-minimization

conditions derived in Camerini-Otero and Felsenfeld 1978. (iii) Our assumptions of evenly distributed superhelical turns, energy independence of the interwound branches, and negligibility of entropic, electrostatic, and excluded-volume effects, may all require revision. (iv) We have nowhere considered the consequences of local denaturation. Benham 1979 presents a pretty analysis of this subject; we will discuss his work in connection with the functional role of supercoiling. For now, we leave the elastic model and begin a new topic: determining C from observable properties of linear DNA. Happily, this independent approach will corroborate our results.

As mentioned previously, the torsional stiffness does not affect the static geometry of linear DNA; yet it governs the molecule's dynamic geometry. On the nanosecond time-scale, DNA conformation fluctuates dramatically, due to torsional Brownian motion. Thermal agitation causes transient duplex twisting, i.e. the relative rotation of adjacent base pairs about the duplex axis. The magnitude of these interesting fluctuations depends entirely on the value of C.

Miraculously, physical biochemists can monitor such tiny and rapid fluctuations by the use of fluorescence spectroscopy. Before examining the values of C derived from studies of DNA torsional Brownian motion, let us pause to discuss this important and very beautiful experimental technique. (Refer to Freifelder 1976 for more details.)

When certain molecules called chromophores absorb quanta of light, they very soon after emit radiation of longer wavelength, i.e. lower energy. This emission is called fluorescence. The physical and chemical environment of a chromophore drastically affects its fluorescence

spectrum - thus a chromophore's sensitivity to its surroundings makes it an ideal chemical probe. Biochemists cherish a particular chromophore known as ethidium bromide (EtBr) because of its convenient intercalation abilities - EtBr wedges itself snugly between adjacent stacked bases and clings to the backbone of the double helix. Now we can actually watch the duplex twisting because a light-emitting chromophore is riding along with it.

More precisely, consider the following experiment. We intercalate EtBr between the bases of a sample of DNA in solution. Then we send a nanosecond pulse of monochromatic polarized light through the sample; choose a wavelength which is efficiently absorbed by the chromophore. The exciting light pulse induces fluorescence. We place a detector at right angles to the incident beam to ensure that we are recording only the emitted light. Even though the exciting light is polarized, the fluorescence will be only partially polarized or completely unpolarized; because of the rapid twisting fluctuations of the duplex (and hence the attached EtBr), the chromophore's orientation changes substantially during the lifetime of the excited state. In this way, the depolarization of fluorescence illuminates DNA's Brownian motion at the nanosecond time scale. We measure this phenomenon quantitatively in the following way: define $I_p(I_n)$ to be the intensity of the emitted light which is polarized in a plane parallel (normal) to the polarization plane of the incident light. Due to the geometry and photochemistry of the chromophore, there is an initial difference between these intensities. Then the emission anisotropy, $R(t)$, is given by

$$R(t) = \frac{I_n(t) - I_p(t)}{I_n(t) + 2I_p(t)}$$

and is measured as a function of time. In practice, R(t) is observed to decay exponentially; the decay rate is related to DNA's rotational diffusion coefficient. Since DNA can twist along its axis or tumble end-over-end, we'd normally observe a decay curve consisting of two connected branches. In our case, the nanosecond excitation pulse serves to single out the decay due to torsion, which is expected to occur much more rapidly than end-over-end rotation. Thus the fluorescence depolarization anisotropy (FDA) decay curve reflects the DNA torsional stiffness; qualitatively, the stiffer the helix, the slower the decay.

Three teams of workers have recently constructed models of DNA oscillatory fluctuations. By fitting a theoretical FDA decay curve to the empirical data of Wahl et. al. 1970, these groups inferred values of the DNA torsional stiffness. In the most recent analysis, Allison and Schurr 1979 model DNA as a chain composed of stiff rods connected by "localized torsion joints". They found two hypothetical rod lengths to be compatible with the data (86 base pairs and 1 base pair) and two corresponding values of C:

$$C_1 = 2.92 \times 10^{-19} \text{ erg-cm}$$
$$C_2 = 1.77 \times 10^{-19} \text{ erg-cm} \qquad [24]$$

Our result [21 b,c] encompasses only the lower C_2 value. Note that only C_2 agrees with the computer simulation result [23b] reported by Vologodskii et. al. 1979.

Le Bret 1978 found an especially low value equivalent to

$$C = (.94 \pm .24) \times 10^{-19} \text{ erg-cm} \qquad [25]$$

In accordance with this result, he writes "The energy that must be given to bend the DNA is concluded to be much larger than the energy which must be given to twist it". We have already argued that, for the general case, such a result is unreasonable, because it implies that an underwound duplex will exhibit only decreased helical winding in lieu of supercoiling. But Le Bret derives his relationship under the explicit assumption that "the number of tertiary turns of the supercoiled DNA is small". So he may be correct in some sense; at first the duplex may accept unwinding more readily than bending, though continued torsion will provoke a writhing reaction. Still we are suspicious of his low value for C. Several authors have criticized his work on methodological accounts (Barkley and Zimm 1979; Allison and Schurr 1979; Vologodskii et. al. 1979).

The contribution of Barkley and Zimm 1979 represents the most mathematically sophisticated treatment of DNA torsional Brownian motion. By fitting their theory to the FDA data, they determined an optimum value of

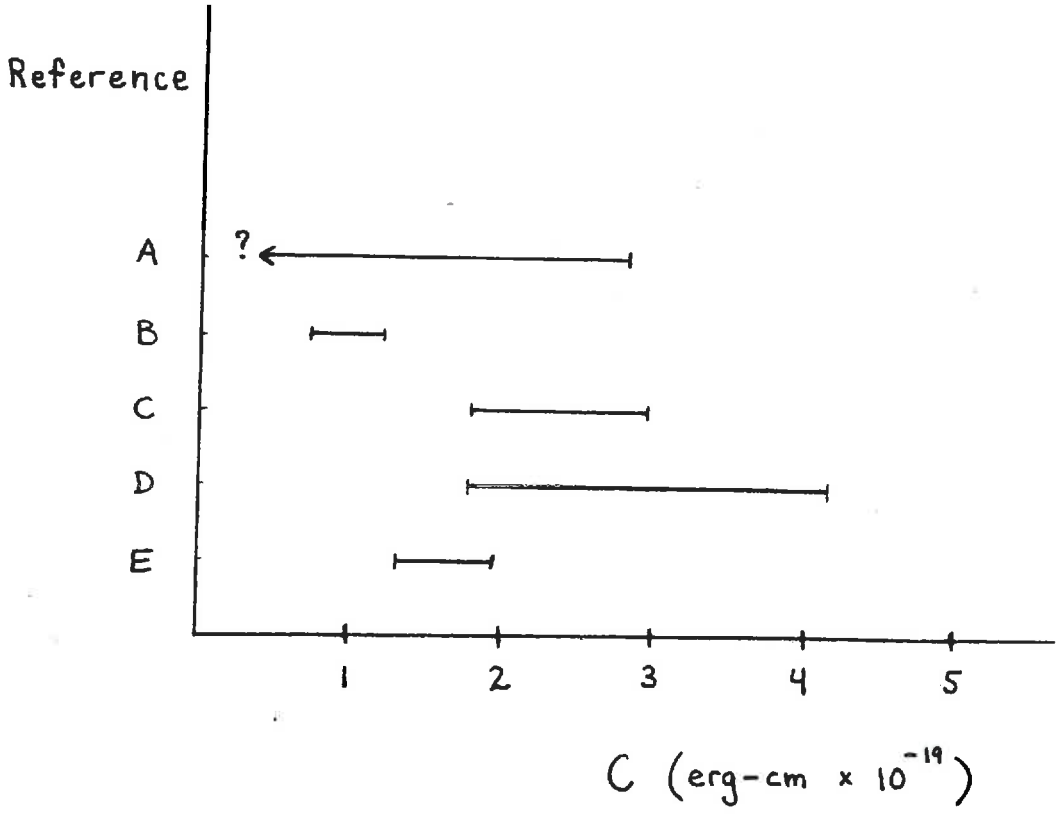
$$C = 4.13 \times 10^{-19} \text{ erg-cm} \qquad [26]$$

A second estimate, $C = 1.75 \times 10^{-19}$ erg-cm, based on the Poisson ratio and bending stiffness of DNA, was also judged to give an acceptable fit. But their third estimate is of most interest to us because it is, as far as we know, the only place in all the literature where C is computed from an application of elasticity theory to the supercoiling data. Before we discuss this computation in detail, let us tabulate the various proposed values of C. (For ease of comparison, all values are reported in erg-cm $\times 10^{-19}$ for an assumed temperature of $T = 293^{\circ}K$). See Table 2, and the accompanying graph.

Table 2. Proposed Values of the DNA Torsion Stiffness C (erg-cm $\times 10^{-19}$)

Reference	Smallest possible value	Largest possible value
A. Present work	(see text)	2.79
B. Le Bret 1978	0.70	1.18
C. Allison & Schurr 1979	1.77	2.92
D. Barkley & Zimm 1979	1.75	4.13
E. Vologodskii et. al. 1979	1.30	1.94

Graph of Table 2



To check their optimum value of C inferred from the FDA data, Barkley and Zimm performed a wholly independent estimate based on the supercoiling data of Depew and Wang 1975, and Pulleybank et. al. 1975. Here is the calculation, taken verbatim from their article:

A third value of C comes from measurements of the properties of supercoiled closed circular DNA.[26,27] If one assumes that all the free energy of the extra supercoiling comes from torsion and none from bending, then we can relate the observed free energy of supercoiling to the torsional rigidity. (For discussion of this point see Benham,[28] Camerini-Otero and Felsenfeld,[29] and LeBret.[30]) The torsional elastic free energy, U, of a twist ϕ in a length l is

$$U=(C/2)\phi^2/l\,. \qquad \text{(V.4)}$$

Pulleyblank *et al.* report their data in terms of the free energy G per molecule with n phosphorus atoms and τ supertwists. To convert G to U, we assume a length l of 3.4×10^{-8} cm per two phosphorus atoms (the B structure of DNA) and a twist in this length of $2\pi\tau/(n/2)$; we also assume $T=293\,^{\circ}$K. The range of values reported by Depew and Wang[26] and by Pulleyblank *et al.*[27] for the quantity $Gn/k_BT\tau^2$ range from 1840 to 3120, giving a corresponding range of C from 0.64×10^{-19} to 1.086×10^{-19} erg cm. Since we have ignored bending energy, these numbers are presumably lower limits. We therefore take the higher value, 1.086×10^{-19}, as our best estimate of C from supercoiling data.

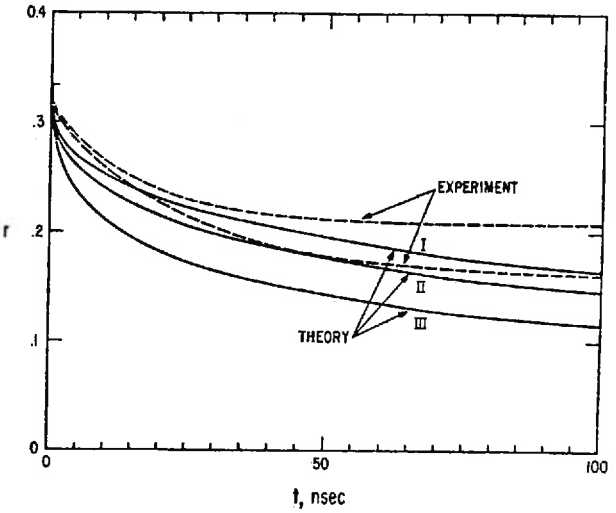


FIG. 2. Emission anisotropy, r, as a function of time after excitation, t. The three theoretical curves correspond to the three cases discussed in the text, with the following parameter values: I, the radius $b=12.5$ Å and the torsional rigidity $C=4.125\times10^{-19}$ erg cm; II, $b=15$ Å and $C=1.753\times10^{-19}$ erg cm; III, $b=12.5$ Å and $C=1.086\times10^{-19}$ erg cm. The two experimental curves correspond to the two equations that Wahl and co-workers find can be used to fit their data, Eqs. (V.11a) and (V.11b).

They later comment that this estimate fails to give "acceptable agreement with the observed anisotropy... though admittedly only a lower limit compatible with the supercoiling data, (it) is surprisingly far from the mark". (FDA data shown above.)

We too were surprised by this discrepancy - it must be possible to reconcile the supercoiling and FDA data. In fact, this very problem originally motivated us to develop the elastic model proposed earlier. In our opinion, the analysis of Barkley and Zimm contains three soft spots (two of which amusingly cancel the third to some extent.) We now examine those spots in order of increasing softness.

Is it valid to assume that "all the free energy of extra supercoiling comes from torsion and none from bending"? Note that the authors cite Le Bret who, as we have seen, made a similar assumption and (coincidentally, as it turns out) obtained a similarly low value of C. However, based on calculations made with our model, the torsional energy is typically 3-5 times greater than the bending energy at $\Delta Lk = -25$; the bending energy is even less significant at smaller values of $|\Delta Lk|$. Thus this assumption is OK, as long as the linking deficit is small. (We retained a bending term in our calculations because we were dealing with values of ΔLk between -25 and -30.)

So the neglect of bending energy is not a soft spot in itself, but it provokes the mistaken claim that "since we have ignored bending energy, these numbers (values of C) are presumably lower limits". In fact, the neglect of the bending energy causes the torsion energy, and hence C, to be overestimated, not underestimated. If the rest of their argument were valid, they would have obtained an upper limit on C. To see this, let C^* be the value of the torsional stiffness obtained by neglecting the bending energy G_B, and let C be the "correct" value. Remember always that Barkley and Zimm are computing C^* as an approximation to C. Then, using the notation in the passage cited, we have

$$G = C^*\phi^2 = C\phi^2 + G_B \qquad [27]$$

where we have neglected unimportant constants. Since $G_B > 0$,

$$C^* > C \qquad [28]$$

In other words, the value of the torsional stiffness calculated by neglecting bending is an upper bound on the actual value.

Now for the second flaw. Because they believed they had obtained lower limits on C, Barkley and Zimm felt free to choose the largest of their lower limits, i.e. they selected the highest value of C^* they could find. (See the last two sentences of the cited passage.) This C^* was derived from the value of G reported by Depew and Wang 1975 for the tiny E. Coli 15 plasmid DNA. Inspection of the Depew-Wang data reveals that this DNA has an exceptionally high G - when Barkley and Zimm cite a range of 1840 - 3120 for $Gn/kT\tau^2$, they should mention that all other DNAs inhabit the range 1840-2440 whereas this plasmid DNA gives values of 2600-3120. Such DNA is far from typical, and selection of its G is unwarranted. Most importantly, since Barkley and Zimm had in fact computed an upper limit for C, this effort to find the largest imaginable C^* will backfire and worsen their overestimate!

But with all this overestimation, why is the ultimate value of C^* so small? Referring to Table 2 and its graphical representation, we see that C^*, which ranges from 0.64×10^{-19} erg-cm to 1.09×10^{-19} erg-cm, is compatible only with Le Bret's anomalous values. See also the case III curve in the diagram from Barkley and Zimm 1979 reproduced earlier, next to the cited passage.

The low value of C^* is due to the third and gravest error of Barkley and Zimm. They assume that a DNA with "τ supertwists" and n/2 base pairs has a "twist" of $2\pi\tau/(n/2)$ in the length between adjacent base pairs. Then there is a "twist" of $2\pi\tau$ along the entire molecule:

$$\text{"twist"} = 2\pi\tau \qquad [29]$$

By "twist" they mean something like "the total number of radians swept out in the course of duplex twisting". We previously used the symbol $\int_0^L (\Delta w)ds$ for this quantity. Another equivalent expression is

$$\text{"twist"} = 2\pi(\Delta Tw) \qquad [30]$$

(Refer to discussions preceding [13] and [2] for a refresher on the definitions.) Now by "τ supertwists "they must mean that

$$\Delta Lk = \tau \qquad [31]$$

since ΔLk is the quantity measured in gel electrophoresis as reported by Pulleybank et. al. 1975. Combining [29], [30], and [31], we see that their assumptions translate into the assumption that $\Delta Lk = Tw$, i.e. all the linking deficit ΔLk is partitioned to twist and none to writhing! In particular, $Wr = 0$.

Such an assumption must lead to erroneous results. If the topoisomers had not differed in writhing number, they would not have been separated into discrete bands on the gel. Moreover, nicked DNA, for which Wr is near zero, migrates far slower than the cd DNA. Supercoiling is observed for cd DNA; $Wr = 0$ is not a good assumption.

From the recent computer simulation studies of Vologodskii et. al. 1979, about half of ΔLk is partitioned to Wr, and half to ΔTw - see equation [19]. With this result, which was chronologically unavailable to Barkley and Zimm, we can understand and resolve their underestimate of C^*. Rewrite [27], again neglecting unimportant constants, as

$$G = C^*(\Delta Tw)^2 \qquad [32]$$

where G is the measured free energy of supercoiling and C^* is the torsional stiffness computed by neglecting the contribution from bending. If we make the correct substitution $Tw = (\Delta Lk)/2$, we obtain $C^* = 4G/(\Delta Lk)^2$;

whereas the faulty substitution of Barkley and Zimm, $\Delta Tw = \Delta Lk$, would yield $C^* = G/(\Delta Lk)^2$. Hence, their computed values should be very roughly too small by a factor of 4. (By overestimating ΔTw by a factor of 2, they underestimate C^* by a factor of 4.) Following this prescription we correct their results more precisely by using [19] and the factor $(.46)^{-2} = 4.73$. Observe that

$$C_1^* = (4.10 \pm 1.07) \times 10^{-19} \text{ erg-cm} \qquad [33]$$

after our first corrective therapy. This number appears high, as expected because of the two overestimates inherent in it. When we account for, and then disregard, the unusually high values of G for E. Coli 15 plasmid DNA, the supercoiling data imply:

$$C_2^* = (3.53 \pm .50) \times 10^{-19} \text{ erg-cm}$$

after our second correction. Finally, because of the arguments preceding equation [28], we expect that the true value of the torsional stiffness is somewhat smaller than C_2^*. Table 2 supports this conclusion.

We have shown that the supercoiling data and the FDA data are fully compatible. If we have seemed savage in our treatment of Barkley and Zimm 1979, it should be noted that our reconciliation of the two sets of data speaks favorably for, and lends credence to, their theory of DNA conformational fluctuations.

Summary

We have developed an elastic model of supercoiled cd DNA in order to evaluate the torsional stiffness of the double helix. Our model explicitly incorporates tertiary structural parameters of DNA, as well as the free energy of supercoiling. The torsional stiffness is found to

be roughly equal to the bending stiffness. This result substantiates our original guess that supercoiled DNA in procaryotes reflects a torsionally sturdy duplex writhing in response to enzymatic twisting. Thus, to package their DNA, the Earth's first organisms needed only to unwind it.

Our numerical estimates for the torsional stiffness agree with those previously obtained from fluorescence studies of linear DNA. The compatibility of the supercoiling and fluorescence data is demonstrated here for the first time; in particular, we have resolved the discrepancy noted by Barkley and Zimm 1979.

Applications of the Mathematics of Supercoiling

II. Chromatin Structure and Linking Numbers

Introduction

The supercoiling of procaryotic DNA accounts for only some of the molecular compactification necessary for packaging. The remaining condensation is now believed to be accomplished by the folding of DNA around small basic proteins (Griffith 1976). These proteins are probably not tightly bound to the DNA - only gentle extraction procedures permit their detection.

However, in the eucaryotic cell, the DNA is very firmly complexed with proteins called histones. Like their evolutionary ancestors in procaryotes, these proteins contain a high proportion of basic amino acids; thus they can neutralize the charged, acidic phosphate groups of DNA (which destabilize the double helix by intramolecular electrostatic repulsions). Through these energetically favorable interactions, histone proteins bolster the DNA in its duplex state and facilitate the bending of the double helix. They are "certainly fundamental to the structure of chromosomes" (Kornberg 1980, whose explanations we are following closely).

Five kinds of histone (H1, H2A, H2B, H3, and H4) are almost always present in the eucaryotic chromosomes. It is astonishing that the amino acid sequence of H4 is identical in the cow, pig, and rat, and differs from that of pea seedlings in only two sites. H3 is also strikingly similar in diverse species. Kornberg 1980 concludes

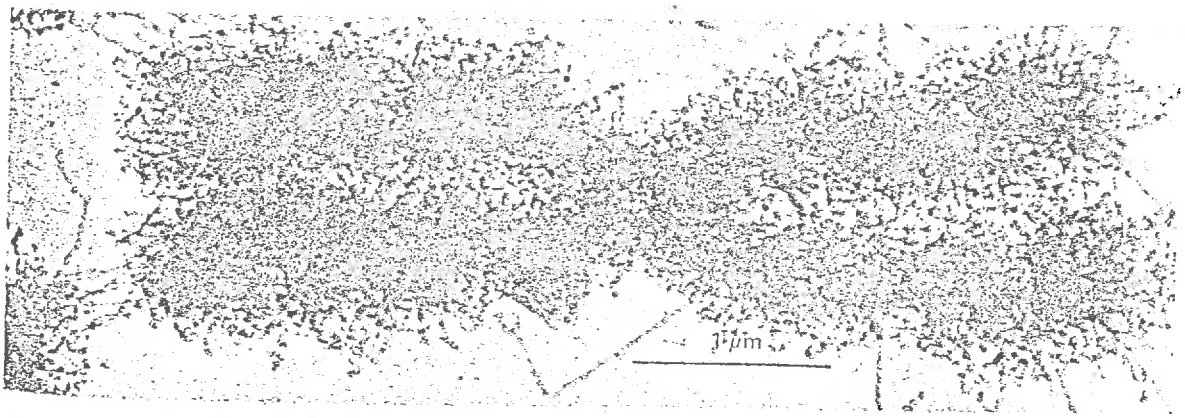
> "This unusual conservation of sequence through widely diverging lines of evolution must have arisen from a structural requirement for multiple precise contacts of H3 and H4 with DNA and with the other histones and proteins involved in replication and transcription. Comparable evolutionary stability is found in the amino acid sequences of the structural protein actin in unicellular amoeba and in muscles of trout and rabbit."

Histones are responsible for the first level in a stepwise compaction mechanism (Chambon 1978) which leads to the packaging of DNA at high concentration in the interphase nucleus or the metaphase chromosome (the extremely condensed form of the chromosome visible at the time of cell division - see photo on next page).

At other times in the life cycle, the genetic material exists in a diffuse, thread-like form called chromatin. Recent studies of chromatin structure have established that eucaryotic DNA is organized into repeating nucleoprotein subunits called nucleosomes (Felsenfeld 1978). A nucleosome consists of a protein core of eight histones (two each of H2A, H2B, H3 and H4) around which about 200 base pairs of DNA are wrapped. One may visualize the chromatin "thin fiber" as a string of DNA wound around nucleosome beads.

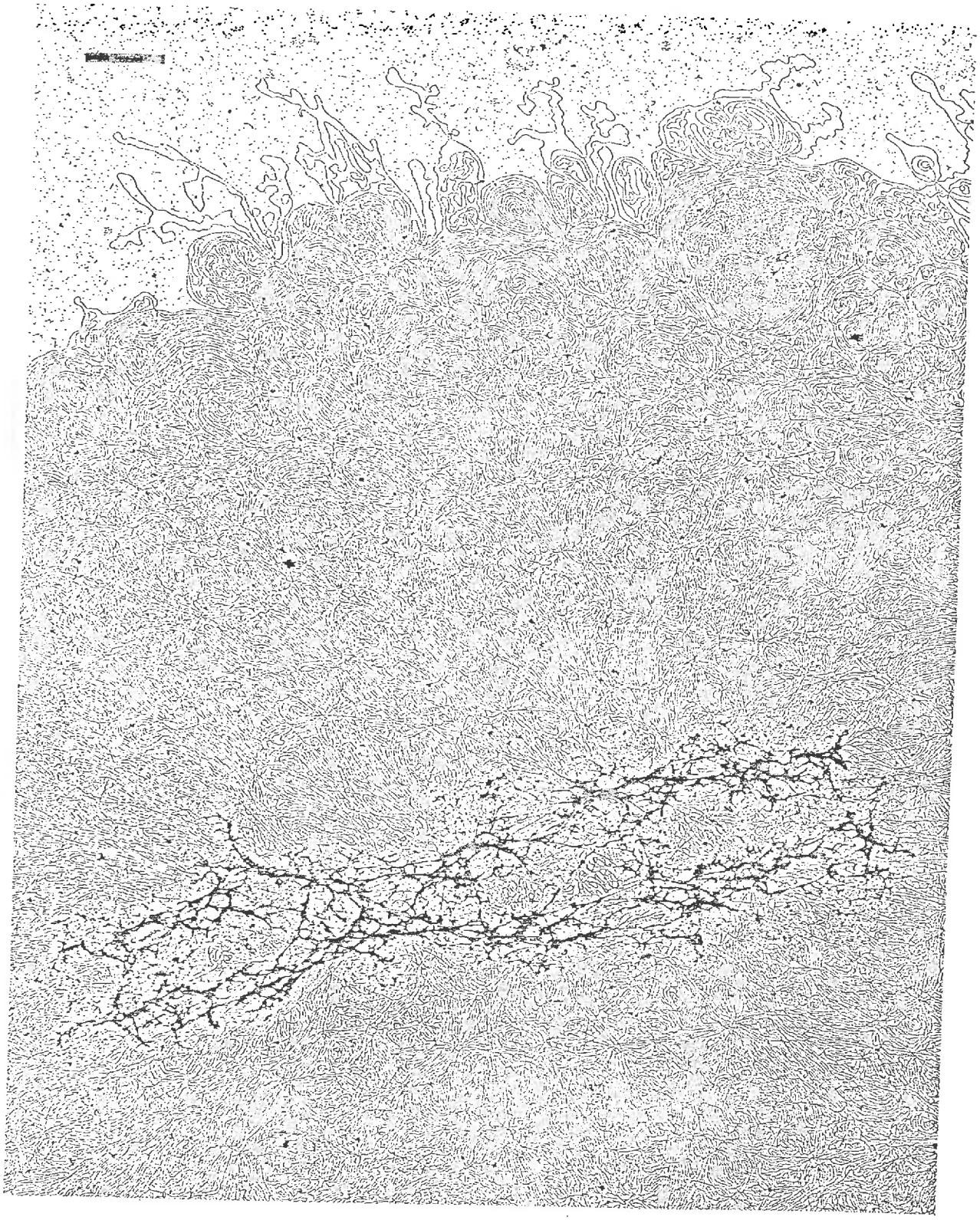
The 200 base pair repeat length is variable, depending upon the organism and tissue from which the nucleosomes are extracted. However, if the nucleosomal DNA is subjected to sustained cleavage by enzymes, a nucleosome core particle is obtained which contains an invariant amount of DNA (140 base pairs) in all tissues and species, regardless of the original amount of DNA in the nucleosome (Chambon 1978). The DNA of variable length which connects the nucleosome cores is termed the linker.

A Human Chromosome. From a dividing lymphocyte. Note the complex folding of the chromatin fiber. [Courtesy of G. F. Bahr, Reprinted from *Fed. Proc.*, **34**: 2209 (1975).]



Before we discuss the nucleosome in more detail, return to the question of packaging. The packing ratio of DNA in nucleosomes is between 5 and 7 (Chambon 1978) whereas the overall ratio in metaphase chromosomes is about 8000 (Laemmli et. al. 1978). Higher-order organization may be achieved by coiling the 110 A° string of nucleosomes into a solenoidal structure of 300 A° diameter - this solenoid further contracts the DNA duplex by a factor of about 6 (Bak and Zeuthen 1978). The 300 A° nucleohistone "thick fiber" is probably the elementary strand of metaphase chromosomes, though we see that it accounts only for a length reduction factor of about 40. U.K. Laemmli, a biochemist here at Princeton, has shown that the thick fiber is itself folded into loops and attached to a scaffold of non-histone proteins (Laemmli et. al. 1978). His remarkable electron micrograph (next page) shows the enormous length of the single continuous duplex of DNA in a chromosome (not all the DNA is shown!) Also compare the morphology of the intact chromosome with condensed DNA, shown earlier.

All these higher-order structures are dependent on, and perhaps dictated by, the structure of the nucleosome core particle. Though its internal architecture is not yet understood, biochemical and physical studies have provided clues about both the shape of the core and the path followed by the DNA as it winds around the nucleosome. In what follows, we focus on the latter problem.



The Path of Nucleosomal DNA

The biochemical experiments of Germond et. al. 1975 elegantly demonstrate the role played by nucleosomes in the supercoiling of eucaryotic DNA. After infection of cultured cells with the mammalian virus SV40, the circular DNA of this viral agent can be isolated from the cell nuclei in the form of DNA - protein complexes. These complexes show the typical beaded-string structure which is characteristic of eucaryotic chromatin; therefore, within limits, the SV40 "minichromosomes" may be regarded as a model of cellular chromatin (Keller et. al. 1978).

The SV40 minichromosome contains an average of 24 nucleosomes (Keller et. al. 1978); when the histones are removed, the DNA is found to be underwound by an amount $\Delta Lk = -25 \pm 1$ (Keller 1975; Shure and Vinograd 1976). This correspondence suggests that each additional nucleosome contributes -1 to ΔLk.

The in vitro reconstitution experiments described next support this conclusion. When relaxed, protein-free, closed duplex SV40 DNA is allowed to associate with purified histones, one observes the formation of a highly twisted "beaded-string". Treatment with a nicking-closing enzyme generates a relaxed minichromosome. Then when the nucleosome beads are removed, the isolated DNA exhibits negative supercoiling. As before, there is a one-to-one ratio between ΔLk and the number of beads.

To interpret these results, note that the original relaxed cd DNA has $\Delta Lk = 0$. When a relaxed circle wraps around the purified core particles, thus imparting some supercoiling to the DNA, the linker DNA must become supercoiled or twisted in the opposite sense because of the topological constraint $\Delta Lk = 0$. This accounts for the observed highly twisted

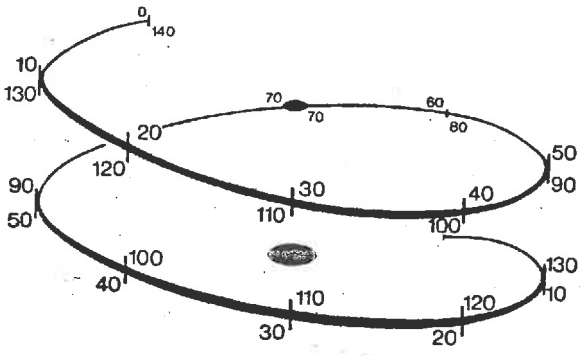
beaded-string. Nicking-closing enzymes relieve the topological constraint by cutting one of the DNA strands - they thus relax the torsionally stressed linker region. Now because the compensatory supercoiling of the linker has disappeared, the removal of the nucleosomes exposes a cd DNA with $\Delta Lk \neq 0$. The observed negative supercoiling ($\Delta Lk < 0$) has been interpreted as evidence for left-handed winding of the DNA on each nucleosome. Germond et. al. 1975 concluded,

> "The above results demonstrate that in vitro the formation of each nucleosome on a negatively supercoiled DNA molecule reduces the superhelicity of the complex by one turn... This could be due to one negative superhelical turn or to an overall unwinding of the double helix by one turn. Alternatively, a combination of intranucleosomal unwinding and supercoiling could cause the torsional alteration."

Two years later, Finch et. al. 1977 published an extremely thorough physical study of the nucleosome core particle. Their X-ray crystallographic data (resolution < 25 A°) show that the core is a flat disk about 110 A° in diameter and 57 A° in height. These results corroborated earlier neutron-scattering studies (see Felsenfeld 1978 for references) of the nucleosomal dimensions; all the studies unambiguously placed the DNA on the surface of the core particle. If the 140 base pair length of DNA is wrapped in a uniform superhelix around the core, the superhelix must have a pitch of about 28 A°, 80 base pairs per superhelical turn, and thus 1.75 superhelical turns per nucleosome.

Such a picture is further substantiated by information obtained from enzymatic cleavage patterns of the nucleosomal DNA. The distribution of cuts made by the enzymes DNase I and II shows a striking regularity: the lengths of the DNA fragments are quantized in integral multiples of about 10 base pairs. Moreover, sites separated by 80 base pairs are

equally accessible to DNase. The 10 base pair periodicity probably reflects the duplex screw - if, as according to Watson and Crick, the double helix makes one turn every 10 base pairs, then the strands will protrude most from the nucleosome surface, thus becoming accessible to enzymes, with the same periodicity. Moreover, the equal accessibility of sites separated by 80 base pairs may be rationalized by the model of Finch et. al. 1977; since this model postulates 80 base pairs per superhelical turn, the local environment, and hence enzyme sensitivity, would be approximately the same for two sites separated by this length (Felsenfeld 1978). See the diagram below, from Finch et. al. 1977.



The pioneering work of Germond et. al. 1975 and Finch et. al. 1977 elucidated the path taken by DNA as it winds around the nucleosome. However, a "discrepancy" between their results has disturbed the biochemical community from 1977 to the present. Finch et. al. propose -1.75 turns of DNA per nucleosome, but Germond et. al. (and numerous subsequent workers) find that each nucleosome accounts for a change of -1 in ΔLk. Finch et. al. write

> The change in linkage number is the same as the number of superhelical turns only if the screw of the DNA double helix does not change in the local coordinate frame defined by the super-helix path... We propose 1 3/4 superhelical turns corresponding to 80 base pairs per turn. The only way to reconcile these results with the linkage number change of -1 1/4 is to conclude that the helical screw of the DNA double helix does indeed change on binding DNA free in solution on to the nucleosome.

For now let us suppose these claims to be correct - later we will have a few words to say with regard to the first sentence cited above. Note that the authors use a "linkage" change of -1 1/4 instead of -1 because at that time, the number of nucleosomes in a minichromosome was underestimated - some beads were never counted because they were lost during a harsh extraction procedure. (Keller 1978)

Concerning the question of the changing helical screw, Crick wrote (Crick 1978)

> It is clear that a reliable and accurate experimental value of the number of base pairs per turn of DNA in solution is urgently required.

In 1979, the brilliant experimentalist J.C. Wang (whom we discussed earlier in connection with the free energy of supercoiling) showed by novel DNA splicing techniques that the double helix in solution has 10.4 $\pm$ 0.1 base pairs per turn (Wang 1979). In the same year, the DNase cutting pattern of nucleosomal DNA was found to exhibit a fragment and site periodicity of 10.4 base pairs (Lutter 1979; Prunell et. al. 1979) rather than 10.0 as thought previously (Noll 1974). Assuming that this periodicity reflects the duplex screw, one must conclude that the helical screw of DNA does not change as it is bound onto nucleosomes.

This recent result seems to invalidate the original claim of Finch et. al. 1977, in which they maintained that an altered screw is "the only way to reconcile these results (-1.75 turns/bead) with the linkage number change of (-1)". The apparently inevitable conclusion: the nucleosome reconstitution experiments of Germond et. al. contradict the X-ray diffraction findings of Finch et. al.

One possible escape from this quandary is to deny that the cutting periodicity precisely reflects the helical screw. In Prunell et. al. 1979, the authors argue that "the periodicity of digestion of DNA coiled in a two-turn nucleosome is expected to be greater than the periodicity of the double helix itself" because of the enhanced DNA exposure to enzymatic attack from various angles. In this way, they cling to the possibility of a change in DNA screw.

At the present, many members of the biochemical community are searching for less artificial explanations - "everyone is breaking their heads on this problem" (A. Worcel, personal communication)

In the following pages, we propose an entirely different approach. No screw change need be invoked. Recent observations suggest a helical zig-zag structure of supranucleosomal chromatin. A remarkable feature of such a structure is that it solves the apparent paradox between the number of DNA turns per nucleosome and the total linking number of a nucleosome-containing cd DNA. We will show that the fundamental repeat unit, composed of two nucleosomes with -1.75 DNA turns per nucleosome, contributes -2 to the linking number of a cd DNA and that, in general, for a cd DNA containing n nucleosomes, $\Delta Lk = -(n+1)$. This formula quantifies the experimental findings of Germond et. al. 1975; in particular, it correctly predicts a value of $\Delta Lk = -25$ for the SV40 minichromosome.

A New Model and a New Formula

Finch et. al. 1977 claim that to account for the discrepancy between ΔLk/nucleosome and the number of superhelical turns/nucleosome one must postulate that the helical screw changes when DNA is bound onto a core particle. Absolutely crucial to their argument is the following assertion, cited earlier (p. 66):

> "The change in linkage number is the same as the number of superhelical turns only if the screw of the DNA double helix does not change in the local coordinate frame defined by the superhelix path".

Scrutinize this assertion by rephrasing it in the language developed in the first part of this essay. "...the screw of the DNA double helix does not change in the local coordinate frame defined by the superhelix path" translates as $\Delta Tw = 0$. Then by equation [9], $\Delta Lk = Wr$, an equality which parallels the first part of their claim. Unfortunately, since Wr does not generally equal the number of superhelical turns, the cornerstone assertion of Finch et. al. is false. The erroneous identification of Wr with N, the number of superhelical turns, poisons the remainder of their analysis. Whereas inequality of ΔLk and Wr would demand a screw change, the observed inequality between ΔLk and N implies no such thing.

Earlier in this essay we dwelled on the differences between Wr and N. For our present purposes, the difference of utmost importance is the global nature of Wr, as opposed to the purely local and additive nature of N. Realize that under the assumptions usually made in the biochemical literature, $N = (-1.75)n$, where n is the number of nucleosomes; thus only those portions of DNA confined to the nucleosome core particle can

influence the value of N. In particular, N is independent of the linker DNA configuration. Now in contradistinction, both the linker and the core portions of the DNA contribute to the value of Wr. Moreover, unlike N, Wr cannot be calculated piecewise - because the integral [6] is taken over all pairs of distinct points, Wr is significantly affected by distant interactions among separated regions of the DNA.

Since it neglects the linker DNA, the parameter N does not precisely reflect the global geometry of a cd DNA wrapped on nucleosomes. Therefore it is totally inappropriate to compare N with the topological invariant Lk. The discussion above suggests that the linker DNA must be taken into account in any analysis of chromatin topology.

Little is known about the in vivo conformation of linker DNA. It is "not extended and therefore not seen in native chromatin, where the nucleosomes appear as individual particles in close apposition" (Chambon 1978). Some inferences may be drawn concerning the bending and twisting of linker DNA. Apparently the linker is flexible enough to allow the higher orders of coiling required to condense chromosomal DNA (Worcel and Benyajati 1977). Because of the intracellular presence of nicking-closing enzymes, it seems likely that native linker DNA is torsionally relaxed. This notion is supported by the results of Germond et. al. 1975, who reconstituted the SV40 minichromosome in the presence of nicking-closing enzyme (thus relieving any linker torsion) and observed its structural similarities with the native minichromosome isolated from infected cells.

Proposed models of higher-order chromatin structure must incorporate these features of the linker DNA. Packaging is fairly well understood at the level of the nucleosome, and at the level of the 110 A^{o} "thin filament" (which is very probably a linear array of nucleosome cores in contact with one another - Felsenfeld 1978); but still unclear is the structure of the 200-300 A^{o} "thick fiber". A. Worcel, of the Department of Biochemical Sciences at Princeton, has recently developed an elegant and suggestive model of the thick fiber. Curiously, should his model of this higher-order structure turn out to be correct, it would neatly explain the -1 vs. -1.75 paradox which arises at the nucleosome level. Before we describe Worcel's model in detail, let us establish a minimal set of prerequisites which must be met by any candidate model of the thick fiber.

(a) The linker DNA is not torsionally stressed.
(b) The nucleosomal DNA is wound -1.75 (left-handed) turns per core particle.
(c) The fiber has a diameter between 200 and 300 A^{o}.
(d) If the duplex axis is a closed curve, it is topologically equivalent to a circle, i.e. it isn't knotted.

Criteria (a) - (c) have already been discussed. We believe (d) is necessary because the SV40 minichromosome moves reversibly, depending on environmental conditions, between a relaxed circular beaded-string and a condensed thick fiber.

Because nucleosome cores have a 110 A^{o} diameter, criterion (c) suggests that the fiber is roughly two cores wide. Also, it is known that the disc-shaped nucleosomes enjoy stabilizing attractions, lid to lid, with other nucleosomes (Chambon 1978). So in the case of the SV40 minichromosome with 24 nucleosomes, one might envision a structure composed of two side-by-side columns of 12 beads stacked lid on lid. Then the DNA path is uniquely determined by criteria (a) and (b). A

schematic diagram of the DNA winding pattern is shown in Fig. 1. The DNA crossovers between columns are a notable feature of Worcel's proposed structure - these crossovers inspired the name "zig-zag chromatin". Crystal packing evidence obtained by Finch et. al. 1977 suggests an offset stacking arrangement of the cores, as shown in Fig. 2. Finally, the molecular architecture may be directly visualized by means of the electron micrograph in Fig. 3. One can actually see the DNA crossovers, originally predicted on the basis of criterion (b) and other structural considerations.

In the winding pattern shown below, the spheres represent core particles and the curve represents the DNA duplex axis. No attempt has been made to show the DNA strands - compare Figs. 7 & 8, where the duplex is identified with a twisted ribbon.

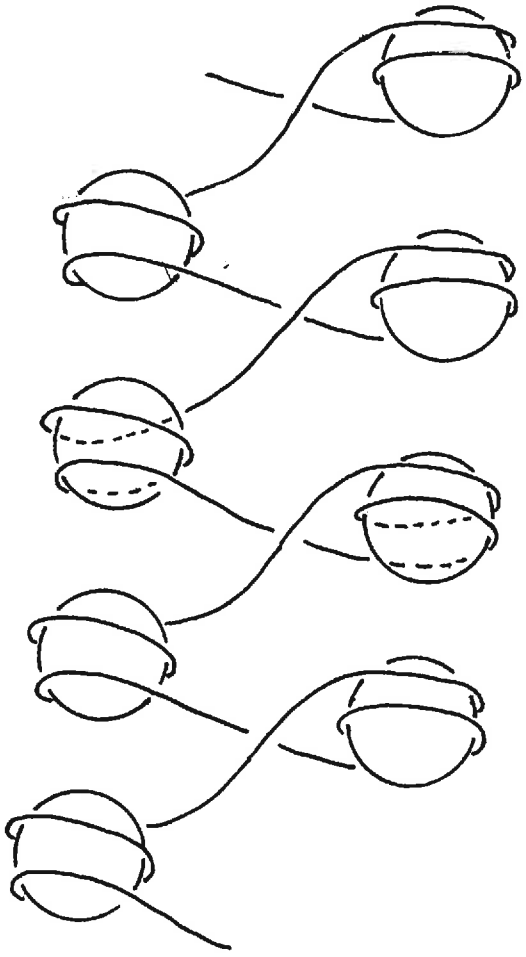
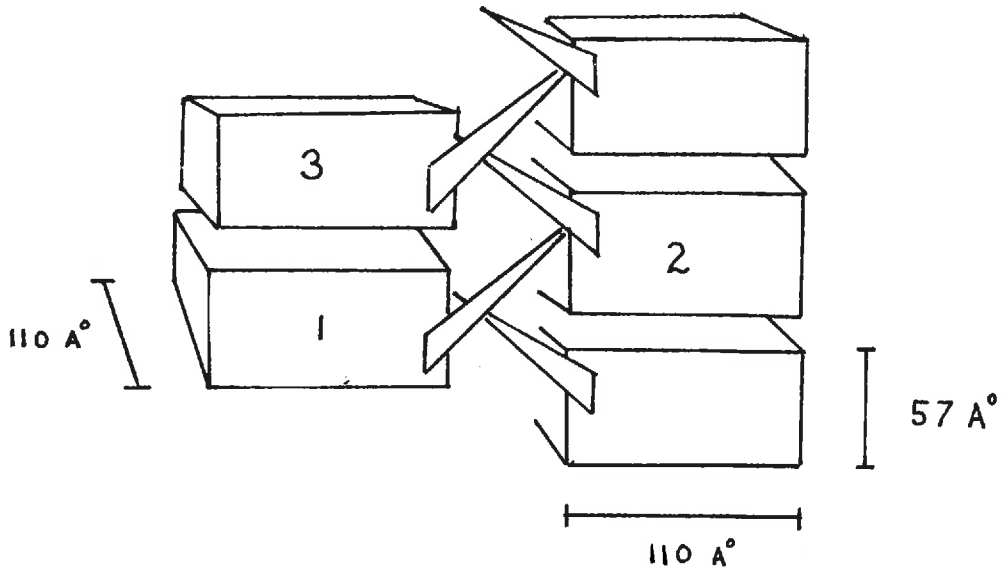


Fig. 1



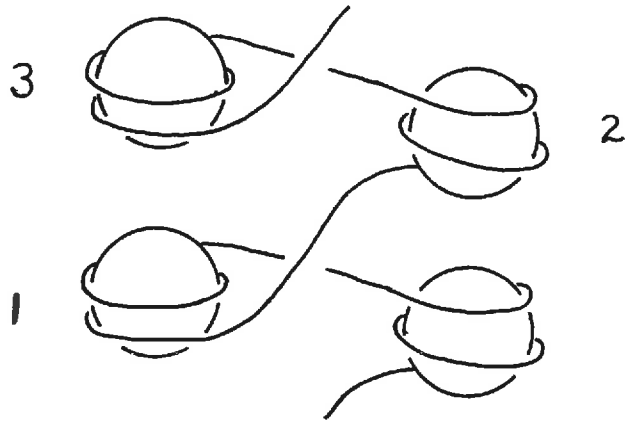
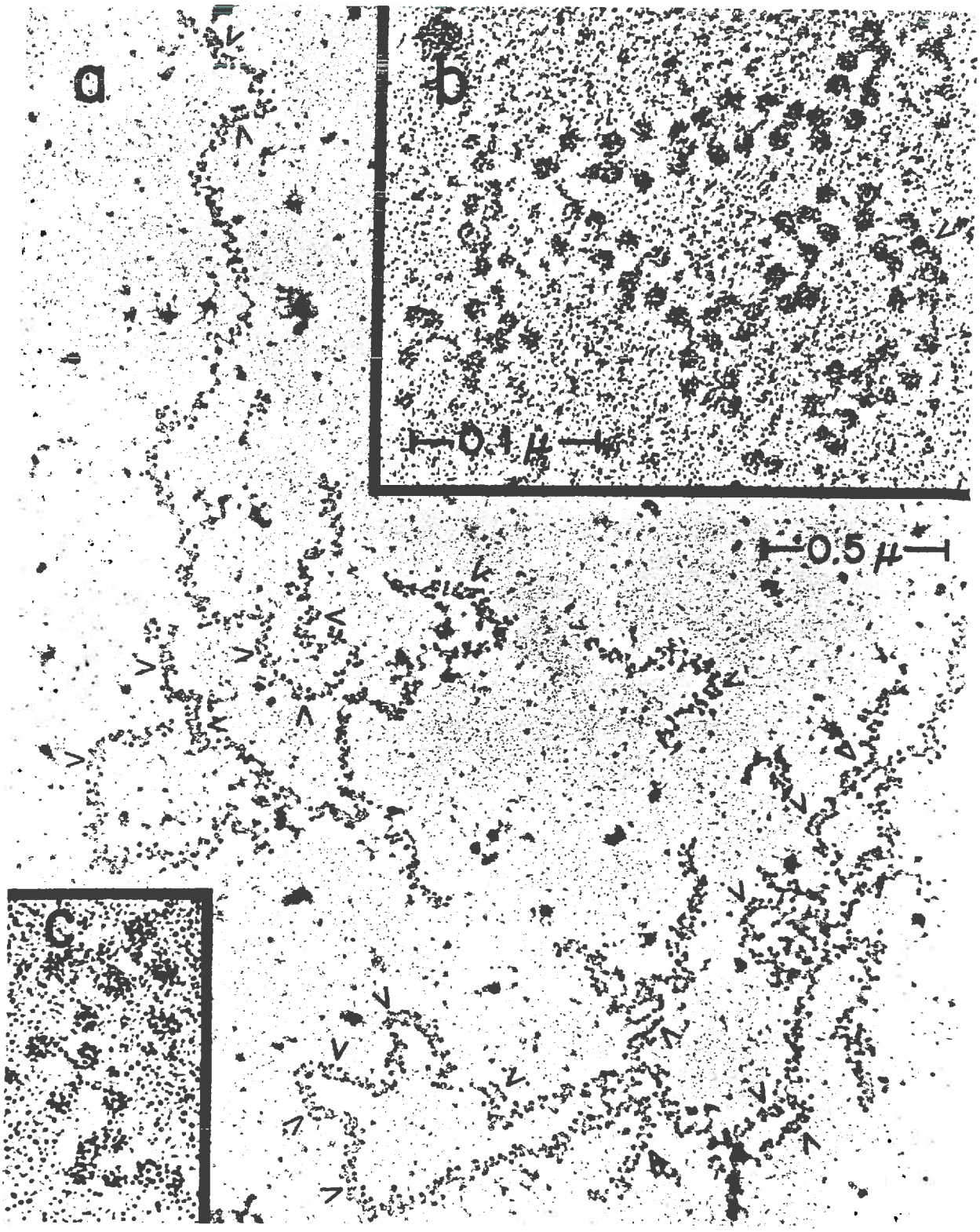


Fig. 2



a: chromatin fibers (250 A°) from chick erythrocytes; arrows indicate ribbon structure

b: higher magnification of fiber; note stacked nucleosomes.

c: zig-zag path of spacer DNA between unstacked nucleosomes.

Fig. 3

To generate the thick fiber from the zig-zag shown in Fig. 1, Worcel has suggested that both 12-bead columns may be twisted helically about the midline parallel to the columns. Experimentation with molecular scale models demonstrates the feasibility and enhanced symmetry of this higher-order coiling. In this way, the flat structure of Fig. 1 could acquire the "volume" characteristic of the thick fiber.

Aside from its elegance, Worcel's model appears reasonable in light of biochemical, crystallographic, and electron microscopic evidence. But until now, we have only hinted at its most splendid virtue: for the DNA of such a structure,

$$\Delta Lk = -(n+1) \qquad [34]$$

where n is the number of nucleosomes. In other words, each nucleosome contributes -1 to ΔLk, although the structure is build with -1.75 turns per nucleosome! Thus the nagging discrepancy is resolved without the ad hoc assumption of a change in DNA screw.

This author discovered equation [34] by studying structures topologically equivalent to the DNA in Worcel's model. Following the suggestion of Crick 1976, we wound twelve feet of ribbon around two wodden poles according to the DNA winding pattern shown in Fig. 1. Before beginning the experiment, we drew a red line along one edge of the ribbon and a black line on the other edge. The ribbon was wrapped flat on the poles, and then the ends were joined in a twistless fashion (the details of the connection are discussed later). The red and black edges of the closed ribbon may be identified with a pair of closed space curves; then the linking number, Lk, is defined for those two curves. Obviously, if the ribbon were merely closed to a circle, the edges would have

Lk = 0. Thus for more complicated closed ribbons, Lk ≠ 0 is due solely to supercoiling, i.e. the deviation from the untwisted circular "relaxed" ribbon. By analogy, we see that

$$\Delta Lk \text{ (supercoiled DNA)} = Lk \text{ (edges of ribbon)} \qquad [35]$$

Having explicitly stated this correspondence, in what follows we will freely confuse ribbons with DNA, and Lk with ΔLk.

It is non-trivial to evaluate Lk for a ribbon wrapped around two poles; so how does this method help us? Since Lk is a topological invariant, it conveniently does not change when we slide the closed ribbon up and off the poles. By then constraining the ribbon axis to lie in a plane (the floor) we force Wr = 0. Thus all of Lk is partitioned to Tw - but it is easy to measure Tw! We just snip the ribbon and introduce countertwists until the ribbon appears "flat" (untwisted). The number of imparted counterclockwise revolutions simply equals Lk. Alternatively, we may calculate Lk by counting edge-crossovers as described on p. 20.

This empirical approach led us to the discovery of [34]. To prove [34], we use a natural repeat unit inherent in the proposed structure. By reference to Fig. 1, we observe that the fundamental repeat is an "open dinucleosome" (see Fig. 4). Several copies of this unit are joined end-to-end in the overall structure. We now seek to isolate the contribution made to Lk by each open dinucleosome. Before doing so, let us clarify the notion of "contribution to Lk".

Roughly speaking, the idea is this: suppose we hypothetically remove one of the open dinucleosomes from the structure in Fig. 1 and replace it with a straight untwisted segment of DNA. What will be the resulting

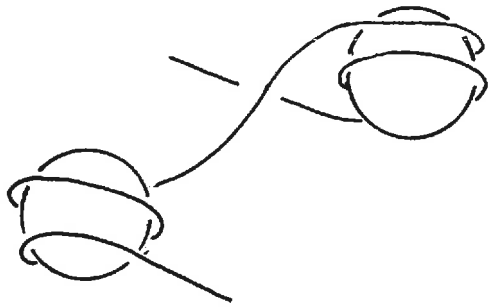
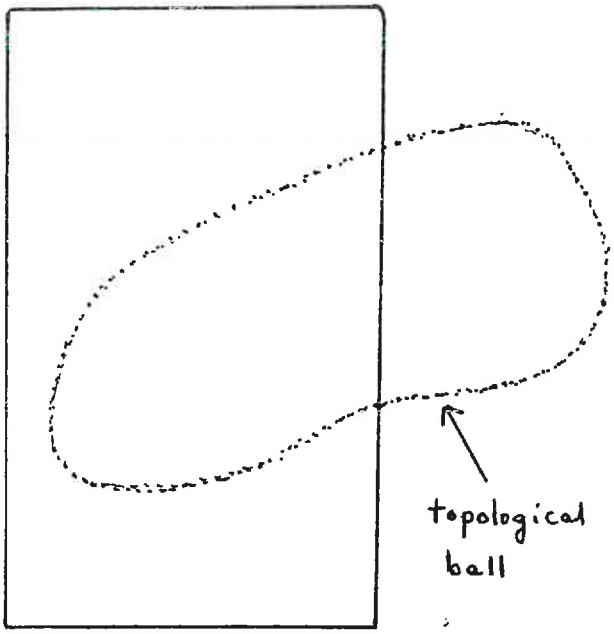


Fig. 4

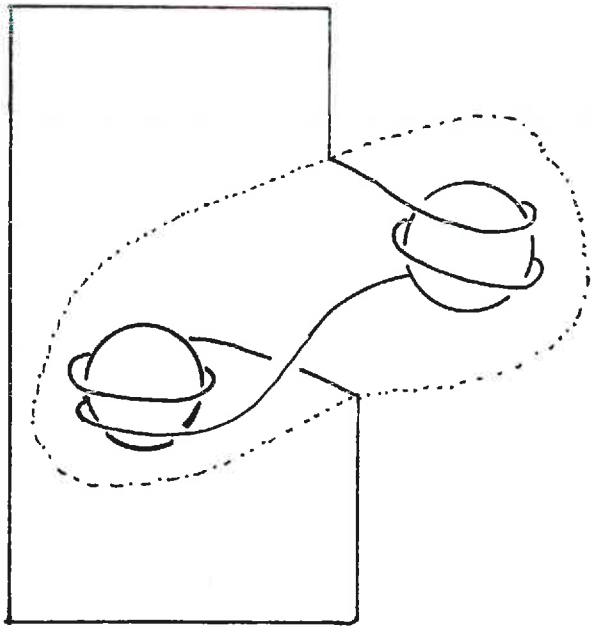
change in Lk? Ideally we'd like to ascribe the change in Lk to the topology of the open dinucleosome. Only in this case would it make sense to speak of the "linking contribution" per open dinucleosome. More to the point, how does Lk change if we remove segments of linker DNA and insert open dinucleosomes in their place? By imagining that the zig-zag of Fig. 1 has been built up through several repetitions of this procedure, we greatly simplify the computation of Lk and lay the groundwork for a proof of [34] by mathematical induction. Such a proof fundamentally rests on the hope that the local structure of the open dinucleosome alone determines its effect on Lk.

This hope will be dashed, in general. Fuller 1978 has indicated some of the pitfalls involved in decomposing Lk into contributions from various portions of a structure. He later announces a very happy special case (See Fig. 5, next page). Suppose a topological ball contains the segment of linker DNA to be hypothetically excised (5a), and in particular this ball excludes all other regions of linker and nucleosomal DNA. Then if the open dinucleosome to be inserted can also be contained in the ball (5b), there is a well-defined "contribution to Lk" associated with the open dinucleosome. Fig. 5c shows the ambiguity connected with an inserted structure which is not confined to the original topological ball - it may disrupt additivity by threading its way around distant parts of the ribbon.

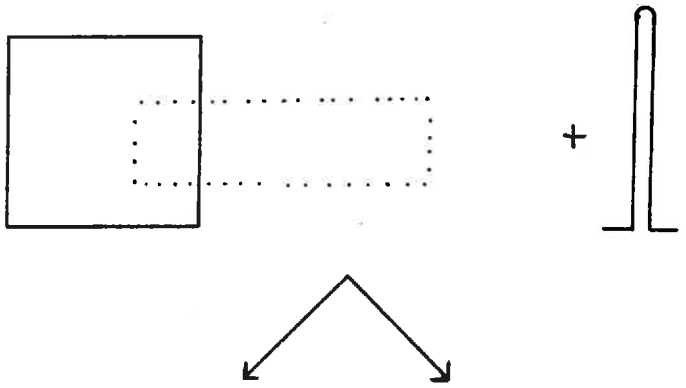
As should be clear from Fig. 1, the open dinucleosomes in the helical zig-zag have replaced segments of linker DNA without any subversive "threading". Hence, for our purposes, the contribution to

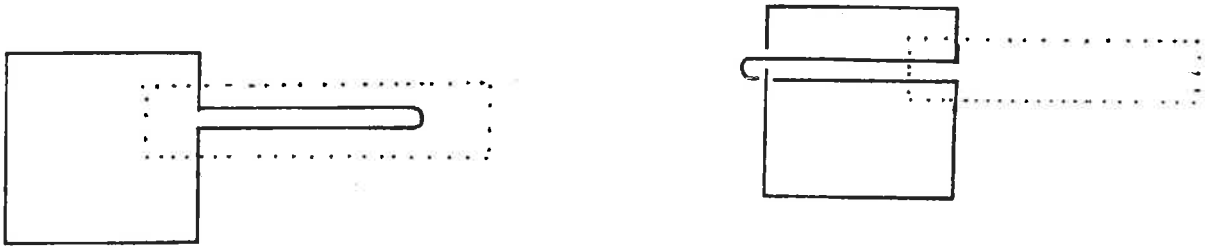


5a



5b





5c

linking number , λ , is a well-defined property of an open dinucleosome. (Fuller 1978 calls λ "the linking difference" δLk)

A simple way to calculate λ is shown in Fig. 5. According to our prescription

$$\lambda = Lk(b) - Lk(a) \qquad [36]$$

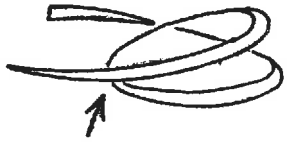
By counting edge crossovers, we find $Lk(b) = -2$ and $Lk(a) = 0$. Thus

$$\lambda = -2 \qquad [37]$$

A sly way to obtain the same result has been pointed out by W. Brodie (W. Brodie, personal communication). This proof by transformation is diagrammed in Fig. 6, and is followed by a page of annotation.

6a

6b



6c

6d

6e

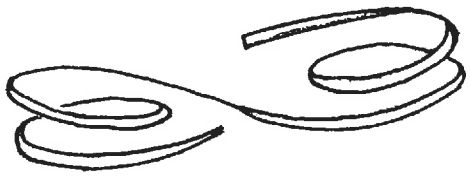


Fig. 6

6a: shows the repeat unit of the structure, the open dinucleosome of Fig. 4. The ribbon represents the DNA duplex wound -1.75 turns on each core particle (core not shown).

6b: We reproduce the right half of 6a to facilitate comparison with 6c, d. The endpoints of structures 6b-d are taken to be fixed.

6c: The portion of the duplex which lies under the DNA exiting the nucleosome (see arrow in 6b) has been imagined to have passed over on top. This double stranded passage changes λ by +2. (See p. 20 for the sign convention at crossovers)

6d: This structure is topologically equivalent to, and hence has the same λ, as the structure in 6c. We have slackened the portion of the duplex indicated by the arrow in 6c, and pulled it up and over the lower ring. In this way, we make it obvious that 6c is equivalent to a structure with 1.75 <u>right-handed</u> turns.

6e: The resulting structure is shown here. Because of its reflection symmetry, this structure would have $\lambda = 0$ ($\lambda \longrightarrow -\lambda$ under an orientation-reversing transformation of the space.) Since it differs from the open dinucleosome in λ by +2, the open dinucleosome must have $\lambda = -2$.

Now we prove the main result.

Theorem: For a cd DNA containing n nucleosomes, the proposed helical zig-zag has $\Delta Lk = -(n+1)$.

Proof: The proof consists of two parts depending on whether n is even or odd. We generate all structures with n odd by repeated insertions of open dinucleosomes into an initial closed mononucleosome. The proof for even numbers begins analogously with a closed dinucleosome. Since each insertion changes n by +2 and ΔLk by -2 (from [37]), it suffices to show $\Delta Lk = -(n+1)$ for the basic cases n = 1 and n = 2.

A constructive proof that Lk = -2 for a closed mononucleosome (n=1) is presented in Fig. 7. The constraints of -1.75 turns/nucleosome and torsionally relaxed linker DNA dictate the structure shown.

Fig. 8 shows the only closed dinucleosome compatible with the constraints on the DNA. (Do not confuse this structure with the hypothetical open dinucleosome of Fig. 4.) Observe that it differs from the mononucleosome of Fig. 7 by having an additional left-handed superhelical turn of DNA. As might be expected, ΔLk for the closed dinucleosome differs by -1 from that of the closed mononucleosome, i.e. $\Delta Lk = -3$ when n=2. A constructive proof similar to the one in Fig. 7 yields the same result.

Constructive Proof that Lk = -2 for Closed Mononucleosome

Consider the places where the bottom strand crosses over the top strand - there are two such places (indicated below). By convention, we orient the strands to run parallel. Then both crossovers are of the type $-\uparrow\rightarrow$ so $\Delta Lk = -2$.

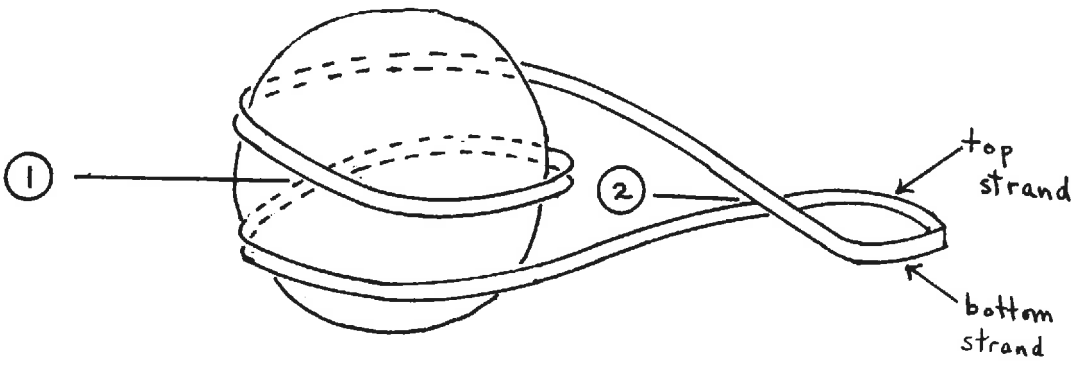


Fig. 7

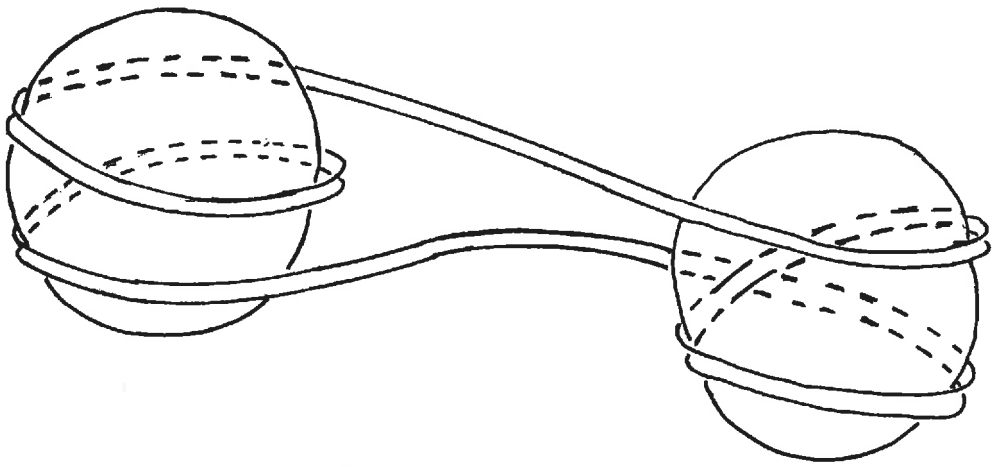


Fig. 8

Implications of $\Delta Lk = -(n+1)$

The formula $\Delta Lk = -(n+1)$ has the following consequences:

(i) It resolves the -1 vs. -1.75 paradox which has disturbed the biochemical community from 1977 to the present.

(ii) It triumphantly reconciles the nucleosome model of Finch et. al. 1977 with the findings of the reconstitution experiments of Germond et. al. 1975. The dubious assumption of a change in the DNA helical screw is thus shown to be unnecessary.

(iii) It quantitates the observation that ΔLk changes by -1 after addition of a nucleosome to cd DNA in the presence of nicking-closing enzyme.

(iv) It correctly predicts a value of $\Delta Lk = -25$ for the DNA from SV40.

We conclude this essay with two sweeping speculations. First, $\Delta Lk = -(n+1)$ is possibly a general law for all chromatin. Across the evolutionary spectrum, from the DNA of the bacterium E. Coli to eucaryotic chromosomes, there seems to be an invariant "linking density": about 200 base pairs for each change of -1 in ΔLk (Finch et. al. 1977). This very mysterious regularity is somehow connected to the DNA packaging problem encountered by all organisms on this planet. Our formula may elucidate the connection - it predicts a change of -1 in ΔLk for each added nucleosome, and as we have mentioned, each nucleosome is associated with about 200 base pairs of DNA! So perhaps the 200:1 pattern reflects the universality of chromatin-like structures.

Our second speculation concerns the number -1.75. This number of superhelical turns of DNA per nucleosome core is no doubt determined by chemical interactions between, and physical properties of, histone proteins and DNA. Yet why were nucleosomes built this way? Since more turns imply superior packaging, might not we expect to find, somewhere in Nature, larger cylindrical cores with many turns of DNA wrapped around them?

Such questions are usually regarded (and rightly so) as scientifically naive and out of spirit with evolutionary thinking; still, by asking we are led to consider hypothetical nucleosomes. What formula would correspond to $\Delta Lk = -(n+1)$ if DNA were wrapped with, say, -0.5 turns per nucleosome?

Let s be the number of left-handed superhelical turns per nucleosome core. Since uninteresting end effects are most prominent when n is small, we rephrase the question above in terms of a function f defined by the ratio

$$f(s) = \lim_{n \to \infty} |\Delta Lk/n| \qquad [38]$$

where we are always assuming the two column structure postulated by Worcel. In the previous pages we have shown $f(1.75) = 1$. Let us investigate only the cases

$$s \in \{0.5, 0.75, 1.5, 1.75, 2.5, 2.75..,\}$$

Notice that the half-integral cases correspond to DNA being wound from column to column "around the outside" without the crossovers in the middle that characterize the remaining cases. There are no other topologically distinct possibilities, given the earlier criteria governing DNA winding patterns.

The behavior of f is shown in Fig. 9a (next page). It is non-decreasing until a sudden plunge at s = 1.75. Although subsequent drops occur, they are ghosts of this first one. Since f is monotonically increasing on the set {1.75, 2.75, 3.75...} the drops in f after s = 1.75 are merely echoes of the one interesting point in the graph. It is particularly bizarre that f(s) is unaffected in going from s = .75 to s = 1.75. In other words, an extra turn of DNA may be wrapped on each nucleosome, thus packaging an entire length of 80 base pairs, with no cost in Lk! Evolution may have noticed, and favored, the enhanced packaging which occurs at s = 1.75.

Fig. 9b, a plot of f(s)/s: s , highlights other peculiarities of s = 1.75. (Observe that if, as Finch et. al. 1977 believed, increased numbers of superhelical turns produced exactly equal increases in ΔLk, then Fig. 9b would show a constant function identically equal to 1.) If, by analogy with procaryotic DNA, we speculate that energy-consuming enzymatic processes are at least partly responsible for increased supercoiling of DNA, then the minimum at s = 1.75 expresses an optimum balance between energetic and packaging considerations.

It is noteworthy in itself that nucleosomes with s = 1.75 have such unusual properties; but what is really stunning is that, even if by coincidence, Nature chose to build nucleosomes in precisely this unusual way.

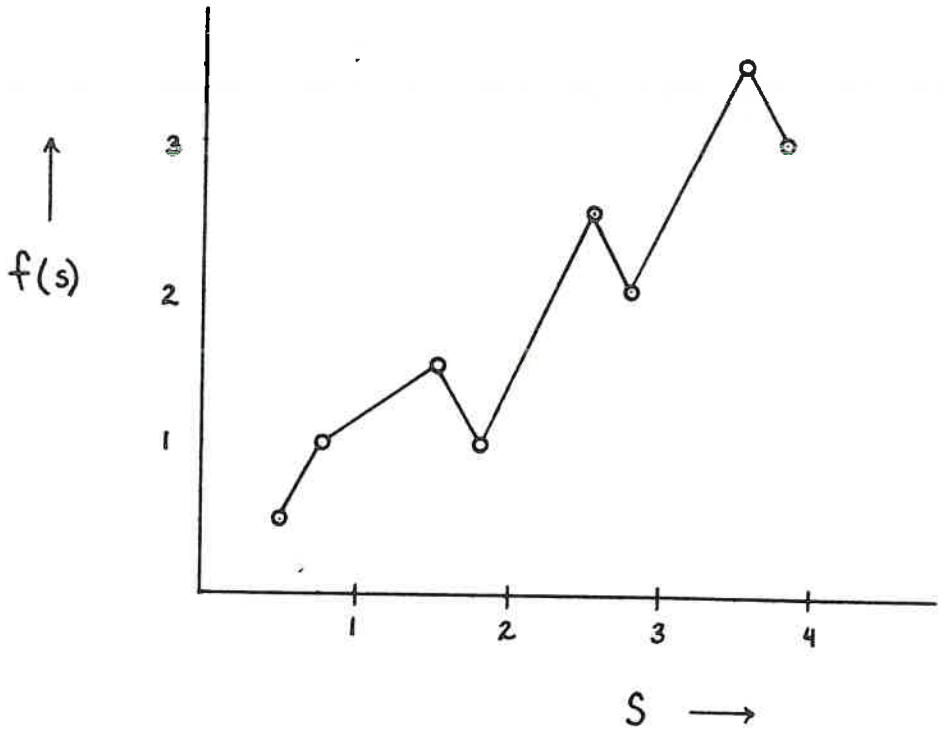


Fig. 9a

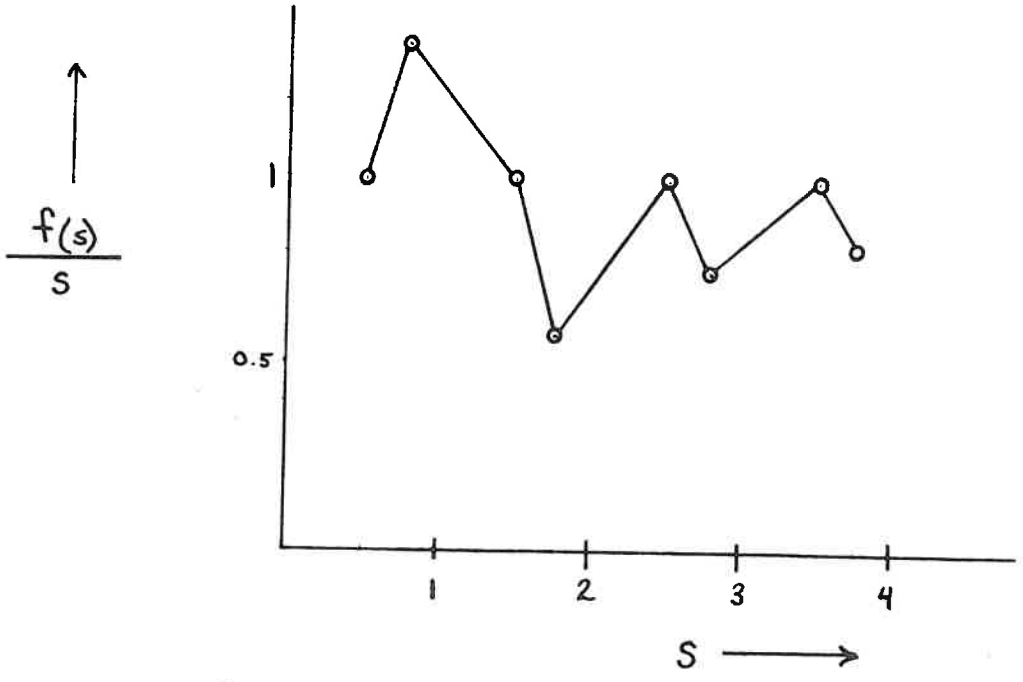


Fig. 9b

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Appendix:

Historical Comments on Lk = Wr + Tw

The formula Lk = Wr + Tw is of paramount importance in rigorous analyses of supercoiled cd DNA. It decomposes the topological invariant of the molecule into contributions from bending of the duplex axis and twisting of the strands about the axis. And as we have seen, the constraint that Lk remain constant imbues cd DNA with all its idiosyncratic properties. Considering the significance of the above formula, one would naturally like to know who discovered it, and under what circumstances. The parentage of important results in science is frequently a matter of some dispute, and as we shall see, this case is no exception.

In 1959, G. Calugareanu in Cluj, Rumania published a study of knots by differential-geometric methods in which he sought an invariant of isotopy analogous to the Gauss linking number of a pair of curves. (Calugareanu 1959). To achieve success, he had to adopt the following very restrictive notion called "non-degenerate isotopy": Let smooth deformation be defined as a C^3 map $H: S^1 \times I \longrightarrow E^3$ such that for fixed t, $f_t(x) = H(x,t)$ is a closed immersed curve. Then H is a non-degenerate isotopy if each f_t has non-vanishing curvature, and is imbedded, i.e. free from double points. Under these assumptions, he showed that

$$Wr + \frac{1}{2\pi} \int_C \tau \, ds = \text{integer} = \text{"K"}$$

where $C = f_t(x)$, t fixed, is a curve parametrized by arc length s and τ is the torsion. Calugareanu proved that this integer is an invariant of non-degenerate isotopy.

In his 1959 paper, he established his results by considering the ribbon about C having edges $C \pm \varepsilon n$, where n is the principal normal and $\varepsilon > 0$ is sufficiently small so that C and $C + \varepsilon n$ don't intersect. In 1960, he found more general results by considering arbitrary closed ribbons about C (Calugareanu 1961). In particular, the third equation from the bottom of p. 613

$$4\pi G(C,C^*) = J + 2\int_C (\tau+\theta') \, ds$$

is the celebrated formula Lk = Wr + Tw, though disguised by Calugareanu's notation. (Note however that his result assumes that the "duplex axis" C admits a Frenet frame everywhere - eight years later White dramatically strengthened the formula by dispensing of this assumption.)

The subject lay dormant until 1967 when W.F. Pohl attacked the same questions in a more sophisticated way (Pohl 1968). He used differential forms, Calugareanu used Taylor series; the result is, according to Pohl, "a new, clearer, and much simplified treatment of (Calugareanu's) results." Yet the paper deals only with the invariant K which Pohl renames SL, the self-linking number. He does not mention the formula Lk = Wr + Tw.

In 1968, a student of Pohl's, J.H. White, generalized his adviser's work to the case of submanifolds in higher dimensional Euclidean space. In so doing, he proved (White 1969) Lk = Wr + Tw in full generality, with no curvature assumptions on the axis curve. In a personal communication to this author, White has stressed the superiority of his result as compared to Calugareanu's, with respect to biochemical applications; biochemists frequently need to consider duplex axes which contain linear segments - Calugareanu's results do not apply to this crucially important case (White, personal communication).

Also during the late 1960s, the biochemist Jerome Vinograd and his colleagues at Caltech were exploring the properties of the newly discovered cd DNA. Knowing nothing of the work just described, Vinograd asked a mathematician at Caltech, F. Brock Fuller, whether a quantitative description of supercoiling was possible. Fuller wrote a beautiful paper, which has now become the classic work on the subject, in which he coined the vivid name "writhing number", investigated its properties, and applied his results to a twisted elastic rod model of cd DNA (Fuller 1971). It should be noted that in his article, Fuller refers to the prior work of Calugareanu and Pohl, citing their Gauss integral as equivalent to his Wr. Though Fuller presents no proof of Lk = Wr + Tw, he has clearly hit upon the key idea of defining Tw in terms of an arbitrary normal vector field based on the duplex axis - by so doing he needn't invoke any curvature assumptions on the axis. In this way he was led to rediscover White's general result.

But the mathematics of supercoiling made real impact on molecular biology only after it was elucidated in a review article by F.H.C. Crick, Nobel laureate and co-discoverer of the structure of DNA (Crick 1976). He explains the concepts of Lk, Tw, and Wr in great detail and with many enlightening visual examples. Crick writes of Fuller's work in an almost reverential tone, calling it "difficult to grasp for the average molecular biologist". After the publication of Crick's article, nearly all subsequent papers on supercoiling adopted Fuller's notation and approach. Unfortunately, in the biochemical community, credit is often given to Fuller for <u>discovering</u> the formula Lk = Wr + Tw.

Fuller himself has helped to rectify this matter of priority; in a later paper he writes "I wish to take the opportunity here to point out that the basic relation $Lk = Tw + Wr$ and a number of the results of my earlier paper had previously appeared in a paper by J.H. White" (Fuller 1978). Two years later, confusion on this issue is still rampant. Meanwhile, Calugareanu's pioneering contribution - the discovery of $Lk = Wr + Tw$ for a large class of "ribbons" - has been downplayed or even overlooked. In our opinion, this forgotten professor deserves as much credit as anyone.