WHAT IF WATSON AND CRICK WERE WRONG?

J.D. Watson and F.H.C. Crick's Nobel prize winning double-helical model of DNA (deoxyribonucleic acid) is widely considered to be one of the great discoveries in biology. It is ranked with Mendelian inheritance, even evolution by natural selection, and seminal to the development of molecular genetics - a discipline which appears to be reaching an understanding so profound and so awesome in its potential that Judson (1980a)* describes it as the dawning of "the eighth day of creation". Watson and Crick's original (1953a) letter advancing their structure is a scientific document whose fame bears comparison with Einstein's first paper on special relativity. The anniversary of its appearance is periodically marked by Nature with special issues. A growing library of works chronicle, debate and analyse Watson and Crick's achievement.**

---

* Bibliographical details of references are provided in the list of Works Cited.

** The key document is Watson's extremely popular (1968) autobiographical memoir. It has recently been republished together with reviews, commentary and the main scientific papers [Stent ed. (1980)]. This is an invaluable collection of primary documents of various kinds, providing an excellent introduction to the controversies which surround Watson's account [on which see also Sayre (1975), Chargaff (1976) and (1978)]. There are three main secondary sources. Olby (1974) is a scholarly historical study which concentrates on the work immediately prior to and culminating in the Watson-Crick model. Judson (1980a), a massive monograph addressed to a more general audience, takes the development of the double helix as its starting point. Both are widely read by scientists. In a less well known work, Portugal and Cohen (1977), themselves scientists, trace research on DNA through a hundred years from its beginnings in the last century.

A nearly universal presumption underlying this literature is that the structure of DNA was discovered by Watson and Crick.* The comments of the the eminent molecular biologist, Gunther Stent, are quite typical:

> I learned in my history class at Hyde Park High School in Chicago that the Renaissance began on May 29, 1453, the day Constantinople fell to the Turks.... Although I eventually managed to appreciate the absurdity of pinpointing the exact start of an historical era, I still hold that the era of molecular biology began exactly five hundred years - almost to the day - after the fall of Constantinople. That beginning came on April 25, 1953, when there appeared an article in the British scientific journal Nature by two young scientists, James Watson...and Francis Crick, reporting the discovery of the DNA double helix [(1980) ed.,p. xi].

Amid all this enthusiasm, the pseudononymous F.R.S. sounded a note of caution:

> And yet. If they were wrong. If their model, like Ptolemy's.... [(1968) emphasis in the original]

In 1976, two groups of scientists published essentially the same radical alternative to the Watson-Crick double helix. The group led by G.A. Rodley and R.H.T. Bates at the University of Canterbury in Christchurch, New Zealand, have priority. Their paper, advancing a Side-By-Side (SBS) conformation for DNA, appeared in the Proceedings of the National Academy of Sciences (U.S.A.) for September [Rodley et al. (1976)]. The other team, headed by V. Sasisekharan at the Indian Institute of Science in Bangalore, had worked quite independently. They were alerted to the New Zealanders' work by the referees of their own first article, published in the 20 November number of the Indian journal Current Science [Sasisekharan and Pattabiraman (1976)].

---

* Hamilton (1968) and Jevons (1979) are rare exceptions.

Of course, the best known feature of the Watson-Crick model of DNA is the sugar-phosphate double helix itself. The alternative conformation for DNA devised by these two groups is not helical. The New Zealanders and Indians suggested that the 'backbone' or, more correctly, the exoskeleton of DNA is composed of two strands which, schematically, look rather like wool that has been unravelled from knitting: sine-curves in three dimensions which change direction after completion of half a helix. Rather than intertwine, as do Watson and Crick's helices, they lie side-by-side.

An important consequence of the double helix is not widely appreciated. Chromosomal bifurcation occurs prior to replication of the nuclear material, followed by cell division. Thus the long, tubular DNA molecule within the chromosome must also divide longitudinally. On the Watson Crick model, this means that the two helical strands of which it is composed move apart. But there is a complication. Because the two strands are intertwined, they can only be parted intact by vertical unwinding. Various means of achieving this have been suggested. Most scientists now believe that that it is done with the aid of enzymatic processes. But unwinding during strand separation is not well understood, straight-forward or unproblematic.

As both the New Zealanders and the Indians made clear in their initial papers, the SBS model of DNA was a structure which offered a simpler account of separation. The two semi-helical stands were not intertwined, and so could move apart laterally, 'unzip', without unwinding. As a result, the SBS

structure was dubbed the 'warped zipper' by one commentator [Arnott (1979)]. The original articles advancing the 'warped zipper' model also make it plain that the structure was developed by both groups in order to circumvent the problem of unwinding.

The question of most immediate interest provoked by the advent of the SBS structure for DNA is straight-forwardly scientific: Is DNA a double helix or a 'warped zipper'? The initial scientific answers to this question are reported here. However, this is not a scientific dissertation, and does not attempt to arbitrate among scientific arguments. My concerns are meta-scientific. Among these, with Sir Karl Popper, "I shall distinguish sharply between the process of conceiving a new idea, and the methods and results of examining it logically [(1972), p.31]." Of the latter, very little is said here - though I have published elsewhere a preliminary study of the methodological implications of the appraisal of the 'warped zipper' [Stokes (1982)]. My interest here is why and how the individuals who invented the 'warped zipper' came to do so.

Unlike Popper, I "regard it as the business of epistemology to produce what has been called a 'rational reconstruction' of the steps that have led the scientist to a discovery [idem., emphasis in the original]." At present, the majority of philosophers of science hold, with Popper, that

> The initial stage, the act of conceiving or inventing a theory, seems ...neither to call for logical analysis nor to be susceptible of it [(1972), p. 31].

So viewed, the invention of the 'warped zipper' is irrational or, at best non-rational, excluded by that from philosophical investigation, instead the proper subject of psychological and sociological inquiry. I will establish that this philosophical dogma is not true a priori, as is usually supposed, and, in the case of the SBS structure of DNA, false a posteriori.

In examining the origins of the 'warped zipper', the criteria of subsequent appraisal by scientists other than those who devised it is strictly irrelevant on temporal grounds. They were assessing what had previously been invented. It is this that makes the community's judgement irrelevant, and not the fact that their appraisal lies within what Reichenbach (1938) termed 'the context of justification', whereas the invention of the SBS model lies within his 'context of discovery'.* However, the argument does not does eliminate all epistemic judgements. Scientists no more want to devise false hypotheses than they want to adopt them. Thus the assessments of the adequacy of the double helix by the inventors of the 'warped zipper' are relevant to their invention, and will be examined.

The great and, as I will show, unnecessary cost of banishing 'discovery' beyond the pale of logical analysis is that theory change in science, minor or major, must be seen as fundamentally non-rational. Successor theories may be better than their predecessors, but their occurrence (as distinct from

---

* The two pairs of terms, 'justification' and 'discovery', 'appraisal' and 'invention', are only imprecise synonyms. See chapter VI, (136)ff.

their adoption) cannot be regarded as the outcome of reasoned thought about the deficiencies they rectify. Examination of the motivation for and development of the SBS structure for DNA reveals that the whole process is best characterized as significantly, though not entirely, rational. The 'warped zipper' is a plausible alternative to the Watson-Crick model because of this. Thus, the study of the motivation and method which produced it constitute a vindication of, and the first steps toward a theory of, the logic in discovery - a theory of the the role of reason in the progress of science.

Newly devised scientific ideas appear, for judgment, in a literature devoted to appraisal. Accordingly, practically nothing is said there about <u>why</u> the hypothesis or theory was devised. Even when the defects of an earlier hypothesis or theory, which are overcome by a later competitor, motivated its development, this is obscured or misrepresented by the preoccupation with appraisal. Worse still for a study of the invention of an hypothesis, nothing at all is said about <u>how</u> it was devised. As philosophers have often pointed out, this is a quite distinct question from that of how it should be assessed. Nevertheless, the inventors of recent new ideas may supplement by correspondance and recorded interview the inadequate published sources. This is the approach I adopted.

What follows is not a fully developed account of the logic in discovery. It is located firmly in a particular example, from which only tentative generalizations made. I do not argue that the invention of new scientific ideas is an

entirely rational phenomenon - indeed an attempt is made to isolate the non-rational elements in the invention of the SBS structure and assess their importance. Moreover, I argue that no algorithmic logic of discovery is possible since creative reasoning must be ampliative.

The whole history of research on the structure of DNA from 1953 to the present is not retold here. I have confined the descriptive material and, especially, its analysis, more or less strictly to what is necessary to demonstrate the possibility of logic in scientific invention, and to establishing its presence a particular case. A certain amount of additional historical material has been included so that the descriptive portion coheres. This has been chosen because it figured in, or is required background to the invention of and early debate over the merits of the SBS model. The result is highly selective, and does not provide an overview unless read in conjunction with more general studies [for example, Olby (1974), Portugal and Cohen (1977), or Judson (1980a)].* By and large, however, these works tell a story with a happy ending. Within the triumph of molecular genetics there lies concealed a history where the question 'what if Watson and Crick were wrong?' is not counterfactual. The hidden history critical of the double helix is told here. That, to a degree, may stand on its own.

The scientific, historical and philosophical strands of this dissertation are not woven into a seamless cloth - partly because I have tried not to presume that any one reader will

---

* Moreover, certain recent reports which favour the Watson-Crick model are not canvassed here because they do not advert to the 'warped zipper' structure, played no part in its invention, and are not referred to in published discussions of its adequacy. Examples are: D. Rhodes and A. Klug, 'Helical periodicity of DNA determined by enzyme digestion', Nature, vol. 286 (1980), pp. 573-578; D. Rhodes and A. Klug, 'Sequence-dependent helical periodicity of DNA', Nature, vol. 292 (1981), pp. 378-380; Satori Iwamoto and Ming-Ta Hsu, 'Determination of twist and handedness of a 39-base pair segment of DNA in solution', Nature, vol. 305 (1983), pp. 70-72 [Robert Olby, pers. comm.].

have a detailed knowledge of all three. The first five chapters are expository. Chapters I and II tell the story of the motivation for and invention of the 'warped zipper' in New Zealand and India respectively. Chapters III and IV take the motivation for the new model and explore its 'prehistory'. Chapter V provides an account of the reception accorded the SBS model up until toward the end of 1981. The view of the specialist community to that time was that the evidence favoured the Watson-Crick model against the 'warped zipper'. Reports since have reinforced this view (cf footenote p. 11).

The remaining chapters are analytical. Chapters VI and VII seek to clear the philosophical ground for an inquiry into the logic in scientific invention. Chapters VIII and IX return to and analyze the motivation for the invention of the 'warped zipper' from this perspective. Chapters X and XI do the same for the invention of the alternative model itself, treating the two groups separately. The last chapter, XII, deals with the SBS model as a multiple invention, a phenomenon hitherto considered only from a sociological and not a logical point of view. Finally, I deal briefly with the relevance of the case study for a general theory of invention and change in science.

Because of the separation of the descriptive and the analytic sections, frequent, chiefly back-references are called for. This has been done as often as seemed necessary in an attempt to avoid repetition. So that material may be located quickly and accurately, the paragraphs have been numbered, beginning with Chapter I.

I THE GENESIS OF THE 'WARPED ZIPPER' IN NEW ZEALAND

(1) In 1971 Clive Rowe worked as a technician for the Chemistry Department of the University of Canterbury in the South Island city of Christchurch, New Zealand.[1] Rowe had an established and wide-ranging amateur interest in science. He was a member of the Royal Society of New Zealand, regularly attending its lecture series. He also participated in an informal lay and professional group which met in its members' homes to discuss biological topics. Rowe called upon a circle of scientific friends and acquaintances to verify his understanding, test his ideas and judge the reliability of the authors whose work he read.

(2) New Zealand academic institutions played host during 1971 to W. Hayes, Professor of Molecular Genetics at the University of Edinburgh, Scotland. In September of that year Hayes was in Christchurch to give guest lectures. One, entitled 'Modern Ideas on DNA Replication', Rowe attended. He came to it having read and been "fascinated" by J.D. Watson's (1968) The Double Helix, A Personal Account of the Discovery of the Structure of DNA. He was familiar, therefore, with the Watson-Crick model. In broad outline, Rowe was also acquainted with the theory of replication which had been developed on the basis of the double-helical structure.

---

[1] I recorded an interview with Rowe at the University of Canterbury on 20/11/79. The transcript is the source of all otherwise unacknowledged material concerning him (including direct quotation).

(3) Watson and F.H.C. Crick's model of the three dimensional molecular structure of deoxyribonucleic acid, DNA, was first advanced in the April 25th. issue of Nature (1953a). It consisted of two right-handed co-axial helices with a radius of 10 Angstroms (A).[2] The helices were composed of two atomic groups; phosphates on the outside and deoxyribose sugars on the inside. Within this two-stranded exoskeleton and perpendicular to it were pairs of flat, nitrogenous bases. Each pair contained one purine (double-ring) and one pyrimidine (single-ring) base; adenine (A) paired with thymine (T), guanine (G) with cytosine (C). Each base in a pair was bonded to a sugar and hydrogen bonded to its partner. The pairs were 3.4 A apart, with 10 nucleotides (phosphate-sugar-base groups) in each repeat (complete helix) of 34 A. (See Figure 1.)

(4) In its base-pair sequence and the complementarity of its two helical chains (each having half the bases attached), the Watson-Crick model of DNA provided a structural basis for understanding genetic coding and replication at the molecular level. According to the theory subsequently developed, replication begins by severing of the hydrogen bonds between the base pairs followed by separation of the exoskeletal strands. Because they are twisted together, this occurs by rapid longitudinal unwinding. Simultanously, new nucleotide chains are synthesized so that, when the process is complete, there are two DNA duplexes having an identical base sequence. Since each of the daughter duplexes has one of the original strands with attached bases, this is known as the semi-conservative theory of

---

[2] 1 A = one ten millionth of a millimetre (10^{-10} metre).

replication. (See Figure 1.)

(5) Although Rowe found Hayes' lecture "concise and lucid" [C. Rowe to M. Probine, 18/9/71], he was puzzled by aspects of what had been said. This puzzlement was not resolved by a conversation with Hayes afterward, and Rowe aired it in a letter written two days later to one of his scientific acquaintances, Dr. M. Probine, then Director of the Physics and Engineering Laboratory in the capital, Wellington [loc. cit.].

(6) Hayes had discussed the process of replication in circular DNA molecules. He had noted that separation of the exoskeletal strands appeared to occur at multiple sites simultaneously, proceeding in both directions from a starting point, so that single-stranded loops were formed. Moreover, there did not seem to be any breaks in the molecule. Rowe wrote to Probine:

> If this 'peeling off' interpretation is true, then it introduces some awkward mechanical difficulties not apparent in the 'open loop' model where free ends are free to rotate without constraint.

For, he explained,

> I have made a model up consisting of two intertwined helices, looped and soldered at the junction. If such a 'double helix' is rotated helically, one part unwinds and the other winds up. It does not seem possible to separate the two loops without breaking them. [idem, q.v., III][3]

(7) Rowe also considered unwinding, taking as his example the common gut bacterium E. coli. For the circular DNA molecule of this organism, he gave figures of "up to 1mm" for length, and

---

[3] An analogue of a double-helical model of circular DNA molecules is a quoit, whose two hempen strands are inseparable unless severed.

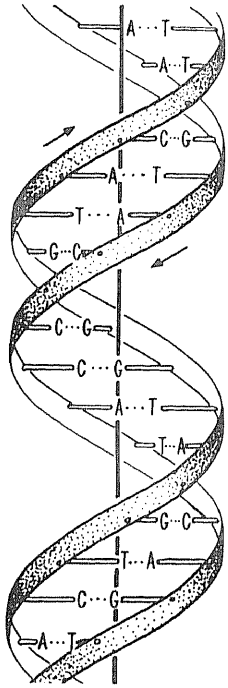


Figure 1(a) A schematic model of the Watson-Crick double helix, showing base-pairing and the direction of the helices.

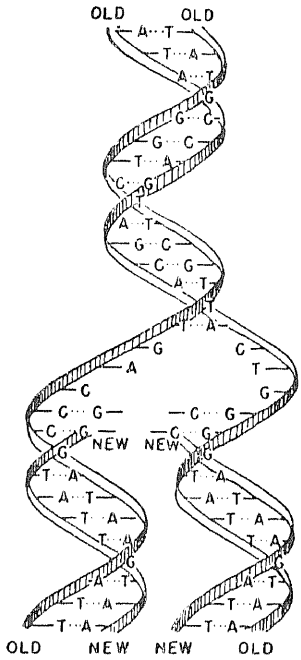


Figure 1(b) A schematic model of semi-conservative replication on the basis of a double-helical structure.

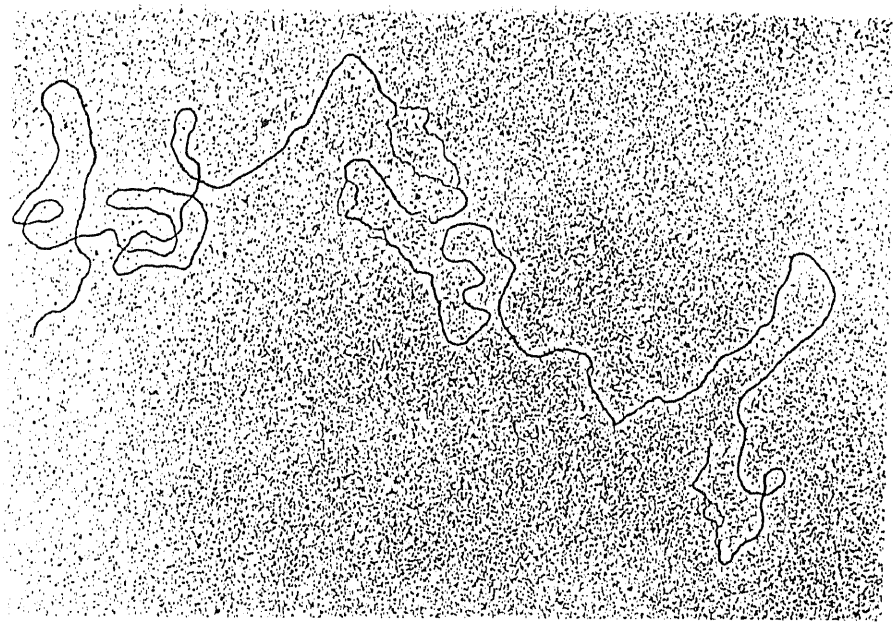


Figure 2 An electron micrograph of DNA, showing single and double strand regions.

"20 minutes or so" for the time taken to complete replication.[idem] From them he calculated that the molecule must revolve around 200,000 times at a rate of order 10,000 revolutions per minute. Although he was subsequently to regard the rate of unwinding required by the Watson-Crick model as a serious difficulty, in his letter to Probine, Rowe laid no emphasis upon the matter.

(8) Probine, to whom Rowe had written because of his work on torsional features of wood fibres [G.A. Rodley to T.D. Stokes, 8/5/81], was unable to offer much help. Accordingly, Rowe delved into the literature. But his reading only served to confirm that the formation during replication of single-stranded loops in unbroken circular DNA molecules was a genuine problem with which the molecular biochemists had been wrestling for some years. Watson, in the second edition of his textbook, Molecular Biology of the Gene (1970), had written:

> Particularly puzzling is the absence of free ends and the dilemma it creates about the unraveling of..[circular] structures demands the presence of a molecular swivel(s) about which the non-replicated material can rotate. Unfortunately this idea is very difficult to translate into a precise molecular form. Particularly difficult to comprehend is the process occurring when replication passes over a supposed swivel region. [p.284]

(9) Rowe returned to his wire models. Realizing that it was the interlacing of the two exoskeletal strands in the Watson-Crick with the resultant necessity for unwinding that was causing the problem, Rowe wondered if there was a double-helical arrangement in which the two chains were topologically independent. His model-building showed that this could be attained by having equal lengths of left-handed and right-handed

duplex helices. [G.A. Rodley to T.D. Stokes, 16/6/81] Rowe's investigations in the literature, however, seemed to eliminate this solution. He noted in his copy of the letter he had written to Probine [q.v., (5)-(8)]: "A pity left and right helices aren't observed!"

(10) With his puzzles unresolved, Rowe raised them at the informal biological discussion group he attended when it next met. [q.v., (1)] He interested one of its members in particular, G.A. Rodley, with whom Rowe worked in the Chemistry Department of the University of Canterbury. Although trained as an inorganic chemist, Rodley had an established interest in organic chemistry.[4] He obtained his doctorate in co-ordination chemistry (on the geometry of metal ions) from University College, London, where he studied under Peter Pauling.[5] Whilst in London and on his return to New Zealand Rodley's work had familiarized him with X-ray diffraction crystallography - which provides the most direct empirical evidence of the structure of the DNA molecule. However, he says, "I never got to the stage where I would claim to be a crystallographer." On sabbatical leave at CalTech, Rodley discovered that the co-ordination chemistry which he had studied in inorganic molecules had been found in biological macro-molecules such as haemoglobin. Intrigued, Rodley had spent several years examining oxygen binding in haemoglobin.

---

[4] I recorded an interview with Rodley at the University of Canterbury on 19/11/81. The transcript is the source of all otherwise unacknowledged material concerning him (including direct quotation).

[5] Who had been Watson and Crick's confident during the development of their model of DNA, the source of their information regarding the work of his father, Linus Pauling, whose own structure for DNA [Pauling and Corey (1953)] appeared a bare two months before theirs.

(11) In order to try and answer Rowe's queries concerning replication in circular DNA, Rodley began to brief himself on the chemistry of the molecule. In the course of this, he "came across the interaction of copper with DNA ...a very unusual effect. Copper and metals like it are those that I'd been involved with in the co-ordination chemistry area, so I got involved in doing some work [on copper/DNA interactions]." This apart, Rodley's interest in DNA was, at this time, "completely peripheral" to his professional scientific activities, and was pursued in his spare time. Nevertheless he was receptive to the critique of various aspects of the Watson-Crick model which Rowe was then developing. Rodley considers that his acquaintance with diffraction crystallography had given him "some feeling for the possibility of ambiguities occurring in the X-ray analysis." Rowe's recollection of Rodley's response is that:

> Gordon said, could we not propose an alternative mechanism [of replication] where the key point would be that both strands [of the exoskeleton] would be at all times topologically independent.

(12) Although Rodley was primarily "interested in the basic problem of whether the [X-ray] data could be explained in some alternative structure" (he observes: "I don't think I thought much about the fact of unwinding being a problem, simply accepting that it probably was."), nevertheless he did not leave the question of the adequacy of the Watson-Crick model entirely to Rowe. In reading the literature of polynucleotide conformation studies, Rodley came across a suggestion by Wu (1969) of a four-stranded helical structure for DNA.[6] Reading Wilkins' (1970)

---

[6]Cavalieri and Rosenburg (1961) made a similar suggestion.

response, Rodley felt that Wu had been dismissed "a little too readily" - an impression confirmed by correspondence with Wu.

(13) Rodley's goal early in 1972 was to produce a model of the structure of DNA, consistent with the available evidence, but differing from the Watson-Crick model in that it would not need to unwind when replicating. He "was working on the idea, at that stage, that any alternative model must look pretty similar to a double helix in order for it to fit the X-ray data as well as the double helix." Because of this requirement, Rodley did not question the Watson-Crick base pairing arrangements or that the sugar-phosphate exoskeleton was two stranded. Neither, at this point, did he consider any but a helical configuration of those chains.

(14) In vivo, DNA is intimately associated in the chromosome with other molecules (e.g. protein) and so cannot be observed in isolation. The structure of in vitro preparations of pure DNA is studied in two main ways: electron microscopy and X-ray diffraction crystallography. Micrographs of replicating material have confirmed fairly unequivocally the two-strandedness of DNA. But, although magnifications of about 10^7 are possible [Rodley and Reanney (1977), p.17], the instrumental resolution is insufficient to exhibit details of the conformation of the atomic groups. (See Figure 2.)

(15) The technique of X-ray diffraction crystallography involves X-ray exposure of a cross-section of hydrated salt fibre crystals and, lately, of fully crystalline hydrated salts of DNA, with the resulting diffraction pattern recorded photographically.

In DNA, these photographs show a characteristic cross-pattern. (See Figure 3.) From a scale model of a proposed structure it is possible to calculate the theoretical diffraction pattern it would produce. The mathematical technique now used to do this is known as Fourier analysis, and the results of its application to a given model are that model's transforms. These transforms are mapped onto electron intensity diagrams prepared from the diffraction photographs so that the fit between the two can be determined. (See Figure 4.) The better the fit, the more highly confirmed a proposed structure is. It should be noted that the fit between the Watson-Crick model of DNA and the diffraction data, though good, is by no means perfect. [q.v., IV]

(16) Before a model of a molecule such as DNA can be subjected to Fourier analysis, a check must be made on its stereochemical viability. A structure is said to be stereochemically viable if its constituent atoms are bonded within the possible range of angles, and if there are no adjacent, unbonded atoms which approach each other too closely (close contacts). Two sorts of molecular model are commonly used; wire models - where the bonds and bond angles are represented by short lengths of soldered wire (the atoms themselves not being shown) - and Pauling-Corey space filling models - whose components are designed so that, roughly speaking, they can only assembled if there are no close contacts. (See Figure 5.) In recent years, computors have been used to a greater or lesser degree to improve the determinations of sterochemical viability provided by molecular models.

(17) Rowe and Rodley mulled over their spare-time project

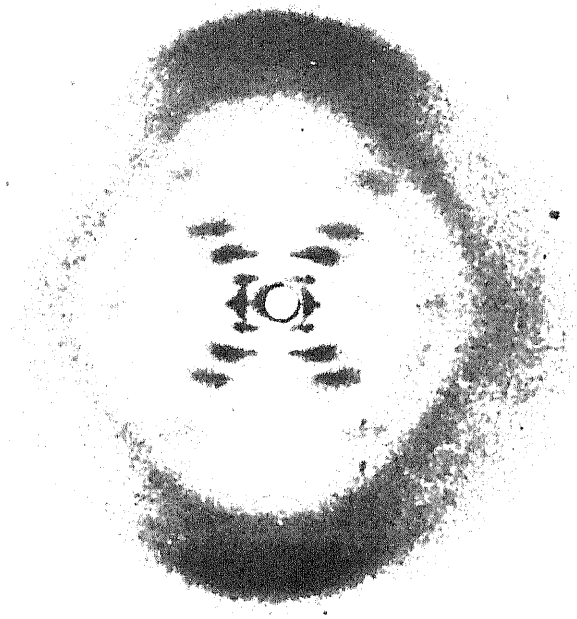


Figure 3 An X-ray diffraction crystallograph of B-DNA taken by Rosalind Franklin in 1952, exhibiting the characteristic cross-pattern.

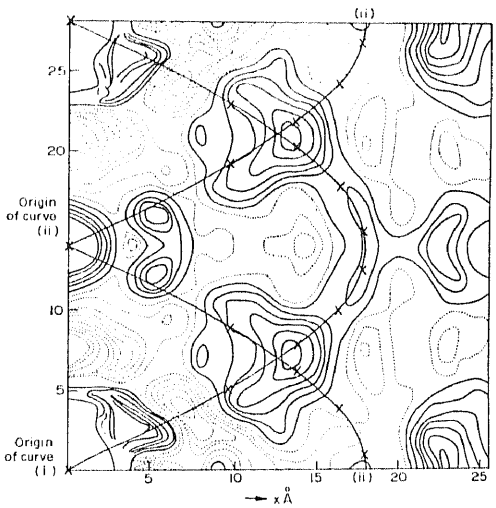


Figure 4(a) An electron density map (cylindrical Patterson function) drawn from diffraction data, with the line of a double helix overlaid.

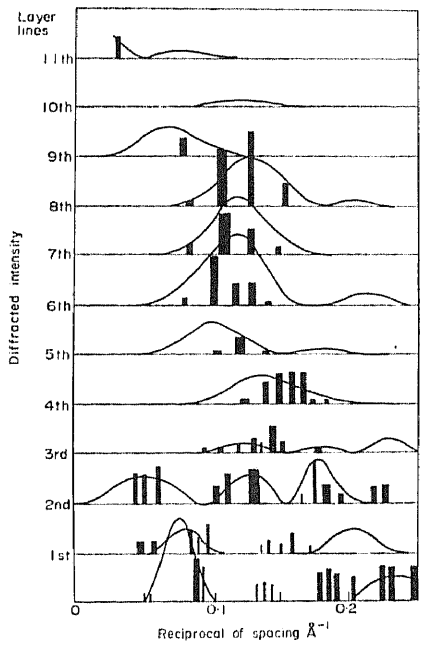


Figure 4(b) Observed intensities of diffraction by NaDNA (rectangles) compared with intensities calculated for the double helix using Fourier analysis (curves).

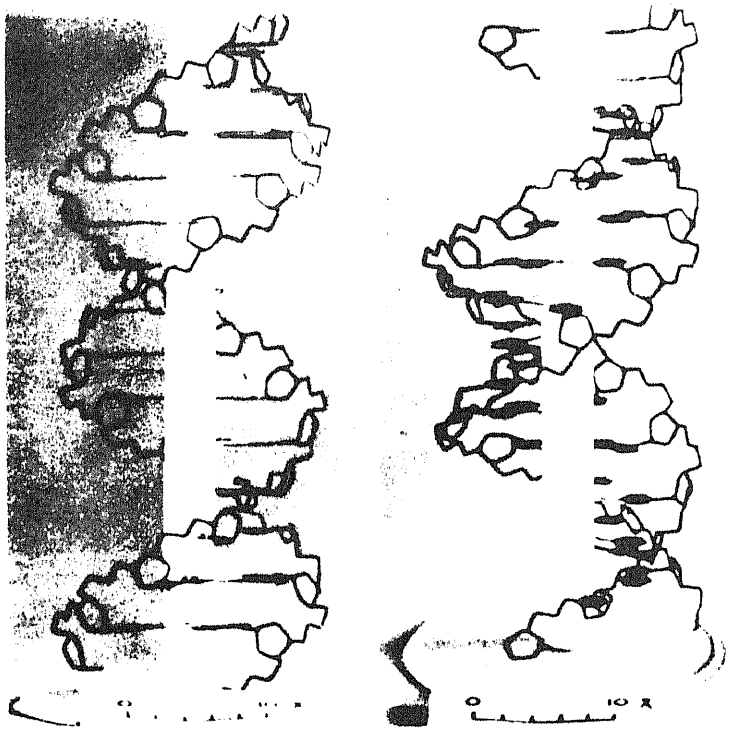


Figure 5(a) Two different views of a wire model of the Watson-Crick double-helical structure.

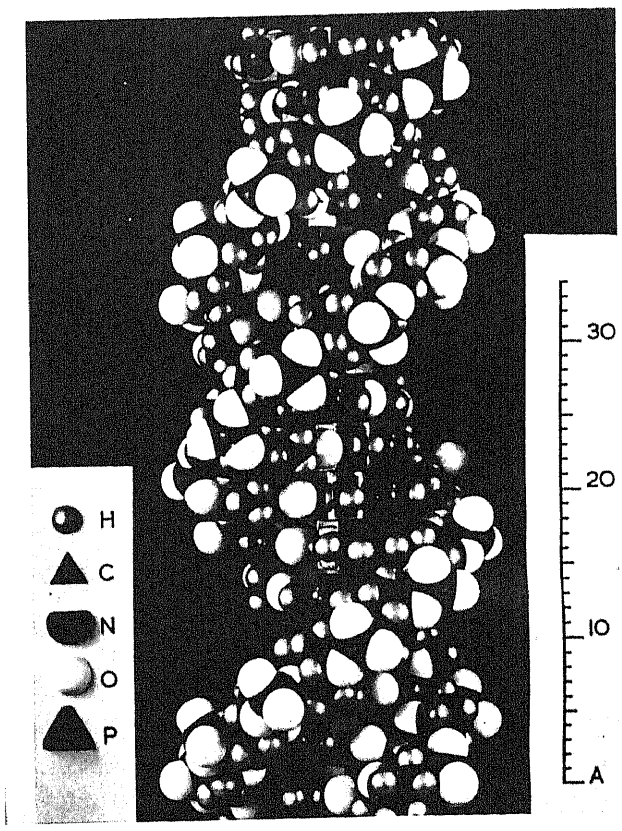


Figure 5(b) A Pauling Corey space-filling model of the double helix.

when they could, the former focusing his attention on developing a critique of the Watson-Crick model and the latter on the possibility of an alternative model of the DNA molecule. Rowe could see no solution to the problems associated with replication of unbroken circular DNA molecules. He was also beginning to worry about other difficulties arising from the necessity for unwinding the exoskeletal strands of the Watson-Crick model during replication. Linear DNA molecules are very long in higher organisms - there is nearly a metre of DNA divided into the twenty three chromosomes in each human cell. Moreover, the molecule is highly convoluted in vivo. In order to take account of these factors, current replication theory had it that linear DNA fragmented prior to replication. Given the short time in which experimental evidence suggested chromosomal duplicates formed, Rowe felt that the rate at which the DNA molecule had to unwind was inordinately high - especially in the light of the low error rate known to occur during recombination. Accordingly Rowe was convinced of the need for a model of the type that Rodley was working toward; viz., one which featured topological independence. In presenting these arguments to Rodley, Rowe found a sympathetic listener, but one who did not really need persuasion since he was absorbed by the challenge of building another model of DNA itself.

(18) Working with simple schematic model building materials such as coloured wire, string and wool, Rodley first built a representation of the double-helical exoskeleton of the Watson-Crick model and then set about trying various arrangements in search of one in which the two strands were not interlaced. In 1974, he came up with a structure that appeared to exhibit the

desired topological independence:

> I found that by putting together two strands, one of which had a right-handed twist and the other a left-handed twist; but putting these on top of one another [laying one chain on the other] they would intermesh to give a structure that superficially looked very similar to a double helix.

Moreover, because of the opposite handedness of the model's strands, they could be separated laterally without unwinding.

(19) Rodley spent the end of 1974 and the beginning of 1975 at the Australian National University in Canberra. He devoted most of his time there to projects in inorganic chemistry, but took the opportunity to explore the literature on the conformation of DNA utilizing the superior library facilities at the A.N.U. Rodley also imported (not without puzzling the Australian Customs officers) wire model-building equipment to explore the viability of the left and right-handed structure he had devised.

(20) On the whole, Rodley's reading discouraged him somewhat. In particular, Rodley's perusal of diffraction interpretation work of Struther Arnott led him to form the "conviction that perhaps the X-ray analysis had completely sewn up the double helix structure."[7] On the other hand Rodley also read the trenchant criticism of the Fourier method as a basis for verification of molecular models of Donohue (1969), and learned of the alternative base-pairing scheme that he had advanced (1956). Wilkins et al.(1970), Crick (1970) and Arnott (1970) had

---

[7] Arnott was a member of the group of crystallographers at King's College, London, who, initially under the leadership of M.H.F. Wilkins, had undertaken the task of testing and refining the Watson-Crick model of DNA. See IV for an account.

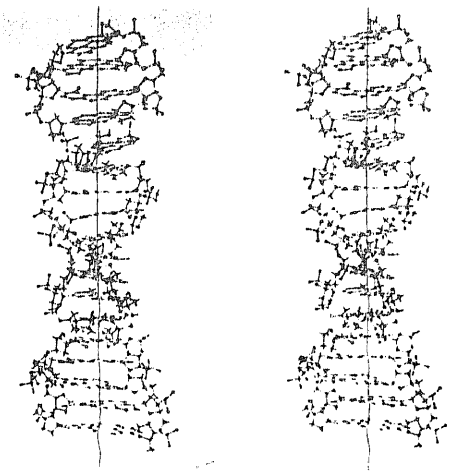
replied to Donohue, but Rodley noted that Donohue, an expert on X-ray studies of the structure of DNA, had, in his rejoinder (1970), been unpersuaded by their defence. [q.v., (92) to (96) for a detailed account.] This argument among the professionals seemed to indicate that there was some room for doubt. But Rodley's enthusiasm was still dampened and, in consequence, he only assembled his model of the left and right-handed structure he had devised "just to a very crude extent, but not to the extent of being able to see very clearly whether this idea really worked or not." In particular, he was still uncertain as to whether his model was stereochemically viable.

(21) Back in Christchurch, Rodley had a research student who was working on the interaction between chromium ions and DNA. At a routine review of his progress, it was suggested that a molecular model would facilitate illustration of the work. Rodley decided to build this himself, asking a senior undergraduate, Ross Scobie, to assist him. Using the wire modelling materials Rodley had taken to Canberra, he and Scobie first built a model of the Watson-Crick double-helical structure. Having finished that, the two men decided to try and build a detailed one left-handed helix, one right-handed helix model so as to check its stereochemical viability. In doing this, they immediately came upon a problem - it did not seem possible to maintain a constant radius of curvature in the exoskeletal curves. Rather, the radius of curvature seemed to have to be constantly changed in order to accomodate a core of Watson-Crick base pairs. A good deal of fairly rough pushing and pulling of the model ensued, in the course of which it occurred to Rodley to try building each sugar phosphate strand of successive half left-handed and half

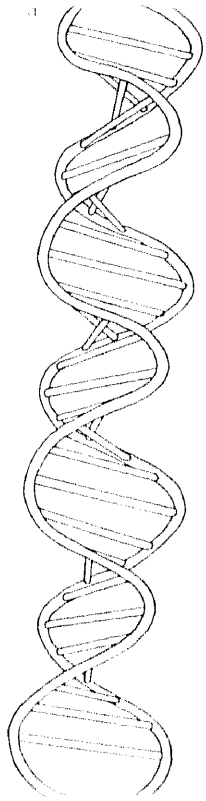
right-handed helices, so that they formed three-dimensional sine curves.

(22) This idea had a number of immediately apparent virtues. It overcame the problem of accomodating the base-pairing whilst closely resembling the single left-handed strand, single right-handed strand model (which, in turn, looked like a Watson-Crick structure). Moreover, like that structure (and unlike the right-handed double helix), the exoskeletal chains of the alternating semi-helical model preserved the topological independence. Rodley's rough calculations had earlier indicated that the one left-handed, one right-handed helical structure would produce the cross-pattern characteristic of both the diffraction photographs and the Watson-Crick model of DNA. The physical similarity of the new sine curve structure to the opposite handed helical model seemed to suggest that the same might be true of it. (See Figure 6.)

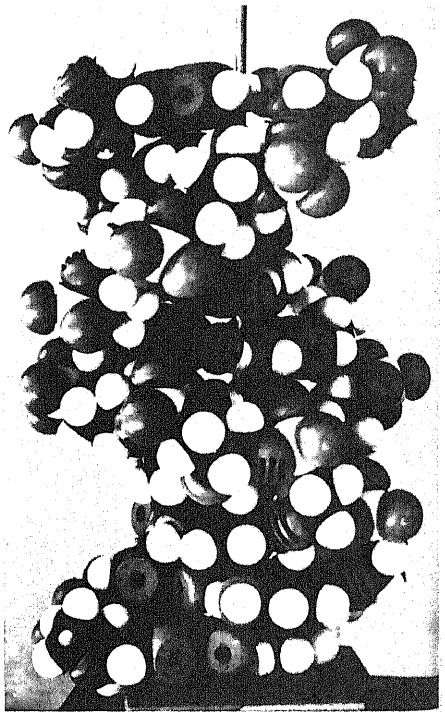
(23) Another member of the informal biological discussion group to which Rowe had brought his problem concerning the unwinding of circular DNA was D.C. Reanney, an evolutionary biologist. At this point, whilst Rodley and Scobie were mulling over their two molecular models of DNA (the double-helical and the semi-helical alternating handedness structures), Reanney gave Rodley a copy of Pohl (1967). There results were reported which suggested "an alteration in the structure of the double helix dependent on ionic strength."[p.616, trans. H. Maxian.] Specifically, Pohl thought this might "signify a reversal of the direction of twist of the helix". [idem, author's emphasis] He then suggested that if parent and daughter molecules were of



Above: Figure 6(a), a stereo view of a wire model of the New Zealand SBS structure.

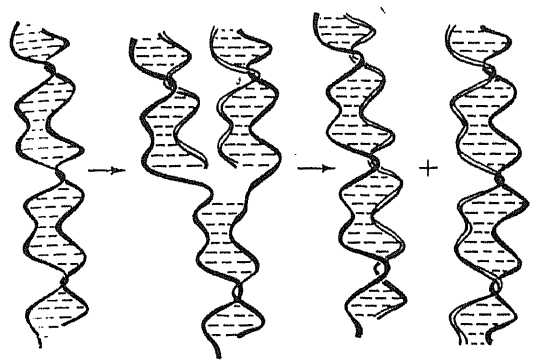


Right: Figure 6(b), a schematic model of the 'warped zipper'.



Left: Figure 6(c), a space-filling model of the Indian Type II SBS structure.

Below: Figure 6(d), a schematic model of semi-conservative replication on the basis of the SBS structure for DNA.



opposite handedness, "topological difficulties in the case of replication of long circular DNA can be avoided".[idem] Pohl noted in conclusion that the mechanism of replication he was advancing was but "one conceivable expansion of the [Watson-Crick] model hitherto suggested but not yet confirmed or excluded by means of direct experiment."[idem]

(24) This last remark encouraged Rodley and Scobie; implying as it did that the structure of DNA was not a cut and dried subject. Furthermore, Pohl's experimental results seemed to indicate that the frequent switches from left to right handedness which their semihelical model of the sugar phosphate exoskeleton required were not implausible. A measure more optimism was provided by a conversation which Rodley had with an experienced protein crystallographer (D. Parry of Massey University) who suggested that the X-ray evidence was not so tight in its confirmation of the Watson-Crick model as to eliminate all doubt.

(25) Their confidence boosted, Rodley and Scobie returned to model-building. They set the length of each half-helix of the exoskeletal chains at five base-pairs, after which there would be a bend and the sense would reverse (right to left, or left to right). Rodley recalls that "we literaly felt at the that stage that we had stumbled on the structure of DNA." Whilst this work on the model was proceding, Rodley read Olby's (1974) account of the genesis of the Watson-Crick structure. This "encouraged us a little more to feel that there may have been something wrong with the [diffraction] analyses." They were struck in particular by Olby's account of R.E. Franklin's doubts concerning the merit of Watson and Crick's firm belief in a helical solution to the

structure of DNA. Seeking to explain this hesitation, Olby had written:

> Franklin was a professional structural crystallographer who distrusted intuitive guessing, and who wanted to solve the structure by direct methods, i.e., without introducing assumptions in the form of hypothetical structures. She was not against helices as such, but against assuming helices when the evidence, in her opinion, was inadequate. [p.374, emphasis in the original]

In support of this interpretation, Olby cites Franklin's comment in lecture notes on the method used by Pauling to develop his model of the polypeptide keratin. Of this approach - which Watson and Crick admired and sought to emulate - Franklin had observed: "the time has come to review evidence and assumptions - have we found the solution or a solution?" [idem, emphasis in the original.]

(26) Arrangements were made for Parry, the crystallographer from Massey University, to visit and inspect the Rodley-Scobie model. Rodley found the detailed questioning of this professional "pretty difficult to answer". Nevertheless, he sought Parry's advice on testing the structure; in particular the possibility of obtaining optical transforms from it for comparison with the X-ray data. A few experiments were subsequently made to this end, but Rodley was insufficiently familar with the technique to be confident of the results.

(27) Clive Rowe now re-enters the story. By this time, he no longer worked for the Chemistry Department of the University of Canterbury. After a period in private industry, he had taken a position as a technician in the Electrical Engineering Department

of the University. He had not, however, lost contact with or interest in the work that Rodley and his student were doing. Rowe knew that the best test of the alternative model they had devised was to calculate the Fourier transforms and compare them with the X-ray data. It happened that the Head of the Department for which he now worked, R.H.T. Bates, was familiar with the mathematical intricacies of the Fourier technique. He also had interests which extended beyond the confines of electrical engineering. So Rowe mentioned Rodley and Scobie's work and, finding that Bates was interested in it, arranged for Bates to see and discuss with them their model.

(28) Bates' first reaction was not favourable: "I thought...this can't be right. On the cover of all the textbooks you see a double helix..."[8] But Rodley's arguments persuaded him that the alternative model was not so easily dismissed, and he offered to help in testing it. Together with one of his students, R.M. Lewitt, Bates calculated the Fourier transforms of the Rodley-Scobie structure and compared them with the best diffraction data they could obtain from the literature. This confirmed Rodley's earlier, rough, calculation that his model would generate the cross pattern characteristic of DNA. Indeed, they found that there was a whole class of structures which did so. Moreover, Bates and Lewitt determined a fit between the Rodley-Scobie model and the crystallographic evidence which was comparable with that for the Watson-Crick model.

---

[8] I recorded an interview with Bates at the University of Canterbury on 20/11/79. The transcipt is the source of all otherwise unacknowledged material concerning him (including direct quotation).

(29) The situation as it now appeared to the New Zealanders was that they had an alternative structure for DNA which was consistent with what was most securely known about the molecule (its chemistry, stereochemistry, Watson-Crick base pairing and semi-conservative replication) whilst resolving the problem of unwinding by eliminating its necessity. They decided, therefore, that the idea merited publication.

(30) Toward the end of 1975 a draft paper was prepared. It contained a critique of the Watson-Crick model with some emphasis upon the problems associated with unwinding. The Rodley-Scobie model was advanced and described as a 'side-by-side' (SBS) structure - thereby stressing the topological independence of its exoskeleton. Rodley felt that there might be difficulties in getting the paper accepted for publication [q.v., (12)]. He was under the (false) impression that papers communicated by members of the U.S. Academy of Science were published in the Academy's Proceedings without refereeing. He happened to know a member, H.B. Gray of CalTech. In the hope that he might recommend the paper and thereby circumvent any problems with referees, Rodley contacted Gray and asked him to look at it. Gray agreed. However he was not a specialist in polynucleotide conformation studies and so sent the paper on to three scientists who were for their informal opinion.

(31) These varied. One informal referee - Jerry Vinograd, also of CalTech - worried whether the SBS model was compatible with the work on circular DNA. Ironically, this work had initially motivated the research which had produced the SBS model. Rodley and Vinograd discussed the question by letter and

tape. Eventually, by split decision, the informal referees decided that the New Zealanders' paper warranted publication. They suggested, however, that its chances would be improved if the criticism of the Watson-Crick model were removed, and if atomic co-ordinates for the SBS structure were supplied.

(32) This latter suggestion posed a problem. The New Zealanders had only wire-model building materials, not the more precise space-filling atomic units. Nevertheless, aided by a computor, it was still possible to do the job. Rodley was not happy with the comparatively primitive techique he was obliged to use but, encouraged by Bates, persevered and a set of co-ordinates was produced. These revealed that there were unbonded atoms rather too close to one another, but they did not seem too severe.[9] Such 'mild' stereochemical problems could be an artifact of the roughish technique and might in any case be eliminated by later refinement of the model.

(33) Thus Rodley, Scobie, Bates and Lewitt prepared and submitted to the Proceeding of the National Academy of Science (PNAS) a revised manuscipt containing these co-ordinates. The critique of the Watson- Crick structure was diminished to a two-line reference to "concern about its intertwined nature"[(1976) p.2959]. The title, 'A Possible Conformation for Double-Stranded Polynucleotides', was matched to a carefully understated and conciliatory conclusion:

> We have constructed a model which demonstrates the possibility of side-by-side association of two intermeshing, anti- parallel polynucleotide strands with the Watson-Crick mode of base pairing. This model has a

---

[9] In fact the wire-modelling approach concealed other short contacts.

> number of attractive features. We present it for consideration as a polynucleotide conformation which may exist at least under some conditions. [ibid., p.2963]

(34) In this revised form, the New Zealanders' paper was refereed and accepted without substantial modification, appearing about twelve months later, in September 1976.

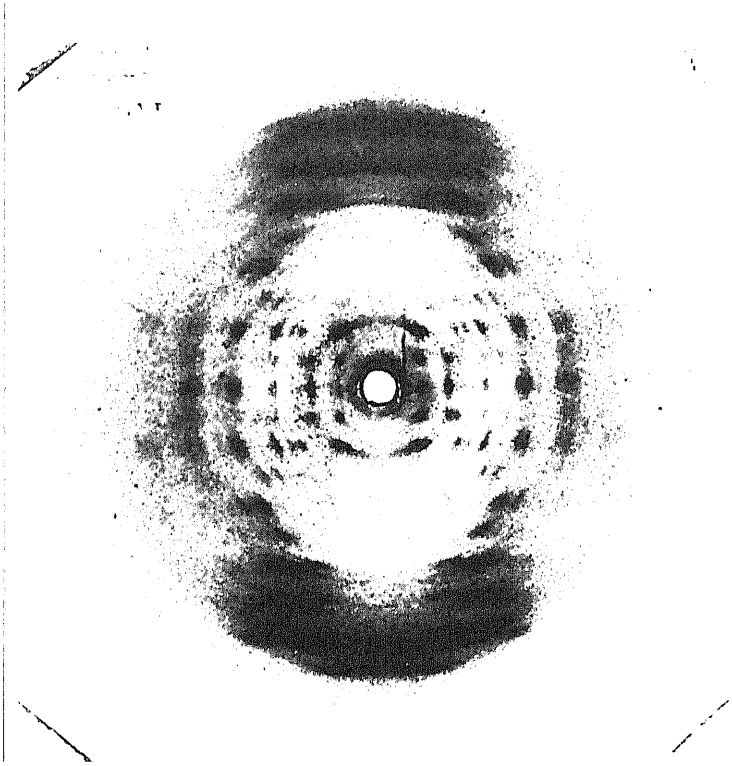
II THE GENESIS OF THE 'WARPED ZIPPER' IN INDIA

(35) In 1970 V. Sasisekharan[10] was Visiting Professor in the Department of Biochemical Sciences at Princeton University. His permanent Chair was at the Centre of Advanced Study in Biophysics in the University of Madras, India. Sasisekharan's field was the conformation of biological macromolecules. [V.Sasisekharan to T.D. Stokes, 7/7/81.] Whilst Sasisekharan was at Princeton, Mitsui et al.(1970) suggested that one kind of DNA might differ from the Watson-Crick model in one important respect, the handedness of its exoskeleton.

(36) The characteristic cross-pattern produced by X-ray diffraction from DNA [q.v., (90)] exhibits sub-types dependent upon the saturation, humidity and cations present in a given specimen. Amongst these are the A-DNA and B-DNA patterns; the latter thought to most closely resemble the in vivo structure (see Figure 7). A sample of DNA can be interconverted from an A-DNA to a B-DNA pattern (and vice versa) by manipulation of one or more of the three variables. Measurement of the spectra of reflected light bands (CD spectra) of A-DNA and B-DNA yield similar results. Fuller et al. (1965) maintained that, although a left-handed double-helical model of B-DNA was not ruled out on stereochemical grounds, a left-handed A-DNA structure involved unacceptably close unbonded atoms. Because of this, and given

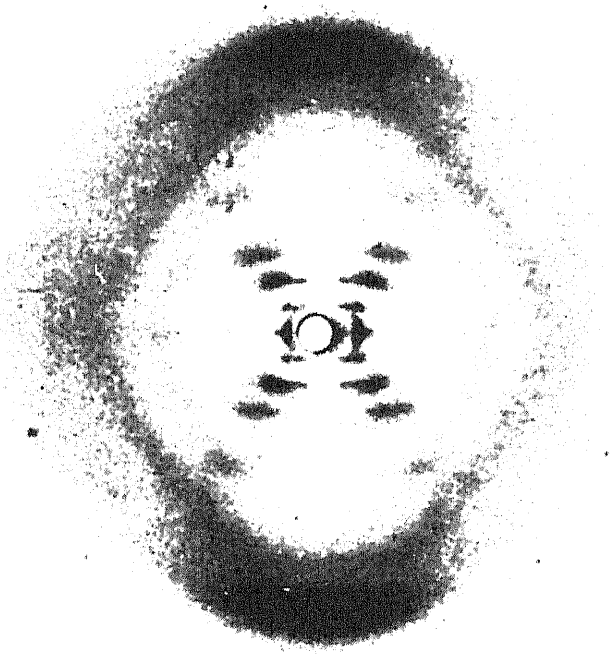
---

[10] I recorded an interview with Sasisekharan at the Indian Institute of Science at Bangalore on 6/12/79. The transcript is the source of all otherwise unacknowledged material concerning him (including direct quotation).



Above: Figure 7(a), an X-ray diffraction crystallograph of A-DNA.

Below: Figure 7(b), an X-ray diffraction crystallograph of B-DNA.



that an alteration of structure so radical as a change of handedness was improbable since the CD spectra were so alike, Fuller et al. concluded that both A-DNA and B-DNA were, as Watson and Crick had suggested, right-handed double helices.

(37) Mitsui et al. (1970) considered an example of another sub-type of DNA, the D-DNA poly d(I-C):poly d(I-C). They reported that they could not interconvert this D-DNA into either an A-DNA or a B-DNA and, furthermore, that its CD spectra was more or less opposite to that of the A and B forms. This led them to suggest that it might be a left-handed double helix.

(38) The response to this proposal was not favourable. Struther Arnott, for example, maintained that an acceptable right-handed structure for the D-DNA could be built which was consistent with the X-ray data. Sasisekharan took an experimental approach. He improved the quality of the D-DNA specimens, obtaining clearer patterns, and found that when he raised the relative humidity to 95% a B-DNA pattern resulted. This seemed to dispose of Mitsui et al.'s suggestion; particularly given the unlikelyhood of a change of handedness resulting from so small an alteration to the sample. Nonetheless, the CD data remained to be accounted for - and many held that the CD spectra indicated the handedness of a helical structure.

(39) Since one of the Mitsui group, R. Langridge, worked at Princeton with Sasisekharan, the two men discussed Sasisekharan's experimental results. Sasisekharan stressed that the difference between the CD spectra of D-DNA and the A and B forms remained to be explained. However, he found Langridge evasive, seemingly no

longer interested in the issue. But Sasisekharan still was; and he began to think about the methodology underlying investigation of the structure of DNA. These thoughts were sparked by the realization that it was "very likely" that Mitsui et al. "had not systematically explored the possibilities." In general, Sasisekharan decided, the procedure had been that a tentative model was built (thus ascertaining its stereochemical viability) and its Fourier transforms were calculated. These were then compared with the diffraction data and, if the agreement was reasonable, the structure was regarded as having been confirmed. But, Sasisekharan realized, "that does not mean that there cannot be another structure consistent with the data [emphasis added]."

(40) Accordingly, Sasisekharan decided to undertake a systematic study of the conformational possibilities for DNA. He had already done some work on polynucleotides (in Madras), from which he had developed an interest in the ring flexibility of the deoxyrobose sugar.[11] This he had pursued, but his enquiries had been limited to single nucleotides by the computing facilities available. The Princeton hardware permitted study of a dinucleotide, even of the polymer structure. Sasisekharan determined to take advantage of this. He took the view that the issue of ring flexibility "would play a very important role in model building of polynucleotides." The argument favouring single

---

[11] Bond angles between atoms may be fixed or flexible over a given range. In a multi-atomic structure (such as the DNA monomer, the nucelotide) the flexibility of the bonds may be reduced or eliminated. Where some flexibility is available, it can be built into a molecular model (giving a number of sub-variants) or one set of bond angles may be chosen. This choice may be based on the overall energetic favourability of the conformation, or it may be arbitrary (say, the mid-point of the range).

right handedness in the DNA monomer was strongly bolstered by assuming a fixed set of angles for its consitituent bonds. And, Sasisekharan reasoned, if "the DNA structure is rigid, then, right from the monomer, the building block itself, it should be rigid. If, on the other hand, [it] is flexible, then you won't be able to arrive at a unique structure."

(41) Sasisekharan's conformational studies were consciously designed to be methodologically superior to those which had been undertaken earlier, notably by Wilkins and co-workers at Kings' College, London and, more latterly, by one of that group in particular, Struther Arnott. (See IV for an account.) In Sasisekharan's mind, Arnott now 'owned' the model-building and diffraction analysis of DNA. His techniques were a benchmark from which to measure and warrant his own work - whether the results were conventional or controversial. In each case it was important to be able to lay claim to an improved approach. If the work were only to confirm Arnott's results, then such a claim would be needed to justify the effort and obtain publication. If, on the other hand, Sasisekharan's inquires were to confound the orthodox view of things, then a clear methodological superiority would be important in establishing their claim to be taken seriously.

(42) Work proceeded at Princeton until Sasisekharan left to take up the Chair of Molecular Biophyisics at the Indian Institute of Science at Bangalore (September, 1972).[12] To that point results "indicated that as far as the backbone conform-

---

[12]The Institute, apart from giving undergraduate and Master's degrees in engineering, is devoted to research. Its students, the cream of the Indian Universities, generally enter with a first class second degree.

ational angles are concerned, both right-handed and left-handed double-helical structures are possible." On his arrival at the Indian Institute, Sasisekharan was determined to continue this work. Accordingly, he sought doctoral candidates to assist him with it. Immediately he struck a wall of (passive) resistance.

(43) Students entering studies in the Molecular Biophysics Unit generally had not been trained in the specialised techniques of structural study of biological macromolecules. Their Master's degrees were normally in a branch of chemistry or physics. It was to the former group, who might be expected to have a greater familiarity with structural biochemistry, that Sasisekharan initially looked for research assistance. But, he found, these students were unhappy at undertaking work for their dissertations which smacked of a potential challenge to established dogma:

> And they refused! ...I tried for two years with two people, and they refused - but they didn't tell me so directly, you know. No progress was made. They told their friends: 'The professor is becoming crazy'.

However,

> Subsequently I tried with two physicists who had no knowledge of DNA... In fact, one of them didn't even know what DNA was. O.K., that was good for me - just so long as they were not programmed.

(44) These two were N. Pattabiraman and Gautam Gupta.[13] Pattabiraman's second degree was in nuclear physics, and Gupta's in particle physics. From the first Pattabiraman and Gupta were both subjected to strong pressure, denegrating the work they were doing with Sasisekharan, from their contemporaries and the more

---

[13] I recorded interviews with Pattabiraman and Gupta at the Indian Institute of Science on 11/12/79. The transcripts are the source of all otherwise unacknowledged material concerning them (including direct quotation).

senior postgraduate students. Pattabirman reports being informed: "'It's a risky problem.' 'It is not worth it - you won't get a Ph.D. out of it.'" His peers "argued that there was already the Watson-Crick model, and they thought it was more than adequate. Gupta was similarly informed that he was burning his fingers with research that would not lead anywhere.

(45) Constantly being informed: "'You can't solve this problem, it's already solved'" was debilitating. At length, Pattabiraman went to Sasisekharan and:

> told him what had been said to me and that I wanted to change my problem. He said that I should try for at least a year. And I agreed...[saying that] if something comes out of it...I'll persevere with it. Otherwise I'll do some other theoretic work and [so] finish my degree.

Gupta's response was a stubborn determination fueled by the opposition. He affected to be blase about the potential effect upon his career. Both men developed a fierce loyalty to, and close working relationship with Sasisekharan who, in turn, took care to provide as much of the encouragement that would normally have come from his students' peers as he could. The three became more of a research team under Sasisekharan's leadership than a Professor supervising two students.

(46) Sasisekharan divided his research on the structure of DNA into two areas. Investigation of the possible stereochemically viable configurations of the exoskeleton he gave to Pattabiraman, whilst Gupta (who began work rather later than Pattabiraman) attended to the base-pair stacking arrangements. Sasisekharan remarks:

> The criteria which we followed were: [1] Watson-Crick pairing must be there because there is no doubt that it is the chemical basis of genetics; [2] models must be stereochemically satisfactory and [3] they should agree

with the data.

Moreover,

> We always had in the back of our minds the various problems associated with the double-helical model.... For example, the unwinding process.

(47) Pattabiraman's enquiries fairly quickly confirmed for the nucleotide polymer what Sasisekharan's work in Madras, and later at Princeton, had suggested was true of the mononucleotide; viz., that the sugar phosphate bond angles of the exoskeletal strands could vary over a significant range. The next task that Pattabiraman undertook was to explore the conformations of sugar phosphate groups allowed by this flexibility. To do this thoroughly was a formidable task - nine or ten variable parameters admitted of 36^{10} - 36^{13} possible combinations. The necessity to utilise a computer to perform this biochemical three-dimensional chess will be readily appreciated. But the facilities at Bangalore, though better than those at Madras, were considerably inferior to those in Princeton. At Princeton, Sasisekharan had hoped to solve for the polymer at once. With the Indian Institute's equipment the job had to be done piece-meal with scale models an essential aid to grasping the whole picture.

(48) For two months after Pattabiraman began work on the exoskeletal chain modeling, the computer generated only right-handed, helical structures. He began, despairingly, to consider the possibility that his peers had been correct and that he and Sasisekharan were unreasonably biased against the Watson-Crick model. Sasisekharan, however, suggested that Pattabiraman go over his computations once more:

> So I analysed the complete theoretical calculations again on the helical generations and, fortunately, I found good left- handed structures coming very

> smoothly.... Then I started concentrating on the left-handed structures...[and] we were able to build a stereochemically viable left-handed structure.

(49) The next stage was to build a left-handed, double-helical model of DNA to see whether such a structure was stereochemically viable and compatable with the diffraction crystallography. The process took a long time. Sasisekharan comments:

> If Struther Arnott had done it, he may have done it much faster. Remember [however] that it took 20 years...to develop even the restricted [refined Watson-Crick][14] model...

Eventually, however, a viable left-handed double-helical model with standard Watson-Crick base-pairing was built and compared with the published X-ray data, first for B-DNA and subsequently for the A, C, and D forms. The comparison proved encouraging. Not only did the left-handed structure fit well, there didn't seem to be much to choose between it and a right-handed Watson-Crick structure.

(50) There is a certain course-work component to doctoral degrees undertaken at the Molecular Biophysics Unit of the the Indian Institute of Science. At the beginning of 1976, when work had proceded to the point so far described, Sasisekharan was giving a course on replication in DNA. One of the students in that course came across a reference whilst preparing a seminar paper which he thought might interest Sasisekharan. McElroy and Glass eds. (1957) contained the papers and discussion of A Symposium On the Chemical Basis of Heredity held in mid 1956. It was one of the papers in this collection, 'On the Mechanism of

---

[14] See IV.

DNA Replication' by Max Delbruck and Gunther Stent, that was drawn to Sasisekharan's attention.

(51) In 1956 the mechanism by which DNA replicated was still very much a moot issue. Delbruck and Stent observed that, given a Watson-Crick model of the structure of the molecule,

> The intertwining of the two polynucleotide chains of the DNA duplex...presents an obstacle to their separation which must be overcome if the macromolecule is to act as a template for replication in the manner proposed by Watson and Crick... [p.700]

One possible way of meeting this difficulty, Delbruck and Stent noted, would be the hypothesis that the "duplex consists of short sections of alternating positive and negative winding numbers." But, they reported, this "possibility has been rejected by Watson and Crick on the grounds that, for stereochemical reasons, they found it impossible to construct a model involving left-handed helices."[p.701- 702, q.v., (69).]

(52) The winding number of a given molecule is the net number of times the two chains are wound around each other. Provided there are no superhelices (overwindings), this is obtained for the Watson-Crick structure by dividing the length of a specimen by pitch of the helices (34 A). Sasisekharan reasoned:

> inasmuch as we have both right-handed and left-handed helical structures, satisfying both stereochemical and X-ray data, why should we not combine the two - which would avoid tangling the two chains.[15]

The question which now arose was whether such a mix was stereochemically viable.

---

[15]From an undated draft reply by Sasisekharan to my intial letter to him (29/6/79). Arrangements were subsequently made for me to visit the Indian Institute of Science where I was given a copy of the corrected draft.

(53) A further consideration in Sasisekharan's mind at this time was the problem caused by the rod-like rigidity of the Watson-Crick model of DNA. It is clear from the sheer length of DNA that the molecule must be highly convoluted *in vivo* - and this is confirmed by *in vitro* electron microscopy. Such a crowded macroconformation necessitates sharp bends and, indeed, this is what the micrographs show. Sasisekharan knew that severe sterochemical difficulties had constantly plagued attempts to construct molecular models of the Watson-Crick structure exhibiting such acute changes of direction in the long axis. Similar, though less severe difficulties confronted construction of a model of the super-helicies (where *both* strands together form a helix - like overwound rope) exhibited by DNA for a Watson-Crick structure.

(54) Thus, Pattabiraman recalls, when considering uniting left and right-handed exoskeletal structures, "it occurred to us, in order to have a flexible model, you need both left and right [handed] helical *sections*" [emphasis added] in each strand rather than complete helices. The most regular example of such a mixture of semi-helical sections in a single strand consisted of half helices, resembling a sine curve on a semi-circular surface. This suggested that the alternation of handedness should occur every half helix and, given the adoption of Watson-Crick base pairing, this meant a switch in helical sense every five base pairs.

(55) A wire molecular model of such a structure was successfully built, suggesting that it was stereochemically viable. In keeping with the aims of their programme, the

structure was then analysed thoroughly, though perforce piece-meal, by computer. Here the base stacking topology that Gupta was investigating proved important, for the Indians had concluded that: "as the conformational energy difference between the various types of backbone [left and right -handed] of the monomer unit of the polynucleotide chain is small, the conformations are mainly determined by the base-base interactions apart from hydrogen bonds."[Sasisekharan and Pattabiraman (1976), p.780].

(56) A DNA base may be regarded as flat, sheet-like atomic groups; one edge bonded to an adjacent sugar, another to the pair-partner. For any base-pair type (A-T, G-C), two of their 'faces' are 'up', and two 'down' (imagine them as two playing cards). Which faces are 'up', and which 'down' is fixed by Watson-Crick base-pairing - though the sequence of base pairs is not. Sasisekharan set Gupta the task of investigating these arrangments and the possibility of varying them by <u>inverting</u> the normal face orientations. This study included examination of the energetic favourability of each conformation taking into account the possible topologies of the surrounding sugar phosphate strands (left-handed at both ends of the base-pair, right- handed at both ends, and opposite-handed at each end - a 'bend region').

(57) First results indicated that (a) on the basis of chemical and stereochemical considerations, certain pairs of bases could adopt inverted stacking, (b) Watson-Crick base pairing seemed compatible with sine-curving exoskeletal chains, and (c) the use of inverted base pairs at the bend regions of the strands was a more energetically favourable arrangement than

using a Watson-Crick orientation. Thus two variants of a structure utilizing alternating left and right-handed semi-helical strands were produced. The Type I model employed conventional Watson-Crick base-pairing and the Type II structure incorporated inverted base pairs at the bend regions. Both variants seemed stereochemically fairly tolerable.

(58) Whilst Sasisekharan was concerned about some close contacts which the Type I model exhibited, he was more worried about the Type II structure. There was, he felt, the air of an artifact of the model-building process about it. It seemed "unnatural", ad hoc in the sense of having been devised to yield energetically favourable bend regions. Accordingly, he asked Gupta to investigate the literature on the conformation of paired structures similar to DNA bases to see whether others had noted inverted stacking occurring. This work yielded an encouraging result: in fully half the structures reported in the literature Gupta examined, inverted stacking was reported.

(59) Although convinced that inverted stacking was present in nature, Sasisekharan moved cautiously. He knew that he was entertaining an idea which ran against the received view of things and that it it is "so easy to be overwhelmed by enthusiasm" for a new idea of one's own.

> In fact we hesitated to publish because I wanted to be sure that I hadn't made a mistake, and that none of my students had made a mistake. One does not want to rush into print when one's whole reputation is at stake. I've been in this field many years. I cannot afford to make a mistake. That is a conservative way of looking at things - maybe.

Certainly this prudent approach seemed overly fastidious to some of Sasisekharan's colleagues:

> They said, on such things, one should come up with [results] very fast. On the other hand, I am in the field and I want to protect my reputation. If, however, I was outside the field, I would be prepared to be more bold. But, having subscribed to...[the discipline's] ideas, I have to be extremely cautious...

(60) The work Sasisekharan, Pattabiraman and Gupta were doing had been aired informally in conversations between the three men and those of their colleagues working or with an interest in the field. But it had not been discussed outside the Indian Institute - partly for the reasons given the in preceding paragraph. In particular, nothing had appeared in print. Early in 1976, Sasisekharan decided that the forthcoming Divisional Review of the Chemical and Biological Sciences at the Indian Institute would provide an occasion to formally present some of their results. However, it was decided to talk about only the Type I alternative structure for at the time the detailed argument favouring the inverted stacking of the Type II model was not yet ready for presentation. The encouraging reception the group received led them to prepare a manuscript for submission. This paper presented both types of alternative model, stressing as their principal advantage that they avoided intertwining of the exoskeletal strands. The results of the work on inverted base stacking was discussed in some detail, but no claims were made concerning the compatibility of the structures with the diffraction data. It was made clear that the results reported were both tentative and preliminary.

(61) This article was submitted to the Indian journal Current Science where, upon receipt, it was forwarded for refereeing. The verdict came as a shock for, in the interim, Rodley et al. (1976), advancing their SBS model - which was identical to the

Indian Type I alternative structure - had appeared, and the referees recommended publication of the Indian paper only on the condition that the New Zealanders' priority was acknowledged in a terminal note. The Indians' initial response was bitter disappointment at having been pipped at the post. Once having seen the New Zealand paper this feeling was somewhat alleviated for it then became apparent to the Indians that their work differed significantly from the New Zealanders' in that not only did they have two alternative models of DNA, their approach to the problem had also been at once more general and more sophisticated than was that of the group led by Rodley. Nevertheless, the demand for acknowledgement of the New Zealand work rankled:

> [W]hat had upset me was that I [had] already submitted the paper before Rodley's article appeared, and it was only when the referee told me that I learned of the New Zealanders' work in P.N.A.S. The referee felt that it was his duty to point out the New Zealand work. I don't think that in any other country a referee would have insisted, as this one did, on that acknowledgement as a condition of publication. He didn't believe, I don't think that I hadn't seen Rodley's work.

All the same Sasisekharan aquiesced, and the paper appeared in the November 20th., 1976 number of Current Science with a terminal note acknowledging the New Zealand work.[16]

(62) Meanwhile work proceeded. The principal upshot of it was to convince the Indian group to plump for the Type II alternative model. In conjunction with computer structural studies, space-filling scale models of the two Types were built. It was then discovered that the close contacts in the Type I structure

---

[16]Given the prior appearance of the New Zealanders' work and the fact that it was noticed by the referees, some acknowledgement would have been customary. However, a more normal procedure would be an editorial interpolation.

were recalcitrant. According to the criteria that Sasisekharan had earlier set [q.v., (46)], this ruled it out of contention. Research now concentrated on supplying the details of the Type II structure that had been promised in the initial paper.

(63) A second paper was prepared, with all three Indians as co-authors. It announced the elimination on steric grounds of the Type I structure (noting its resemblance to the New Zealander's model). The chemical and structural details of the Type II structure were supplied, along with a stereoscopic picture of a wire model and a photograph of the Pauling-Corey space-filling model. A schematic diagram of the semi-conservative mode of replication based on a Type II structure was provided; as was a photograph of a schematic model of super-helical conformation. The ability of the Type II structure to avoid tangling of the exoskeletal strands was once again emphasized. The greater flexibility of the Type II model as compared with the double helix was stressed - particularly its ability to easily conform super-helically where close contacts resulted when the same was attempted with a Watson-Crick structure. This second paper was submitted to P.N.A.S. - where, it will be recalled, the New Zealnders' had published their model - appearing in September 1978.

III THE UNWINDING PROBLEM

(64) It will be apparent that problems stemming from the need for rapid unwinding during replication were important in motivating the research that led to the development of the SBS alternatives to the Watson-Crick model of the structure of DNA.[17] However the Indians and New Zealanders were by no means alone in worrying about unwinding. Difficulties associated with it have concerned scientists working on the structure of DNA from 1953 to the present.

(65) When preparing the initial paper advancing the double-helical model of DNA, Watson and Crick were drawn on the horns of a dilemma. On the one hand they wanted to indicate that they realized the potential of their model for explaining genetic coding and chromosomal replication. On the other they they did not want to over- commit themselves for fear that they had made some fundamental error. Consequently they contented themselves with the observation that the base-pairing and strand - base complementarity of their model "immediately suggests a possible copying mechanism for the genetic material"[(1953a), p.737].[18] A month later, their confidence bolstered by the reception of the

---

[17] q.v., (5), (6), (8), (9), (11), (12), (23), (33), (46), (50), (51) and (52). The terms 'side-by-side' and 'SBS' were coined by the New Zealanders to describe their alternative model of DNA. To begin with the Indians referred to their alternative structures as the Types I and II. Latterly [e.g., Sasisekharan (1981)] they have used the initializm 'RH' (left and right-handed). Discussion in the literature has tended to favour the New Zealanders' term or that of Arnott, the 'warped zipper' [q.v., (115)]. Here I follow this trend.

[18] See: Crick (1974), p.137-138.

model, Watson and Crick took up its "genetical implications". This second paper contained the first statement of semi-conservative theory of replication (as it was subsequently called); an idea quite as momentous as the structural hypothesis that had inspired it. Watson and Crick made it clear that since "the two chains in our model are intertwined, it is essential for them to untwist if they are to separate." They acknowledged that "a considerable amount of uncoiling would be necessary", admitting that "it is difficult at the moment to see how these processes occur without everything getting tangled"; yet they did "not feel that this objection will be insuperable." [(1953b), p.966]

(66) In their (1953c) Watson and Crick raised again the "fundamental difficulty" posed by the necessity for unwinding. There they explained that there are "two main ways in which a pair of [same- handed] helices can be coiled together...[,] plectonemic coiling and paranemic coiling" [p.128]; their model employing the plectonemic or anti-parallel option wherein the exoskeletal strands wound into one another and, in consequence, are "interlaced". Paranemic or parallel coiling, they said, "is found when two separate helices [of the same sense or hand] are brought to lie side-by-side and then pushed together so that their axes roughly coincide."[idem] But, Watson and Crick note, "[t]hough one may start with two regular helices the process of pushing them together necessarily distorts them."[idem] Thus, they conclude, "[i]t is impossible to have paranemic coiling with two regular helices going around the same axis."[idem] And, Watson and Crick warn, the "point can only be grasped by studying models[idem, q.v.,(70)]."

(67) Watson and Crick displayed the size of the problem posed by unwinding by calculating the number of turns required to complete separation of the exoskeletal strands. With a pitch producing one complete helix every 34 A, there would be about 150 helices every 5000 A along the molecule. Thus they give a figure of 1000 turns as a minimum - assuming fragmentation of the molecule in vivo. On the assumption of intact separation, Watson and Crick fix an upper limit at 20,000 revolutions for viruses and up to three orders of magnitude greater for higher organisms.

(68) The problem would, Watson and Crick observe,

> be more simple to resolve if successive parts of a chromosome coiled in opposite directions. The most obvious way would be to have both right and left handed helices in sequence but this seems unlikely as we have only been able to build our model in the right-handed sense. [ibid., p.129, emphasis added]

Simultaneous separation and replication (no single-stranded stage) and tension relieving breaks (fragmentation) in the molecule during unwinding Watson and Crick saw as ameliorating somewhat the difficulties inherent in the process. Nevertheless, they admitted, "the difficulty of untwisting is a formidable one"[idem]. Given their objections to paranemic coiling,

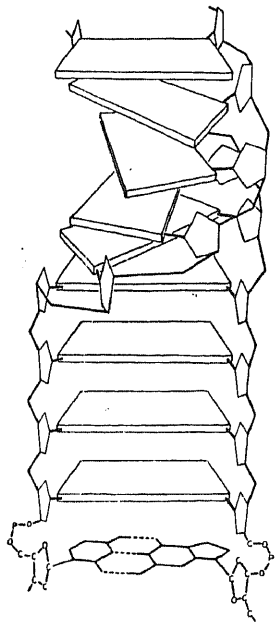
> We should ask...whether there might not be another complementary structure which maintains the necessary regularity but which is not helical. One such structure can, in fact, be imagined. It would consist of a ribbon-like arrangement in which again the two chains are joined by specific pairs of bases...but in which the sugar-phosphate backbone instead of forming a helix, runs in a straight line at an angle of approximately 30° off the line formed by the pair of bases. [idem, emphasis added]

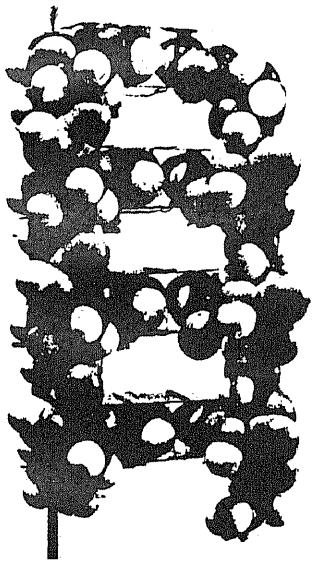
Such a model, Watson and Crick noted, would yield some but not all of the features exhibited by the X-ray diffraction photographs of DNA. They were, however, "not enthusiastic" about

the proposal although "it has not yet been disproved."[idem]

(69) There is, as far as I have been able to ascertain, no further reference in the literature to this non-helical structure, nor any other completely non-helical proposal until 1978. Cyriax and Gath (1978) advanced an alternative model of DNA motivated by the unwinding problem which is frequently grouped with the SBS proposals [for example by Arnott (1979) and Crick et al. (1979)]. However Cyriax and Gath do not advert to the warped zipper structures. Most interestingly, their 'cis-ladder' model resembles closely the description given by Watson and Crick above - though, one presumes, it was independently derived. Cyriax and Gath provide drawings and photographs of space-filling models of their structure exhibiting its principal advantage - great flexibility - but they do not comment on its compatibility with the diffraction data. The model was intended as an intermediate conformation, occurring during replication (to facilitate separation) only - the presumption being that the usual conformation of DNA would be the Watson-Crick structure [see Figure 8].

(70) Gamow (1955) and Linser (1955), concerned by the unwinding problem of the Watson-Crick model for DNA but evidently unaware of Watson and Crick's (1953c) defense of plectonemic coiling on stereochemical grounds, independently proposed paranemic coiling. Gamow also suggested that plectonemically coiled, right-handed DNA could retain topological independence if compensated by left-handed super-coiling [see Figure 9]. Commenting on these proposals, Crick (1957) recalled his and Watson's earlier discussion; adding:

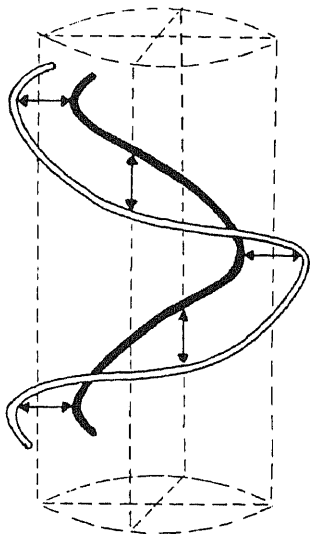




Above, left: Figure 8(a), a schematic model of the conversion of a double-helical structure into the 'cis-ladder' non-helical conformation for DNA suggested by Cyriax and Gath.

Above, right: Figure 8(b), a space-filling model of the 'cis-ladder'.

Below: Figure 9, Gamow's paranemic (parallel) double helix, exhibiting topological independence.



B.

> The two authors putting forth this idea [paranemic coiling] are apparently unaware of the convention that for a structure to be given serious consideration it must be possible to build a scale model of it having acceptable bond distances and angles - inspiration by itself is not enough. Until a satisfactory model has been presented this idea must be regarded as incorrect.[p.533]

(71) Delbruck and Stent's (1957) [q.v., (50) and (51)] discussion of potential mechanisms for replication reiterates much of Watson and Crick (1953c), agreeing that:

> The chief reason for hesitating to accept the topologically simple solution of unwinding the two threads [sic!]is that it involves a very large number of turns,

adding:

> and, ...for each turn to be unwound, essentially the whole mass of the duplex has to be rotated one complete turn around its axis. [p.702, author's emphasis]

they also provide an estimate of the rate of unwinding of 200 revolutions per second, pointing out that it has to take place in a "bent, folded, and coiled" molecules where torque effects were likely to complicate matters still further [p.703].

(72) A decade later, Cairns and Davern (1967) reported that:

> In the intervening years a variety of experiments have shown that the two strands do indeed separate and pass, apparently intact, to the daughter molecules. But the unwinding process has remained a mystery [p.65].

By that time a number of enzymes had been discovered in the chromosome which, in vitro, demonstrably cut and spliced DNA so that it was no longer necessary to suppose that the molecule replicated intact. The degree of fragmentation in vivo, however, had not been established. But, Cairns and Davern noted, there was no reason to believe that "every nucleotide link of the parental molecule is broken and rejoined during the replication process [ibid., p.67]." The greater the fragmentation of the molecule,

the more difficulty there was in understanding the low error rate during replication. The consensus was that the fragments were fairly long. Thus unwinding still had to be postulated.

(73) The 'driving motor' of unwinding had, Cairns and Davern observed, been variously located at the replication fork, the joint between parent and daughter molecules, and the movement of molecules associated with DNA. Still, they held that the source of energy powering unwinding remained unknown. And, concerning the key question of "whether DNA can be shown to rotate during replication", they considered that:

> As yet, no experimental approach has been devised that might answer this question and thereby distinguish between break- reunion [total fragmentation] and the various models of... unwinding [ibid., p.69].

But, Cairns and Davern noted in conclusion,

> Ignorance of the forces that cause (or accelerate) these movements in vivo has not been a limitation in elucidating the sequence of events during retrieval of genetic information [idem].

(74) The unwinding problem was perhaps most acutely and widely felt in studies of closed circular DNA; an example being the discovery of single-strand loops without strand breakages or superhelical formations in partially separated circular duplexes [q.v., (6)-(8)]. Cairns (1963) proposed the molecular swivel solution which Watson [(1970) q.v., (8)] found sufficiently dubious to raise the possibility that the problem itself might be an artifact of specimen preparation and not truely representative of the in vivo situation.

(75) Górski, a Polish biologist, provided an extended and thorough examination of the unwinding problem in his (1975). He

holds that:

> the source of the difficulties are two properties of nuclear DNA: (1) the considerable length of the genophores or double helices included in the chromosome; (2) the circumstance that the double helices (including the genophores) many times coiled in the chromomeres or bacterial nuclear spaces. To these structural properties of nuclear DNA a third should be added, namely the length of time during which the strand separation should be performed. Since these times are short, and the double helices relatively long, the angular velocities of the revolving helices must be large (several thousand revolutions per minute) [p.104].

Görski takes as his example the DNA of the common gut bacterium E. coli (as Clive Rowe had independently done, reaching remarkably similar conclusions [q.v., (7)]). He gives its typical length as 960 microns.[19] From the pitch of the Watson-Crick helix, 34 A, Görski calculates the number of complete helices in an E. coli's DNA as approaching three hundred thousand (2.82×10^5). He notes that replication of this bacterium's genetic material takes place in between thirty and forty-one minutes. Thus strand separation must take place at a rate of between 6900 and 9000 revolutions per minute (115 - 150 revolutions per second). This, Görski observes, is a significant fraction of the velocities achieved by high speed centrifuges [ibid., p.90ff.].

(76) Görski notes that synthesis of new strands and bases to form daughter molecules is held to accompany separation, which entails that the various molecules other than DNA associated with this process must revolve concommitantly with it. Moreover, he points out, this all takes place in an aqueous environment, introducing frictional and peturbative factors. Viscus drag, which some have thought would moderate events, must, Görski

---

[19] 1 micron = one thousandth of a millimeter; thus 960 microns = .96mm.

observes, be overcome by the unwinding motor to achieve the needed rotatory speed. Turning to examine strand separation in higher organisms (Eucaryota), Görski argues that a minimum rate of unwinding (assuming a typical fragment length of not less than 600 microns) of 500 revolutions per mimute is required with there being reason to believe that it might well be much higher.

(77) These considerations led Görski to conclude:

> that the separation mechanism of the strands of double helices based on their unwinding is a succession of processes or events characterized by low probabilities of realization. Therefore their result, namely the replication of DNA double helices based on the rotatory unwinding of their strands, is a complicated operation with the probability of realization so low that it should rather be envisaged as an impossibility [ibid., p.94].

(78) Görski then turns to consider proposals for separation of the exoskeletal strands other than by unwinding, rejecting each in turn. After dismissing Brownian motion as a possible mechanism [ibid., pp.94-98], Görski considers Pohl's (1967) proposal, employing left-handed helices; an idea which had encouraged Rodley when he had come across it [q.v., (23) and (24)]. Pohl's proposal is ruled out, Görski contends, because such left-handed helices are a "stereochemical impossibility" [Görski (1975), p.99]. Görski then turns to Gamow's (1955) suggestion of off-setting right-handed coiling with left-handed supercoiling [q.v., (70)]. This possibility Görski finds doubtful on two main grounds: the very large number of super-helices which must be formed at the time of replication and the difficulty of making a Watson-Crick double helix conform as a superhelix. A proposal by Blatt (1955) Görski rejects too, because it employs a separation mechanism essentially the same as that of Gamow.

Görski then discusses the idea advanced by Platt (1955) whereby replication occurrs at two sites, both perpendicular to the long axis of the parent molecule whose two strands rotate in opposite directions. But, Görski argues, Platt's proposal involves exoskeletal strands that are not complementary - a crucial feature of Watson and Crick's model of DNA. Moreover, Görski calculates that Platt's mechanism still requires an improbably large number of revolutions to achieve separation. Thus Görski rejects it also.

(79) Görski notes that he could find no experimental evidence in the literature to support the claim that strand separation occurs by unwinding. Having argued that such a mechanism, and all the hitherto published alternatives to it, are untenable, Görski concluded that:

> This being so, it becomes necessary to look for other mechanisms based on new principles. The present study was undertaken with the aim of stimulating researches in this direction.

(80) None was forthcoming, partly, no doubt, because Görski had published his work in the Polish journal Folia Biologica. In his (1976), Görski returned to the unwinding problem. There he reported that, even before his (1975):

> The failure of our search for a more plausible separation mechanism finally suggested [to] us the idea that its principle should be sought in a local deformation, or curvature, of the cellular space-time. [ibid., p.158]

The paper then went on to elaborate the proposed mechanism in great detail (replete with appendices full of metric tensors). But Görski's tongue was firmly in his cheek. His (1976) concluded:

> The author of this study is perfectly aware that the proposed separation mechanism of the strands of a DNA

> double helix *in vivo* and based on space curvature will be received as an extravagance perhaps not deprived of some inventiveness. In his opinion, however, a still greater extravagance is the hypothesis that the separation mechanism consists in rotatory unwinding of the strands of DNA double helices. As ...he attempted to show [in Görski (1975)] that... operation ...is characterized by a probability of realization so low that it should be regarded as an impossibility [p.171].

(81) Pohl and Roberts (1978) made an even stronger claim, namely that separation of the exoskeletal strands of DNA by unwinding was in principle impossible. They argue that, assuming that there is an unwinding mechanism, the motor, energy source, enzymatic controls and precursors for synthesis of new strands and bases must be *local* – and this is the usual view taken. When unwinding has taken place, any net rotation of this mechanism *opposite* to the direction of the unwinding itself (due to torque effect) will mean that the parental strands remain linked to some degree. Even if torque does not produce such linkages, the topological changes in the orientation of the hypothetical local unwinding mechanism as it follows the convolutions of the DNA molecule *in vivo* will do so. This establishes what Pohl and Roberts call the alignment problem – i.e., how to eliminate the coiling due to rotation of the unwinding mechanism so as to achieve topological independence of the exoskeletal strands. And it is this problem which they believe to be unsolvable.

(82) The received view is that the alignment problem is resolved by the action of an enzyme present *in vivo* (in addition to those which cut and splice DNA). Denhardt's (1979) model for the action of the enzyme gyrase is this:

> on binding the DNA wraps around the enzyme...ATP then interacts with the enzyme to induce a conformational change that results in the translocation of the DNA relative to the enzyme and the formation of a positive superhelical loop of DNA. [A] nicking and closing cycle

> (or breakage and reunion if a double-strand break is transiently introduced) removes the positive superhelical turn in the isolated section of DNA. ATP hydrolysis returns the enzyme to its intial conformation and leaves the DNA with a net negative superhelical twist. The end result is that the DNA has been partly unwound [p.197].

(83) Pohl and Roberts maintain that, even given that there is an enzymatic action of the sort just outlined, it could not reduce the linking number to zero (topological independence). For, assuming that the linking events are all movements of the local unwinding mechanism, it would be necessary for the enzyme system to store, process and act on information concerning such events. But this would be to postulate a capacity for detection and response of such complexity that it is "almost intelligent". Moreover, Pohl and Roberts contend, the linking number is partly a product of events taking place other than at the local unwinding mechanism - for example the effects of thermal agitation - which, if they occur after the unwinding mechanism has passed a given point along the molecule, cannot be detected by that mechanism. According to Pohl and Roberts, it is at least highly implausible that the information required to correct for linking movements of a DNA molecule could be obtained from some feature of that molecule, or even from some feature of the nucleus.

(84) Having been led to the conclusion that DNA is not double helical, Pohl and Roberts observed that the SBS model of DNA advanced by Rodley et al. (1976) is, because of the topological independence of its exoskeltal strands, not subject to the alignment problem which they held to be an insuperable difficulty facing the Watson-Crick structure. Thus, they say, "we hold that

it [the SBS model] is the simplest interpretation of the evidence that the two strands of the bacterial chromosome are unlinked [(1978), p.400]."

(85) In his (1974) Crick observed:

> Looking back, I think we [i.e., he and Watson] deserve some credit for not being inhibited by the difficulty of unwinding which we clearly recognised and for out forthright stand against paranemic (as opposed to plectonemic) coiling. In this instance our grasp of X-ray diffraction was invaluable [p.141].

It is to the evidence of the structure of DNA provided by X-ray diffraction crystallography that I now turn.

IV TESTING AND REFINEMENT OF THE DOUBLE HELIX

(86) In their initial paper advancing the double-helical model, Watson and Crick wrote:

> The previously published X-ray data on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. [(1953a), p.737]

As they then remarked, some of these were provided in the communications of the King's College crystallographers following hard upon their own.

(87) Wilkins, Stokes and Wilson held that, whilst their interpretation of the X-ray photographs "is not without ambiguity", "in general there appears to be reasonable agreement between the experimental data and the kind of model described by Watson and Crick". [(1953), p.739] Franklin and Gosling agreed - though they were even more cautious - maintaining that their diffraction work was "not inconsistent with the model proposed by Watson and Crick".[(1953a), p.741]

(88) In the view of the King's College group, whilst:

> The approximate diameter and pitch of the nucleic acid helix were derived directly from the X-ray diffraction pattern, and it was clear that two or more polynucleotide chains were twisted around one another in the nucleic acid molecule, and that the chains were bound by hydrogen bonds between bases. [Feughelman et al.(1955), p.834]

Nevertheless

> Proof of the structure was necessary because the preliminary rough agreement between the X-ray data and the model was not so good as to exclude the possibility

> that a structure of some other kind might correlate better with the data. [Hamilton (1968), p.634]

It was a task they proceeded to undertake. One of this group, Hamilton, has described the method employed:

> The analysis was...conducted by atomic groups. Scale models of DNA were built with the stereochemical features of the nitrogen bases, sugar and phosphate groups determined from published structures of simpler compounds. No conformation was allowed in which non-bonded atoms approached one another at less than their van der Waals's distances. The intensity pattern produced by a crystal incorporating such a model was calculated, as well as the contributions made by the three component groups; all were compared with the observed diffraction. The groups were then adjusted in position to improve the agreement between the observed and calculated diffractions. [(1968), pp.634-635]

(89) The first fruit of the programme was to establish that the fit between the Watson-Crick structure and the diffraction evidence was not good enough. In particular, the diameter of the model was too large. [Wilkins, Seeds, Stokes and Wilson (1953), and Franklin and Gosling (1953a)] A modified structure was advanced in Feughelman et al. (1955) and, in his (1957), Wilkins felt able to report that "further work has shown that the correctness of the structure is established with reasonable certainty [p.14]." However still further work was in progress, of which Wilkins said:

> It might seem that such elaborate X-ray diffraction approaches are not necessary now that the structure is fairly firmly established but, in view of the importance of the molecule, it is desirable to eliminate all possible doubts and ambiguities concerning its structure.[ibid., pp.14-15]

(90) As has been noted, the characteristic cross-pattern produced by X-ray diffraction from salts of DNA exhibits variations which are dependent upon, and interconvertable by alterations in the saturation and humidity of the specimens. Early conformational studies concentrated on the B-DNA pattern.

Studies of A-DNA and C-DNA followed. Hamilton's (1968) review of the work of the group at King's College, London, reports that they established that, in each case, "the diffraction data are satisfactorily accounted for by the basic double helical structure incorporating the same Watson-Crick base pairing scheme."[p.634] In his view, this had "gone a long way to establishing that the double helical feature and the base-pairing scheme is unique, and is a much more convincing demonstration of the correctness of the general structure hypothesis than had one conformation alone been studied."[p.636, emphasis added] Indeed, Hamilton argues, "X-ray diffraction and model building have elevated the Watson-Crick pairing hypothesis to the level of experimentally established fact."[p.636, emphasis added]

(91) The crystallographer Donohue (1956) pointed out that the stereochemical constraints, by themselves, did not determine the Watson-Crick base-pairing scheme; and he suggested another mode. Hoogsteen (1959) also proposed an alternative base-pairing scheme. Hamilton (1968) conceded that evidence other than the diffraction data was indecisive. However he maintained that the Fourier method of calculating the theoretical diffraction pattern of a model (transforms) which had replaced earlier techniques had "excluded any significant participation of base-pairs other than those of the Watson-Crick type from the DNA structure."[p.636] In reply to this and similar claims, Donohue mounted an attack which sought to demonstrate that:

> the published X-ray diffraction data from DNA are incapable of being analyzed by the Fourier method for the purpose of obtaining evidence as to what the structure of that substance is, not to mention refining a proposed model. [1969, p.1095]

To support this contention, Donohue argued that Fourier analysis

did not exclude as inconsistent with diffraction evidence a structure known on other grounds to be incorrect. He characterised Fourier analysis as "'pulling-yourself-up-by-your-bootstraps'"[p.1094], citing other workers' view that "Fourier synthesis...is not a good test of a proposed structure; it always tends to support the hypothesis upon which it is based."[Pinnock et al.(1956), cited in Hamilton (1968), pp.1094-1095]

(92) Donohue's claims stimulated a flurry of responses, published together with a rejoinder. Wilkins et al.(1970) conceded to Donohue that "it is undesirable to use Fourier sytheses to prove directly that a structure is correct." However, they asserted that Fourier methods "can readily show that the alternative structures are incorrect, thereby providing proof by elimination."[p.1693] Furthermore, Wilkins et al. believed that both the Donohue and the Hoogsteen base-pairing schemes could be discarded on stereochemical grounds, without resort to Fourier methods of diffraction interpretation.

(93) Crick, though admitting to being "a biased witness",[20] thought that Donohue had over-stated the weaknesses of Fourier analysis:

> If Donohue thinks that an equally effective model for DNA could be produced with some alternative base-pairing, let him build such a model and publish the coordinates. The fit of this model with the observed X-ray data could be compared with that of the models

---

[20]The reason, of course, being his co-authorship of the double-helical model and purine-pyrimidine restricted base-pairing scheme. But every participant in this controversy had an interest to defend: Donohue his base-pairing scheme, Wilkins et al. (which, note, included Arnott [q.v., (94)]) their testing and refinement of the Watson-Crick model.

> described by Wilkins and his colleagues. We would then all see which model fits the data better, or whether there is nothing to choose between them. [(1970), p.1694]

(94) Arnott (1970) replied to Donohue by arguing that Fourier analysis "can result in the rejection of incorrect structural hypotheses even when only low resolution data are available." Although he supported "many of Donohue's general, cautionary statements on the use of the Fourier syntheses approach", Arnott held that "Donohue has ignored the one component of the Fourier method (difference syntheses) which clearly shows that the alternative base-pairing schemes are sufficiently different from one another for the DNA data successfully to arbitrate between them in spite of...[its] bias."[p.1699]

(95) In his rejoinder, Donohue noted that Wilkins et al. referred to the model of DNA that they had refined as "'generally accepted'", commenting: "as if such an important matter could be decided by an opinion poll." He contended that the difference synthesis approach to Fourier analysis which Wilkins et al. had favoured and Arnott had elaborated did not, as they had suggested, eliminate the "distressing tendency always to give back what was put in." Contesting Arnott's view that difference synthesis Fourier analysis would, in the example he had earlier considered, lead to the rejection of an independently eliminable structure, Donohue re-asserted his claim that it would prove acceptable. Of the view "that it is sufficient to consider various models, and then choose as correct (after adjustment) one that gives satisfactory best agreement with experiment", Donohue remarked: "one can never be certain that a model sufficiently close to the true structure has been constructed."

(96) The debate did not continue. Crick, however, discussed it briefly in his (1974) review of events since 1953, published in a special celebratory issue of Nature entitled 'Molecular Biology Comes of Age'. He wrote:

> On the crystallographic side Donohue, whose advice had been crucial to our understanding of base pairing, was a persistent critic of the validity of the later X-ray work, but in recent years he carried it too far, refusing, for example, to admit as evidence the great accumulation of data showing that the two chains are anti-parallel. (In 1956, he had rashly published, with Stent, a quite erroneous structure having like-with-like pairing.) I hope the recent papers...have to some extent reduced his doubts, which at times had some justification.[p.143][21]

(97) Hamilton (1968) conceded that diffraction evidence alone could not determine the helical sense of the exoskeletal strands. Relying on Fuller et al.(1965) [q.v., (36)], Hamilton points out that although a left-handed model of B-DNA was not ruled out on stereochemical grounds, in "the A form, however, it seems impossible to build a left-handed structure without impossibly short interatomic distances, whereas right-handed models can be built with ease." Still following the reasoning of Fuller et al., Hamilton suggests that: "Because the conformation of...DNA may be readily changed reversibly from A to B, it is most unlikely that the transition involves such a radical change in structure as alteration of helix sense." Though he concludes that "DNA in the B form, [that] found in vivo, consists, like the A form, of right-handed helices", Hamilton still introduces a note of caution:

> The determination of the precise structure of DNA has not yet clarified the confusion in the textbooks about whether the model is the actual structure of DNA or

---

[21] Donohue's assistance is acknowledged in Watson and Crick (1953a), p.738.

still a structural hypothesis.

Adding, finally,

> It is nevertheless relevant to current research. The derivation of the A conformation of DNA greatly aided the elucidation of the RNA double helix which is very similar. [p.636]

(98) In the response to Donohue's critique of the value of Fourier analysis, Wilkins et al.(1970) sought to stress that the crystallographic diffraction interpretation begun at King's College, London, and pursued there and elsewhere as the group dispersed had "frequently emphasized the limitations of the X-ray data from DNA, in particular that the resolution is insufficient to resolve single atoms." They also commented that, "as we have often said before,...because DNA is only available in microcrystalline fibers, conclusive proof of the correctness of the DNA structure has not been obtained as directly as is generally the case with single crystals." But, echoing Hamilton's argument [q.v., (90)], they felt that "the disadvantage of having only fibre data for DNA is to a fair extent overcome by DNA having several conformations - all providing data in good agreement with the WatsonCrick scheme."[p.1693]

(99) An account of Crick et al.'s (1979) defense of the Watson-Crick model of DNA against the SBS structures developed in New Zealand and India will be given in the next chapter. However, certain aspects of that paper are generally relevant to the question of how strongly the diffraction crystallography supports the Watson-Crick model. Crick et al state that:

> we consider it unwarranted to rely solely on the details of exact model building, our knowledge of stereochemistry, though now fairly good, may not be adequate to provide firm answers, nor is it advisable to put one's faith completely on the fine details of X-ray diffraction patterns. That of the B form has always been

rather poor...[p.451, q.v., (120)ff.][22]

(100) One issue which Crick et al. did not think they could settle decisively was the handedness of DNA. As has been noted at various points, the evidence supporting a right-handed view of the configuration of the exoskeletal strands had always been based on stereochemical considerations. But these, Crick et al. now conceded, "might not be strong enough to convert a sufficiently obstinate skeptic [ibid., p.456]." Nevertheless, they held that the experimental data was likely to decisively establish the right-handed view of the DNA molecule very shortly for:

> Above all,...[the SBS model] has underlined a need that has been apparent now for some time, but which seemed perhaps less urgent than it does now. This is the solution, to high resolution, of single crystal structures of short lengths of the DNA double-helix having a defined base sequence. This is now technically possible, both from the supply side and from the X-ray side, given a little luck. From these we could obtain more exact perameters than we could ever hope to obtain from fibres. .... In addition the diffraction data could be used to show that the helix is right-handed... [idem, emphasis in the original].

(101) A few months later, Wang et al. (1979) reported the results of diffraction studies at atomic resolution of a single crystal consisting of six base pairs having a known sequence. These revealed a left-handed double-helical fragment whose phosphate atoms, unlike those of a Watson-Crick structure which form regular helices, followed a zig-zag course along the outside of the molecule; a characteristic that led Wang et al. to christen it Z-DNA. They considered there to be some evidence that Z-DNA could exist in vivo, along with double helical

---

[22] B-DNA being the form of diffraction pattern thought to most closely resemble the in vivo structure.

conformations, for, in their view, Z-DNA could be linked to the B form of DNA. Indeed Wang et al. went a step further, suggesting that the Z configuration "is likely to be used in biological systems at one point or another [p.685]."

(102) Raising the question of with which of the fibre patterns of DNA their section of fully crystalline material was to be compared, Wang et al. suggested the obvious candidates were the D-DNAs. They noted that Mitsui et al. (1970) had interpreted one D-DNA as left-handed [q.v., (37)-(39)]. Though this D-DNA was distinctly similar to the specimen Wang et al. studied, it also had significantly different dimensions. Wang et al. also observed that Z-DNA exhibited changes in its CD spectra "similar to the changes found in the high salt form of the alternating dC-dG polymer", and that "CD studies in high salt solutions also suggest that segments of [that] molecule may convert to Z-DNA [idem]." They also noted that:

> Interestingly, self-annealing of single-stranded circular plasmid DNA results in the formation of a double-helical structure which appears to be intact over 70% of its length.[23] This is associated with changes in the CD which are consistent with the formation of left-handed segments of Z-DNA in the annealed circular DNA [idem].

Speculating that:

> In this case, the topological constraints of annealing single-stranded circles with each other has forced the establishment of left-handed segments which may adopt the Z-DNA conformation as judged by the changes in the CD [idem].

(103) Arnott et al. (1980) reported a left-handed diffraction pattern for a synthetic polymer fibre, poly d(GC).poly d(GC), in

---

[23] Here two single-stranded circles of DNA are induced, in vitro, to reform a double strand [q.v., (126)].

which both A-DNA and B-DNA patterns had been previously observed. The bases involved, guanine (G) and cytosine (C), were the same as those in the Z-DNA described by Wang *et al.* the year before. Arnott *et al.* also found similar (i.e., left-handed) structures for other base sequences. These results made it clear that left-handed double helices were not restricted to short crystals, but were also exhibited by fibre specimens. Arnott *et al.* shared with Wang *et al.* the view that "[i]t would be surprising if this novel conformation were not used biologically [Arnott *et al.* (1980), p.743]." They speculated that left-handed segments of *in vivo* DNA "might help render moot many topological problems thought to be associated with some DNA activities", specifically,

> The known DNA conformations that are substantially overwound or underwound compared with B-DNA could be used to store or shunt locally the rotations needed to wind or unwind a double helix. For example, a segment of left-handed DNA, maintained in a region where the preferred [base] sequences were sufficiently common, could be a store of negative windings. In appropriate conditions these could be used to compensate positive windings and produce a region of melted [i.e., topologically independent] DNA available for poly-nucleotide synthesis [p.745].

(104) Asked in a radio interview with the Editor of *Nature*, John Maddox, whether the discovery of the left-handed Z-DNA had surprised him, Crick commented:

> I was surprised, because although we tried to build left-handed models before, we were never able to build a plausible one. But [then] this model is so peculiar, I was going to say so kinky, and so unusual that I think it is to be expected that we won't be able to guess it [*sic*, Crick (1981)].

There were, Crick went on to say, two important features of the Z-DNA. Firstly, only a couple of base sequences were known to exhibit the left-handed structure and, secondly, it was necessary to artificially modify natural DNA chemistry in order to induce the Z-DNA conformation. Indeed, in Crick's view,

> ironically, this is the first really good evidence we've got that DNA really is right-handed...[idem, emphasis added]

Crick's reasoning was as follows: since most native DNA is known to have a handedness opposite to that of Z-DNA, and since Z-DNA had been shown to be left-handed, the normal configuration of DNA must be right-handed. Returning to the subject of the evidence for the handedness of DNA prior to the Z-DNA results, Crick observed that it had all been "very indirect"; adding:

> You could have put your money on it, a bottle of champagne to an ice cream cone it was right-handed; but if you were really nasty and looked at the evidence you'd have said, well, it wasn't absolutely convincing.

V THE APPRAISAL OF THE WARPED ZIPPER

(105) Robson (1977), in the course of an essay in Nature discussing the potential of a new technique in molecular biochemistry, took the opportunity on what he described as "the current heated debate concerning the 'zipper' model for DNA as an alternative to the classical double helix [p.578]." This carefully balanced early appraisal of the SBS structures was cautiously favourable. Robson observed that the SBS model seemed to be in good agreement with the X-ray data which was, he noted, more "ambiguous" for polynucleotides than for proteins. Although, according to Robson, "the double helix has the advantage of being, in some sense, aesthetically more pleasing, particularly because many other biological macromolecules form helical structures, it has to be remembered that nature does not work under an Arts Council grant [idem]." Rather, he thought, any "DNA structure, however bizzare, is likely to coexist in solution alongside the double helix [idem]" if energy calculations showed comparable results to those for the Watson-Crick model, and if the novel structures were as accessible to biological interactions. Robson conceded that there appeared to be interatomic clashes (close contacts) in the SBS structure, but considered that "there is a very real possibility that these can be dynamically relaxed [idem]." Moreover, he added, the SBS model seemed to be able to co-exist and interconvert in vivo with double helical structures. He speculated that "while chromosomal DNA may be always double-helical, short sections of DNA in solution may show dynamic fluctuation to regions of zipper form, and greatly modify certain solution properties such as the

kinetics of unwinding of short DNA *in vitro* (J.P. Day, personal communication) [*idem*]."

(106) Day was another of the members of the biological discussion group at which Rowe had raised the problems he had seen in the replication of circular DNA [*q.v.*, (1) and (10)]. Like Reanney, he had become interested and followed the progress of Rodley's research. After the development of the SBS model Day had become actively involved, preparing a paper with Rodley entitled 'On the Structure of DNA: Implications of an Alternative to the Double Helix'. It dealt with the bubble formations exhibited in previously published electron micrographs of partly denatured DNA. Day and Rodley sought to show that on the Watson-Crick model it was difficult to understand how the windings in the duplex sections of the molecule between the bubbles could be moved through these single-strand regions toward the extremities. In contrast, they argued, the SBS structure did not confront the problem just because it had no need to postulate unwinding at all.

(107) The Day-Rodley paper was submitted to *Nature* near the end of 1976 and rejected. The Biological Manuscripts Editor of *Nature*, Peter Newmark, wrote in his letter informing Day of the rejection:

> It is inevitable that some choice is made from the great number of papers that we received in our opinion your paper is of insufficiently wide interest to compete successfully for our limited publication space [20/1/77].

However this might be (see the comments of Referee 'A' in the following paragraph), Rodley wrote to Newmark that it seemed to him from reading the referees' reports that:

> the main response to this paper seemed to be one of questioning the basic feasibility of the SBS model rather than the evidence presented [G.A. Rodley to P. Newmark, 29/6/77].

Rodley added that he and Day had prepared a detailed commentary on the referees' reports, offering to forward it.

(108) This is what was done, but to no avail for in reply Newmark wrote:

> Despite your plea I am afraid I am not prepared to reconsider your manuscript. It will no doubt not surprise you to learn that most of the people to whom I have talked are not persuaded by your alternative model. That has already caused us a good deal of trouble, and a long delay for you, in finding referees who did not disqualify themselves by their prejudice. I believe the comments that you received were fair and that your response, though reasonable, is not convincing. As far as I am concerned the onus has to be on you to produce a really convincing case for publication in Nature, given that your ideas have already had widespread exposure. In all probability that would require you to confirm experimentally a critical prediction of your model [P. Newmark to J.P. Day, 2/10/78].

The following passages, drawn from the referees' reports for the Rodley/Day paper, summarize their general view of it:

Referee 'A'

> This paper deals with a topic which could be considered of sufficient immediate interest to be published in Nature, but it does not contain any original experimental work, and is purely based on the results of others, which may be being misinterpreted.

Referee 'B'

> I very strongly object to the arguments in favour of the side-by-side model of double-stranded DNA brought forward by the authors of this manuscript. Although the side-by-side model is an interesting alternative to the double-helix, and its possible occurrence should be considered in the analysis of DNA under topological restrictions and in interaction with proteins, most experimental evidence is consistent with a double-helical structure for pure DNA in solution. Only a very superficial look at the electron micrographs of partially denatured DNA could suggest strand separation without unwinding.[24]

---

[24] From copies supplied by G.A. Rodley.

(109) Concurrent and slightly antedating this exchange between Day, Rodley and Newmark was another attempt, equally unsuccessful, to publish work supporting the SBS model. Bates and two of his students, G.C. McKinnon and R.P. Millane, prepared three papers which re- examined the published X-ray diffraction data for B-DNA in the light of the SBS structure. The first introductory article sought to show that a "wide class of structures, of which the double helix is a special case, is found to be compatible with the broad features of the observed diffraction by B-DNA [Bates (1978), p.5]." It went on to argue that a technique of interpreting crystallographic evidence known as the angularly averaged Patterson function was "particularly suitable" for DNA structural studies of DNA. In the second paper, Bates, Millane and McKinnon (1978) maintained the the symmetry of the B-DNA molecule is "apparently significantly non-helical", and that a comparison of the SBS and Watson-Crick models with their diffraction data using an "axial variation of the cylindrically averaged Patterson" for B-DNA showed that the "SBS model satisfies the data rather better than does the double helical model [p.22]." Whereas the second paper utilized data from para-crystalline specimens, in the third Bates and McKinnon (1978) dealt with fibre diffraction photographs. Again they argued their "results combine to show that the SBS model appears to fit the presently available fibre diffraction data somewhat more satisfactorily than does the double helic model [p.49]."

(110) These three papers were submitted to Acta Crystallographica, and another brief paper summarizing their contents was sent to Nature. Writing on behalf of the New Zealand

group as a whole, Rodley said in a letter to the Editor of Nature which accompanied the article sent to that journal:

> We consider this work could represent a decisive step in our attempts to have our model considered because it does to us provide reasonably unambiguous evidence both against the double helix and for our side-by-side proposal [G.A. Rodley to D. Davies, 10/3/77].

Nature's referees did not agree, and the summary paper was rejected. The letter from Newmark (Biological Manuscripts Editor) to Rodley informing him of this decision [16/5/77] was typed ready for his signature when he received a letter from Rodley asking for the paper's withdrawal because Acta Crystallographica had rejected the three papers whose results it reported - again on the advice of referees.

(111) Bates and Rodley wrote to J.M. Cowley, Joint American Co- Editor of Acta Crystallographica, protesting that in their view his referees' remarks betrayed a prejudiced attitude toward the SBS structure. Cowley's reply is of sufficient interest to warrant quotation in extenso:

> I am certainly not very happy over the way these papers were treated or over the rejection. The suspicion was inevitable that the referees were being overly critical of ideas which conflicted with their own. As one who is not at all familiar with the subject matter, I had to rely on recommendations of referees from persons whom I respected but I was not personally acquainted with them or their scientific and professional reputations. However being assured that these people were among the foremost authorities in the field I could not refuse to recognize their seemingly unanimous opinions (expressed rather more forcibly in accompanying letters to me than in their offical referees' comments). Your remark regarding the method of presentation has suggested the possibility to me that much of the adverse reaction (and much of the delay in refereeing) may have been in reaction to the receipt of three papers at once. It may be that...[Bates (1978)] by itself would have had more chance of acceptance. Then the other papers, as sequels of a published paper, may have been accepted more readily. It might be worth trying that, preferably through another co- editor and so another set of referees although, as I explained [in a letter] to Professor Bates, it would be appropriate in that case

> for me to communicate the history of the submissions to the other co-editor, giving the reasons why I support such a resubmission which is normally considered undesirable [J.M. Cowley to G.A. Rodley, 11/10/78].

But Bates and his co-workers did not take up this offer; choosing, rather, to publish privately the three papers concerned together with the referees' reports and a rebuttal of them [Bates, McKinnon and Millane (1978)]. One of the reasons they gave for taking this course was "that the referees have been so outrageous that we think their comments should be recorded, which would not be permissible if the papers were under [further] review...[ibid., p.4]." A reworked version of the papers rejected by Acta Crystallographica also appeared in the Indian journal Pramana two years later [Bates, McKinnon, Millane and Rodley (1980)].

(112) Whilst the New Zealanders were contending unsuccessfully with the anonymous negative appraisals of the referees of Nature and Acta Crystallographica, they had a paper acccepted by the local Journal of the Royal Society of New Zealand [Bates et al. (1977)]. As well, in order to expose their alternative structure for DNA to a wider, including a non-scientific audience, Rodley and Reanney prepared their (1977). Published by the University of Canterbury, this booklet was begun in a spirit of high optimism. But the final manuscript reflected the battles with the referees of the technical work. For example, Rodley and Reanney remark:

> We are at a stage of development in DNA chemistry where it is very unlikely that current workers [will] consider the possibility of interpreting their results in terms of any other model other than the double helix [ibid., pp.49-50].

(113) The Indian experience was rather different to that of the New Zealanders as far as obtaining publication of material

supportive of their respective versions of the SBS structure for DNA was concerned. The New Zealanders, because they had neither the training nor the facilities to perform experimental studies, were obliged to re-interpret others' work. These re-interpretations were vulnerable to criticism qua re-interpretations as well as qua interpretations of evidence. By contrast, the Indians based their follow-up papers on experimental work of their own for which, under Sasisekharan's experienced leadership and with the facilities of the Molecular Biophysics Unit of the Indian Institute of Science, they were well qualified and equipped. As a result their papers escaped much of the criticism that was levelled at the New Zealanders' work. And when, as did occur, referees produced comments which, in the view of the Indians, were indicative more of bias against the SBS model than flaws in their argument, they were able to address their answers to peers. Moreover, the Indians - because of their professional expertise - made fewer errors of presentation and argument in the first place (some of the criticisms made of the New Zealanders' work, as they would admit, were legitimate if arguably carping). Paradoxically, whilst the New Zealanders found their local journal (of the Royal Society of New Zealand) more amenable to their ideas than the major international scientific press, the Indians perceived resistance to their work from their national journals which, however, ceased when their papers on the SBS structure were accepted by international periodicals. In the circumstances, it was as well that the situation was not reversed. In any event, during 1977 and 1978 the Indians published five papers. One appeared in the local Current Science [Sasisekharan et al. (1977)], two in Nucleic Acid Research [Gupta and Sasiskeharan (1978),

Sasisekharan and Gupta (1978)], one in Nature [Sasisekharan and Pattabiraman (1978)] and one, previously discussed [q.v., (62) and (63)], in PNAS [Sasisekharan, Pattabiraman and Gupta (1978)].

(114) During the period 1976 to 1978 the leading figures in polynucleotide conformation studies did not respond in print to the Indian and New Zealand SBS models of DNA. In private correspondance, however, they expressed strong reservations. In a letter to Bates [25/3/77], Aaron Klug set out a number of objections he had to the diffraction re-interpretations that Bates and his students were then trying to publish. Crick endorsed Klug's arguments in a letter to Rodley [11/5/77] in which he began by saying:

> Well, you are certainly trying hard but you'll have to do a lot better before most people will believe it, if only because your structure is so ugly (though ingenious) and ours is so pretty![25]

Crick also said that he was unimpressed "with a series of weak bits of evidence or plausibility arguments." Rather, he thought, what was neeeded was "a striking experiment that everybody will be able to recognize as correct....a completely convincing demonstration of the general truth of your idea and a disproval of our structure." And, Crick added, "I must stress...that without this evidence no hard- headed molecular biologist is likely to believe your ideas so I suggest you promote this type of experiment...[idem, emphasis in the original]." Crick concluded: "I will be most surprised if your model turns out to be correct but you have done a useful job (more useful than Donohue did) in pointing out the rather fragile nature of some of the X-ray evidence [idem, q.v., (91) - (96)]."

---

[25] q.v., (105) for another view of this argument.

(115) Published responses to the challenge to the Watson-Crick structure of DNA posed by the SBS model from those professionally concerned with the conformation of polynucleotides appeared regularly during 1979. Struther Arnott aired his views in a Nature editorial [Arnott (1979)]. He noted that the "warped zipper" model of DNA (as he dubbed it) "abolished at one stroke...the problem of unwinding extensive lengths of cohelical DNA for replication...[though in consequence] new roles will have to be postulated for relaxase, gyrase, helicase and so on [ibid., p.780]." Arnott conceded to the SBS structure that had "such a model been available in 1953, it would have been regarded as a serious competitor for the original bihelical model [idem]." However Arnott said that he thought the fit between the Watson-Crick model of DNA as refined by Wilkins' school (of which he was a member [q.v., (88)-(90)]) and the X-ray data was considerably superior to that he had determined for the SBS structure.

(116) The measure of this fit that Arnott used the reliability (R) index. According to Donohue,

> The function R is the average percentage discrepancy between the observed structure amplitudes and those calculated on the basis of a proposed structure...[(1969), fn. 26, pp.1095 - 1096].

The lower the R value the better the fit. Arnott gives as the best value that he and his colleague R. Chandrasekaran had obtained for the double-helical Watson-Crick model of B-DNA was R = 0.28. He argued that this figure was "reasonable when one considers that fixed standard bond lengths and angles have been imposed on the model... [Arnott (1979), p.780]." For purposes of

comparison, Arnott provides the value R = 0.45 for a double-helical structure with an unacceptable (non-Watson-Crick) base pairing scheme. The figure for the New Zealand SBS model (calculated from data supplied by Bates in a preprint), according to Arnott is R = 0.48. He adds:

> These less regular warped zipper models have ten times the degrees of freedom allowed to regular helices and would have to have R < 0.20 to be regarded as being significantly superior to the best bihelical model...[Arnott (1979), p.781][26]

Nevertheless Arnott thought that it was "not inconceivable to me that a warped zipper model could be contrived with R rather less than the present value of 0.48...[although the] effort required would be considerable...[idem]."

(117) Arnott then turned to give an account of the argument of Crick et al. (1979) against the SBS structures [outlined below, (120) - (123)], an argument which he saw as being sufficiently powerful to obviate any need to try and improve the R value fit of the SBS model. Arnott's final objection to the SBS structure turned on the symmetries apparent in the X-ray diffraction photographs of DNA. These, he held, were difficult to explain except on the assumption of regular double-helices. Arnott concludes:

> For many years attention was concentrated on the base pairing of DNA. It was right that the backbone should be subjected to similar scrutiny. It is unlikely that any

---

[26] Whereas the bond angles and distances of the Watson-Crick model of DNA arbitrarily fixed within the stereochemically acceptable range (see above, this paragraph), those of the SBS model are not. Moreover the SBS structure is more flexible in that bond angles and distances may in many cases vary through a greater range than is possible in the case of a Watson-Crick double helix. Arnott's argument here is that it is harder, therefore, to achieve a given R value for the Watson-Crick model than it is to obtain the same degree of fit for an SBS structure. Consequently, in his view, a better R value fit must be demanded of the SBS models. [q.v., (118)]

major modification will be needed [idem].

(118) In a letter to Rodley [15/5/79], Arnott remarked:

> The unwinding problem was certainly sufficient reason for the paradigm to be reviewed, but a new model must solve this problem and satisfy the other physical data at least as well as the duplex models of plectonemic type. If the X-ray intensity data from polynucleotide fibers are as suspect as some have alleged, then many models would give as good (or bad) a fit as bihelical models. No warped zipper model yet provides this. [Emphasis in the original]

But the reliability index which Arnott had used as a measure of fit has been criticised. For example Donohue (1969) maintained:

> Unfortunately, apparently acceptable R values of around 20 per cent have been obtained for grossly incorrect structures, and values as low as 7 per cent have been reported for structures with incorrect details, that is, 'partially correct' structures [p. 1095].[27]

In view of objections such as this, Greenall et al. held that "it is not clear that a single parameter such as the reliability index is the best way of comparing models of this kind...[p.880]." Rather they compared "the full molecular Fourier transforms of the SBS and Watson- Crick models [idem]."

(119) But the results of this comparison were hardly more encouraging to the proponents of the SBS model than Arnott's had been. They found that:

> there are major and quite unacceptable discrepancies between the observed diffraction from the B form of DNA and that calculated for the SBS model proposed by Rodley et al. Although some of the more serious discrepancies can be removed by the simple distortion of the original model described here, the degree of agreement between observed and calculated diffraction is still very poor and very much inferior to that reported for the best models of the Watson- Crick type [p.882].

The New Zealanders responded to these negative conclusions in a

---

[27] Arnott's best R for B-DNA [q.v., (116)] was 0.28. Donohue gives as the best R for A-DNA (at 1969) 0.39.

letter to Nature [Bates, Rodley and McKinnon (1980)].[28] Directing attention to the paper in Pramana [Bates, McKinnon and Millane (1980), q.v., (111)], they observed that they found "roughly equal disagreement [with the diffraction evidence] for both models [Bates, Rodley and McKinnon (1980), p.384]." Parenthetically, the New Zealanders observed that they had detected what they thought was an anomaly in the double-helical account of the diffraction data, but, they said sarcastically, they did not want to "make too much" of it "because it could well be an artifact of excessive refinement based on inadequate data [idem]." Bates, Rodley and McKinnon noted, too, that Crick et al. (1979) had acknowledged the equivocality of the X-ray evidence for the structure of DNA.

(120) Both Crick and Newmark, it will be recalled [q.v., (114) and (108)] urged upon the New Zealanders the necessity for a 'crucial experiment'. The same view is repeated in Crick et al. (1979). They argued:

> In science ten weak arguments do not add up to one strong one. We are not concerned with the question whether the double helix or the SBS structure is plausible. We wish to know whether there is any very hard evidence which decisively favours one structure over the other [p.451, emphasis added].

And it was

> For this reason we consider it unwarranted to rely solely on the details of exact model building; our knowledge of stereochemistry, though now fairly good, may not be adequate to provide firm answers, nor is it advisable to put one's faith completely on the fine details of X-ray diffraction patterns. That of the B form has always been rather poor and may not yield a clear, unambiguous decision between the two types of structure. One must turn to evidence of a quite different type [idem].

---

[28] Extended negotiations with Nature over the possibility and form of this reply preceded its appearance.

Crick et al. believed that they had found hard evidence which decisively favoured the Watson-Crick structure in experimental measures of the net number of times that each chain of the exoskeleton of a closed circular DNA molecule was wound around the other, the linking number (Lk).

(121) For a circular molecule of 5000 base pairs, the Lk expected on the basis of a double helical Watson-Crick model with a right- handed (+) helix every ten base pairs is +500. A 'pure' SBS model would have Lk = 0. But, because the New Zealanders variant of the SBS structure has a long-range, right-handed twist resulting in one helix every 100 base pairs, for a 5000 base pair molecule Lk = +50.[29] Linear DNA can be denatured (the exoskeletal chains with attached bases separated intact) in vitro by raising the temperature of the specimen. In circular molecules too, if one strand is nicked, then on heating the chains will denature yielding one closed circular and one linear strand. However, the exoskeleton of un-nicked closed circular DNA molecules will not denature intact. Thus, Crick et al. argued, $Lk \neq 0$, eliminating a 'pure' SBS model. They also held that there was evidence which distinguished between Lk = 500 (Watson-Crick) and Lk = 50 (New Zealand SBS) models.

(122) This data was of two kinds. The first concerned interpretation of the discrete bands formed by electrophoresis of

---

[29]The same is true of the Indians' Type I SBS model since it is essentially similar to the New Zealanders' structure. The Indian Type II model however, having a number of sub-variants, can be built without any long-range twist (Lk = 0). Alternatively, it can be constructed to exhibit long-range left or right-handed coiling from Lk = -50 to Lk = +50.

circular DNA on gels containing ethidium bromide.[30] Crick et al. maintained that Lk for adjacent bands differs by unity and that "no other interpretation of the bands is even remotely plausible [ibid., p.452]." Because Lk for closed DNA molecules must be an integer, and since the bands produced by electrophoresis were always distinct and without intermediate formations, Crick et al. reasoned that the Lk value represented by the bands must be discrete. And if that value were greater than one, "say two, this would only increase our estimate of the linking number of a circular DNA molecule, and not decrease it as required by the SBS structure [ibid., p.454, emphasis in the original]." Crick et al. then consider experimental techniques for estimating the Lk of circular DNA. The most recent and precise method they suggest is that of one of their number, J.C. Wang. This utilizes the gel electrophoresis approach and interpretation outlined above. It yielded 10.4 $\pm$ 0.1 base pairs per turn for DNA in solution (the Watson-Crick model predicting 10 base pairs per turn). Moreover, Crick et al. note, earlier methods produced a similar though slightly less exact figure.

(123) Crick et al. concluded:

> The SBS structure is thus incorrect, but this is not to say that the proposals have not served a useful purpose. They have shown rather clearly that while certain general features of the classical double-helix are established beyond reasonable doubt (special cases aside), other features need more careful scrutiny. The SBS model was ingenious because it incorporated the well-established features while altering the less certain ones. It has undoubtedly made us sharpen our arguments for the double helix. It has raised the question of how far a structure can depart from a double-helix and still give the very striking ...diffraction pattern [of DNA]. More calculations here would be of value [ibid., p.456].

---

[30] In electrophoresis, an electric current is passed through a suspension to achieve differential precipitation.

Yet, for all that, they were certain that they had decisively eliminated the SBS models with a 'crucial experiment' distinguishing them from the Watson-Crick conformation, and despite their confidence that future work would establish the right-handedness of DNA [q.v., (100) - (104)]; Crick et al. ended their discussion on a tentative note. "It might be", they wrote,

> sensible to build and calculate the energy of the best left- handed structure and of the best SBS one, since it is by no means certain that, under certain conditions, DNA cannot be forced into such configurations. DNA is such an important molecule that it is almost impossible to learn too much about it [idem, emphasis in the original].

(124) Crick et al. (1979) had a considerable impact on the New Zealanders. When I interviewed Rodley late in the year, he said:

> I think that [their] argument is a very strong one which as far as I can see definitely indicates that there is some double-helical DNA in circular molecules...

He also felt that the "Crick et al. paper has probably had a fairly major effect in suggesting that the side-by-side model is not now a possibility." Others in the group (for example Bates) made similar remarks. A recent review of neoteric work on the exoskeleton of DNA supports Rodley's assessment of the reaction among the scientific community. Citing Arnott (1979) and Crick et al. (1979), Cohen (1980) summarized the advent and evaluation of the SBS models this way:

> The old question: 'is DNA really a double helix? has recently been re-hashed, and, not surprisingly, the Watson-Crick double-helical B structure for DNA has been re-affirmed [p.58].

(125) Sasisekharan was less impressed by the Lk argument of Crick et al. When I interviewed him in December, 1979, he said of it:

> The linking number argument depends on the assumption that the [DNA] molecule is 100% one handed so that even

> if you have in a molecule 5%...which prefers left-handedness... the linking number argument is out.

And, in his (1981), an invited summary of the SBS / double-helix controversy from the Indian group's perspective, Sasisekharan remarked:

> When...[Crick et al.'s] results were reported, left-handed DNA fragments at atomic resolution were not known. Absence of such structures was also taken as an evidence against the possibility of an RL model.[31] But the left-handed DNA structures are now observed. Further, the electrophoretic measurements give no direct estimate of L_k; it is therefore doubtful whether one can rely solely on such measurements to decide in favour of one model over the other model [p.110].

(126) Although it has not proven possible to denature double-standed closed circular DNA molecules intact, Stettler et al. (1979) were able to renature two closed circular single strands into an intact duplex molecule without breaking either of the chains. As they pointed out, such a result can only be explained on the basis of a Watson-Crick configuration if the right-handed intertwining of the exoskeletal strands is compensated for by an appropriate (and large) number of left-handed superhelical twists. Stettler et al. did find evidence of supercoiling, but only in the degree expected on the basis of the New Zealand or Indian Type II structure. That is, the evident superhelices were an order of magnitude too few to permit topological independence (and, thereby, renaturing or annealing of unbroken circular strands) given the Watson-Crick model. Stettler et al. advanced to possible ways in which their experimental data could be explained structurally: either the molecule was composed of approximately 50% Watson-Crick right-handed double-helices associated with left-handed double-helices (having no hydrogen

---

[31] RL = right and left-handed, i.e., SBS.

bonding between the bases) and left-handed superhelices, or an ordered mixture of left and right-handed structures (both with hydrogen bound base pairs). One such ordered arrangement, as Stettler et al. noted, is the SBS conformation. But, they said,

> Our estimate of the extent of ordered structure is not sufficiently reliable to distinguish between the two possibilities. Moreover, it is clear that intermediates between the two models are also possible [ibid., p.39].

(127) Both the New Zealanders and the Indians regarded the work of Stettler et al as encouraging. [See: Sasisekharan (1981), p.110; and Rodley, Bates and Arnott (1980), p.232. Rodley, Bates and Arnott (1980) consists of two articles, one by Rodley and Bates, the other by Arnott, published parallel as a forum discussion of the SBS proposals.] It may be seen as a counter-point to Crick et al. (1979). However Crick himself remained convinced that the SBS models had been eliminated:

> If it were not for the power of the scientific method to falsify suggestions it would seem to me highly likely that the SBS structures would now be the new wave and double- helical structures passe. Such changes of opinion appear to me to happen rather often in literature, music and the other arts [F.H.C. Crick to T.D. Stokes, 15/2/80].

(128) In his contribution to Rodley, Bates and Arnott (1980), Arnott remarks:

> The roles of DNA duplexes in vivo require on occasion that their two polynucleotide chains become separated. Few would dispute, therefore, that base paired duplexes with no net winding might exist at least as local or fleeting precursors. The model of Rodley and Bates...is...[one] of an infinity of conceivable models [of this. pp.231-233]

He then gives an account of the diffraction testing and refinement process which is particularly interesting when read in conjunction with IV, and especially the views of Hamilton [q.v., (88) and (90)] and Donohue [q.v., (91) - (96)]:

> Diffraction analyses of polynucleotides in fibres has

> drawbacks. .... [The] problem has to be solved subjectively by creating a plausible model, then refining it. Modern methods of refinement make it relatively easy to find the model of the kind postulated that best fits the intensities of the X-ray diffraction pattern. Of course this provides no assurance that a different kind of model might not provide an even better solution. But resolution of this dilemma is quite simple albeit tedious. Each new kind of model which gives an encouragingly good account of the X-ray diffraction has to be refined to determine whether its best version is significantly superior to other optimized models as judged by the fit to X-ray intensities, lack of steric compression etc. [p.233]

(129) In accordance with the programme outlined above, Arnott reiterates the claim of his (1979) that the R for the SBS model needs to be improved by refinement to a point where it is favourably comparable with the double helical structure. As was noted in the account of Arnott's (1979), it is his view that, because of the greater flexibility of the SBS structure, a favourably comparable reliability index would be much lower than his best for the Watson- Crick model [q.v., (116)]. Arnott states he is not personally prepared to "embark on this costly excercise [idem]" of refinement because he takes the view that the crystallographic symmetries evident in the DNA diffraction data weigh so strongly in favour of a helical structure [q.v., (116)]. He observes that he has been unable to duplicate the Fourier transform calculations which Bates and co-workers had claimed demonstrated a commensurable fit between the SBS and Watson-Crick models of DNA without introducing "systematic errors".

(130) It will be recalled that the Indians abandoned their Type I SBS structure, in essence the same as the New Zealand SBS model, because of recalcitrant close contacts [q.v., (62)]. Because the New Zealanders had priority of publication, most discussions of the SBS model have concentrated on their version.

Thus the Indian claim to have shown that their Type II SBS structure was entirely stereochemically viable has been given rather less prominence than might otherwise have been expected. Arnott, for example, does not advert to it in Rodley, Bates and Arnott (1980). He does, however, maintain that the New Zealanders have "an obligation to produce a version of their model of B-DNA free of steric anomalies [ibid., p.233]". The New Zealanders acknowledged both the problematic interatomic clashes and the need to eliminate them. In order to do so they turned, as had the Indians in developing their Type II SBS structure, to consider the Watson-Crick base pairing that they had adopted for their model. Whereas the Indians' solution lay in inverting the bases at the bend regions where the exoskeletal strands of the SBS model change handedness, the New Zealnders' came from considerations of vertical base alignment. In Watson-Crick base pairing, the pairs of bases are vertically congruent in certain respects. Just as the Indians discovered that there was no stereochemical reason why the customary face (and thus edge-bond) orientations needed to be preserved, so the New Zealanders concluded that congruent stacking was not warranted by stereochemical considerations. Thus they developed another version of their SBS model in which the base pairs fanned slightly. Rodley and Millane (1981) provided the stereochemical details of this staggered stacked SBS structure. The bond angles and distances utilized in this model are those standardly accepted and it exhibits no close contacts.

(131) At the time of writing, towards the end of 1981, both the Indian and New Zealand groups are confident that their respective versions of the SBS model of DNA are sterochemically

viable, and that their fit with the diffraction data is at least comparable with that of the Watson-Crick model. Neither group believes that the arguments that have been marshalled against their proposals have decisively eliminated the possibility that DNA might exhibit an SBS configuration in vivo. However, judging by the published responses of specialists in the conformation of polynucleotides, whilst the expert scientific community is not as firmly disposed against the warped zipper as it was initially, it nevertheless remains highly skeptical. The deliberate search for experimental evidence favouring the SBS structures against the Watson-Crick double helix is, consequently, largely if not entirely restricted to those who had a part in the genesis and development of the warped zipper.