ON THE MACROMOLECULAR STRUCTURE OF DEOXYRIBONUCLEIC ACID: AN INTERRUPTED TWO-STRAND MODEL*

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Introduction.—A model of the macromolecular structure of deoxyribonucleic acid (DNA) has recently been proposed by Watson and Crick[1, 2], primarily on the basis of the X-ray diffraction studies of Wilkins *et al.*[3] and Franklin and Gosling.[4] The purpose of this communication is to assemble the existing data and present new evidence concerning the physical and chemical behavior of DNA, and to consider the results in terms of the Watson-Crick structure and other potential models.

As we hope to show, most of the data do support a hydrogen-bonded, intertwined, two-strand model of the type proposed by Watson and Crick, but it appears that their proposed structure requires modification because of certain existing data, especially those pertaining to chain length. A model more compatible with all the data is one in which the individual polynucleotide strands, rather than being continuous, are interrupted about every fifty nucleotides, with the breaks in the two strands staggered relative to one another. This implies that the macromolecule, DNA, is an aggregate of much smaller molecules held together in a specific configuration by hydrogen bonds between the purine and pyrimidine rings.

Molecular Size and Shape.—During the last ten years there has been disagreement over the molecular weight of DNA,[5, 6] but it now seems evident that the discrepancies were due either to variations in the preparations of DNA or to limitations in the methods of measurement used at the time. From a variety of physical-chemical techniques, including light-scattering, ultracentrifugation, diffusion, viscosity, streaming birefringence, and electron microscopy, we can now conclude that the DNA preparations obtained by mild methods such as that of Signer and Schwander[7] contain molecules with molecular weights of about 5×10^6. Probably the best values for the molecular weight result from light-scattering measurements,[8] since no assumptions need be made regarding the shape of the molecules in solution. Such assumptions must be made to determine the molecular weight from hydrodynamic methods in view of the absence of reliable diffusion data. It should be pointed out, however, that molecular weights obtained from hydrodynamic data are in the neighborhood of 10^6–10^7 for those models which have been considered.[9]

From the results of extensive studies of synthetic high polymers, it is known that these macromolecules can assume a great number of different configurations in solution because of the possible rotation about the single bonds in the polymer chain. When there is free rotation at each segment in the chain, the macromolecule assumes a configuration called a "random coil," which is described in terms of the distance between the two ends of the chain. This distance is, of course, much smaller than the over-all contour length. At the other extreme is the completely extended or rodlike macromolecule with length equal to the contour length. Intermediate configurations exist depending upon the freedom of rotation about the single bonds, steric factors such as bulky side chains, and electrostatic repulsion due to charged groups. In DNA there is a high density of negative charge along the

backbone structure, and some stretching of the molecule toward a rodlike configuration should be expected, due to electrostatic forces. However, most physical-chemical work on charged macromolecules shows that such repulsion is fairly well damped out at ionic strengths of about 0.2, and entropic forces should dominate, leading to coiled macromolecules (see Sadron[10] for a critical discussion of the methods of determining the form and dimensions of particles in solution). This is shown very clearly in studies of dilute solutions of synthetic polyelectrolytes like polyvinylpyridinium bromide. In water the repulsive forces dominate and the molecules are in an extended form, whereas they appear to be highly coiled in solutions of 0.1 ionic strength. If the bulk of the physical-chemical evidence indicates that the more appropriate shape for DNA under the latter conditions is rodlike, we must look for some structural feature in any proposed model to account for the stiffness of the molecule.

Despite careful studies[11, 12, 13] of the variation of the intensity of scattered light as a function of angle, there is still some indecision as to whether the scattering curves are more representative of rodlike or coiled macromolecules. This uncertainty arises chiefly because of lack of knowledge of the polydispersity of the material with regard to molecular weight. A recent study[8] suggests that the molecule in solution is a stiff coil whose end-to-end distance is about one-fifth the contour length calculated on the basis of a molecular weight of 5×10^6 and two nucleotides per 3.4 A along the chain. Had the molecule been randomly coiled with free rotation at every nucleotide, the end-to-end distance would be of the order of 500 A, rather then the 5,000 A found by these workers. Recent sedimentation studies,[9] at lower concentrations than had been employed previously, showed that the sedimentation coefficient at infinite dilution is about 21*S*, a value almost 50 per cent larger than those reported earlier. Using this value of the sedimentation coefficient, together with the value of 5×10^6 for the molecular weight and 57 $(\text{gm}/100\ \text{cc})^{-1}$ for the intrinsic viscosity,[10] we can calculate the shape of the effective hydrodynamic unit according to the theories of Flory and co-workers[14, 15] and Scheraga and Mandelkern.[16] The results of these calculations[9] are incompatible with a random coil model and show that the hydrodynamic unit is rodlike, with an axial ratio of 300 to 1. Further, the thickness of the particles estimated from sedimentation measurements is about 25 A. This view gains additional support from direct observation in the electron microscope,[17, 18] which shows that the particles are threadlike, with a thickness of 15–20 A. Although the micrographs are of dried preparations of DNA, it seems unreasonable to suppose that the particles would appear almost straight when studied in the dried form on the substrate films used for electron microscopy if, in solution, the molecules had been highly coiled. Further evidence for the rigidity of DNA stems from viscosity studies. Experiments at moderately high concentrations of DNA show that the viscosity in salt solutions is much lower than the viscosity in the absence of salt. There are two ways in which these results can be interpreted. Either the molecules are flexible, like the synthetic polyelectrolytes, and change shape with ionic strength, or the molecules are unaltered in shape but interact with one another in the absence of salt to give high viscosities. Addition of salt would reduce the interparticle interaction and cause a lower viscosity. Evidence favoring the latter alternative, involving no alterations in the shape of the molecules, comes from the recent studies of Pouyet and

Sadron[19, 10] and Conway and Butler,[20] who were able to demonstrate that the specific viscosity of DNA solutions at extremely low concentrations (below 0.001 per cent) is independent of ionic strength. Since the concentrations at which they worked were sufficiently low to preclude contributions to viscosity from particle interaction, it is reasonable to conclude that the shape of the molecules does not change with ionic environment and that the DNA molecules are rather rigid. Studies of the birefringence of flow of dilute solutions of DNA also indicate that the particles are relatively stiff and very elongated.[21, 22]

According to hydrodynamic theories for rodlike molecules,[23] the viscosity of a solution of rods is proportional to the 1.7 power of the length (or molecular weight). and the sedimentation coefficient is almost independent of molecular weight, actually varying with the logarithm of the molecular weight. For randomly coiled molecules, however, the intrinsic viscosity is proportional to the 0.66 power of the molecular weight, while the sedimentation coefficient varies with the 0.44 power of the molecular weight.[14] To discriminate further between these two shapes for DNA, data were obtained at different stages of the digestion of DNA with DNase. It was found that the reduced viscosity, η_{sp}/c, where η_{sp} is the specific viscosity and c is the concentration in gm/cc, decreased rapidly to low values, while the sedimentation coefficient varied only slightly. Although there are limitations to this type of study of particle shape, the results do provide strong evidence against the view that DNA exists as a random coil in solution. Further studies are necessary, however, because the change in sedimentation rate with time would undoubtedly have been faster had the measurements been made at concentrations lower than those used in our studies. There is a compensating factor, however, since the "native" DNA exhibits a shear-dependent viscosity. Thus the zero-time results for the viscosity are too low, whereas the values after enzyme action would be more nearly correct. If the experiments were conducted at zero shear gradient, we would expect the viscosity to decrease more rapidly with time than is indicated in Figure 4.

In summary, the physical-chemical evidence indicates that the DNA molecules in solution are stiff and elongated, perhaps in the form of curved, threadlike molecules. It is important, therefore, to examine various structural features which would account for such stiffness.

Changes in Intensity of Absorption of Ultraviolet Light.—When DNA is hydrolyzed by DNase, treated with alkali to pH 11 or acid to pH 3, the intensity of absorption of ultraviolet light at 260 mμ increases by 32 per cent, with only a very small shift in the spectrum itself.[24, 25, 26] Heating DNA in neutral solutions and subsequent cooling also produces a marked increase in optical density, although the increase is less than that observed from other treatments. The full increase of 32 per cent is observed, however, after heating for 2 minutes at 85° C. in 6 *M* urea. Urea at 20° C. has no effect on the optical density of DNA.

Thomas[27, 28] and Cavalieri[29] pointed out that a composite absorption curve calculated from the individual absorption curves of the nucleotides and the composition of DNA shows a greater absorption than does DNA as commonly isolated. The difference between the so-called "theoretical" curve and the observed curve vanishes when DNA is subjected to the treatments mentioned above. This indicates that the organization of the nucleotides in the "native" DNA in some way causes a reduction in the absorptive capacity of the various purine and pyrimidine rings.

Various explanations have been offered for this reduction in absorptive capacity. It may be π electron interaction between "stacked" purine and pyrimidine rings in the "native" highly organized DNA[30] or the change in resonance within the rings as a result of the specific hydrogen bonding between rings. Both acid and alkali would be expected to rupture such hydrogen bonds, and it has been observed that the anomalous titration behavior[31, 32] of DNA disappears after treatment with these reagents. This has been attributed to the rupture of hydrogen bonds linking titratable groups on the purine and pyrimidine rings. Explanations for the effect of heat and enzyme action which likewise involve rupture of hydrogen bonds will be considered in later sections. It seems clear that the change in optical density is a measure of the breakdown of the specific organized structure characteristic of native DNA.

Effect of Heat.—Many workers have examined the effect of heat on the physical properties of DNA and have observed that dilute solutions of DNA which are heated for brief periods of time to about 85° C. and subsequently cooled no longer exhibit the high viscosity characteristic of "native" DNA preparations.[33, 34, 27, 35] Zamenhof and his co-workers[36] also studied the effect of heat on the transforming activity of the DNA isolated from *Hemophilus influenzae*. They found that the biological activity decreased in exactly the same temperature range over which the large reduction in viscosity occurred.

This decrease in viscosity of solutions of DNA upon heating can be interpreted in two ways. It is possible that the thermal energy has caused a depolymerization of the macromolecules into much smaller particles. On the other hand, the reduction in viscosity upon heating can also be attributed to a coiling of the macromolecule into a more compact form without a change in molecular weight. In order to distinguish between these alternatives, we have also studied the change in sedimentation coefficient produced by heating solutions of DNA. If a macromolecule is changed from an elongated into a tightly coiled form (or from a stiffly coiled into a more compact form) without a change in molecular weight, the hydrodynamic frictional resistance will decrease with a consequent increase in sedimentation rate. Thus paired measurements of viscosity and sedimentation rate of dilute solutions of DNA and heated DNA could differentiate between a disorganization of the DNA molecule without a change in molecular weight, on the one hand, and a process in which there is a degradation into much smaller pieces, on the other hand. For such studies it is important to work at concentrations sufficiently low to minimize intermolecular interaction. The reduced viscosity of a 0.005 per cent solution heated at pH 7 for 15 minutes at 100° C. decreased from 30 $(\text{gm}/100\ \text{cc})^{-1}$ to less than 1.0 $(\text{gm}/100\ \text{cc})^{-1}$. Instead of an increase in the sedimentation rate, which would be expected from the amount of coiling necessary to produce such a large drop in viscosity, we found that measurements at 0.005 per cent DNA showed that the heated material had a sedimentation coefficient of about 6*S*, whereas the unheated preparation had a value of nearly 20*S*. These experiments provide proof that the molecular weight of DNA changes from about 5×10^6 to 5×10^4 as a result of this mild heating procedure. Since it seems likely that such treatment is incapable of breaking phosphodiester bonds, it is necessary to look for structural features of the macromolecule which include either secondary forces, like hydrogen bonds, holding smaller molecular entities into some well-organized structure, or

easily hydrolyzed covalent bonds, such as deoxyribosyl-1-phosphates, spaced at roughly periodic intervals along the main chains.

The heating experiments of Zamenhof *et al.*[36] and Goldstein and Stern[33] indicate a large temperature dependence for the rate of decrease of viscosity. The inactivation of the transforming principle, furthermore, exhibited about the same temperature dependence. From the data of Kurnick[35] on the change of viscosity with time at different temperatures, we can estimate an activation energy of at least 60,000 cal. A similar value can be obtained from the data of Thomas[28] on the rate of increase of optical density, at 260 mμ, at different temperatures. Such high values for the activation energy are comparable to the values observed for protein denaturations. Activation energies of that magnitude are suggestive of co-operative phenomena involving the simultaneous rupture of many weak bonds such as hydrogen bonds. If the energy of an average hydrogen bond in DNA is taken as 3,000 cal., then the activation energy could be considered as the reflection of the simultaneous rupture of twenty such bonds. Evidence to be presented later makes it appear unlikely that covalent bonds are being broken by the heating procedure. There is reason to conclude, therefore, that the observed degradation is the result solely of rupture of hydrogen bonds. This implies, of course, that DNA is an aggregate of fragments of smaller molecular weight, held together by the co-operative action of many hydrogen bonds.

When concentrated solutions of DNA (1.0 per cent) are heated, the viscosity does not decrease as mentioned above for dilute solutions, but instead the solutions become gel-like upon cooling. Zamenhof and Chargaff[37] found that heating a 0.3 per cent solution of DNA at 86° C. for $1^1/_2$ hours yielded a solution, upon cooling, of much lower viscosity. Upon standing at low temperature, the viscosity of this solution gradually increased until it became nearly equal to the unheated DNA solution. Similar behavior has been reported by others.[33] Zamenhof and Chargaff also pointed out that the solution which had been subjected to heat was thixotropic, whereas unheated DNA solutions are not. The contrast between the two solutions was demonstrated further by measurements of the viscosities at different temperatures. In the range 17°–50° C. the decrease in viscosity of the native DNA solution with temperature is almost that found for water. In contrast to this behavior, the viscosity of the DNA solution, previously heated to 86° C. for $1^1/_2$ hours, exhibited no apparent change in viscosity from 17° to 30° C., despite the fact that the viscosity of water decreases considerably over that temperature range. Between 30° and 40° C. there was a marked decrease in viscosity, much greater than that found for water, and, finally, above 40° C. the viscosity change with temperature was again similar to that of water. These interesting results can be interpreted[37, 38] as the behavior of a gel-like structure made up of molecules held together in a network through secondary forces. No change in the apparent viscosity is observed up to temperatures of about 35° C., at which point the thermal energy becomes sufficient to rupture the secondary forces and the gel melts. Above 40° C. the solution shows the behavior observed for solutions of ordinary molecules.

End-Groups.—In view of the evidence pointing to the stiffness of the DNA molecule and the results of the heating experiments, which suggest that the macromolecule is an aggregate of small pieces, it seems pertinent to examine the data for the number of terminal groups per molecule.

A polynucleotide chain presumably terminates at one end in a nucleoside residue containing one unsubstituted hydroxyl group on the sugar and at the other end with a monoesterified phosphate. The detection of the free sugar hydroxyl groups presents difficulties, inasmuch as the reagents which can be used to react with these groups would probably lead to degradation of the polynucleotides. There has been considerable study of the other terminal group exhibiting both primary and secondary phosphoryl dissociations. Careful titration measurements by many workers,[31, 32, 5] under conditions which would not produce hydrolysis of phosphodiester bonds, have yielded the important result that an appreciable fraction of phosphate radicals possess both primary and secondary titratable groups. The most recent studies of Peacocke and co-workers[32] indicate that there is one such terminal phosphoryl group for about every thirty nucleotides. This value is only approximate, since the interpretation of the titration curves requires accurate knowledge of the ionization constants of the dissociating groups of the bases and also the composition of the DNA in terms of the constituent nucleotides. Furthermore, DNA is a polyvalent electrolyte, and it is also necessary to take into account the influence of neighboring charges on a particular dissociating group. Despite these limitations, the results of the titration studies constitute good evidence for the existence of at least one terminal phosphate group for every thirty to fifty nucleotides.

Further evidence for the presence of terminal phosphate groups comes from a study of the binding of the cationic dye, rosaniline, to DNA.[39] From the binding curves Cavalieri and Angelos inferred that two different sites were involved in binding the dye and that these sites were the mono- and diesterified phosphoryl groups of the polynucleotide chains. From the number of sites of each type, it has been concluded that there is on the order of one monoesterified phosphate for every thirty to one hundred nucleotides.[39, 5] It should be emphasized that this is an equilibrium study and conclusions about the absolute amounts of the different types of groups cannot be made. Identification of the less frequently occurring sites as monoesterified phosphoryl groups is not certain, especially in view of the apparent insensitivity of both the binding constant and the number of sites to pH, just in the region of ionization of secondary phosphate groups.

A third type of evidence concerning the amount of terminal phosphoryl groups is obtained from the study of the action of magnesium ions on DNA as measured by the liberation of hydrogen ions and by changes in the ultraviolet absorption spectrum.[29] This interaction was regarded as a complexing of magnesium ions to both the monoesterified phosphoryl and nuclear amino groups of the DNA. Furthermore, it was found that addition of magnesium ions displaces the dye molecules, rosaniline, bound to the ionized phosphate groups of DNA.[40] From measurements of the liberation of hydrogen ions caused by the addition of magnesium ions, we can again estimate that there is one terminal phosphoryl group for every twenty to thirty phosphorus atoms.

Further studies toward the quantitative estimation of end-groups in DNA seem imperative, despite the apparent agreement of the three independent methods. Enzymatic methods such as those used in the study of the end-groups of ribonucleic acids seem to offer one of the best approaches to the unequivocal solution of this problem. Tentatively, we may conclude from the different types of end-

group analyses that there are between three hundred and five hundred terminal groups per DNA molecule of 5×10^6 molecular weight.

Kinetics of DNase Action.—Figure 1 shows representative ultracentrifuge patterns from a series of runs at different stages of enzyme action (DNase). The boundaries observed are consistent with the view that the enzyme acts on the substrate in a random manner. Early in the reaction there is very little material present with the characteristics of DNA and, similarly, there are almost no low molecular weight end-products. The boundaries in the intermediate stages indicate polydispersity, as would be expected from a random attack by the enzyme. Low molecular weight products appear only near the end of the reaction. In

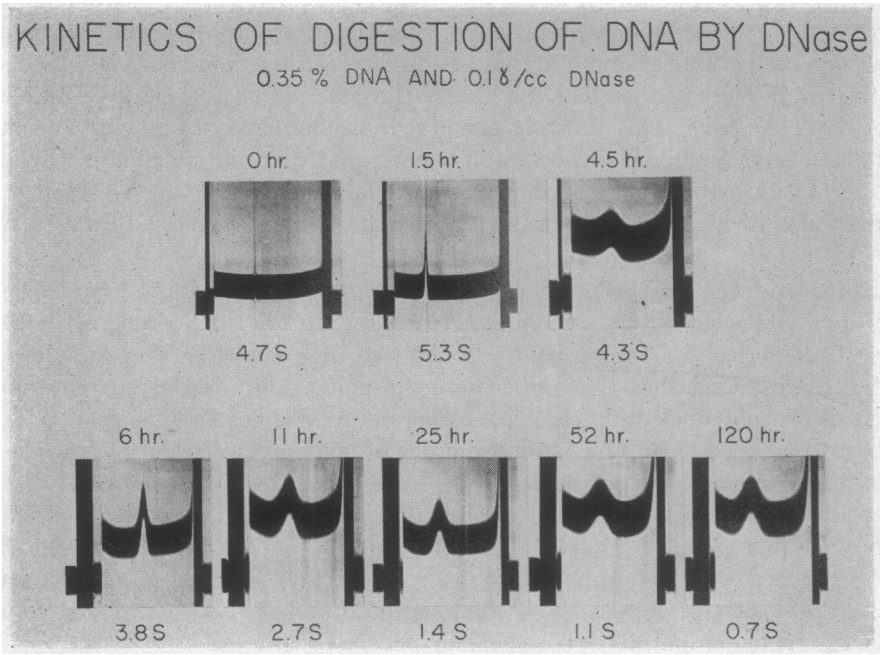

FIG. 1.—Ultracentrifuge patterns at various stages of the digestion of DNA (0.35 per cent) by DNase (0.1 γ/cc). Each picture is a representative pattern about $^1/_2$ hour after reaching speed, 59,780 rpm. The studies at 0, 1.5, and 4.5 hours were performed in conventional ultracentrifuge cells and the remainder in a synthetic boundary cell. All boundaries are moving toward the right.

order to demonstrate the resolving power of the ultracentrifuge in this type of study, some untreated DNA was added to the digestion mixture at various times, and the resulting patterns, shown in Figure 2, demonstrate that the "native" DNA can be readily detected when present. The sedimentation coefficients and reduced viscosities indicate that the molecular weight decreases from about 5×10^6 to 10^3 during the course of the enzymatic digestion.

In Figures 3 and 4 are shown the viscosity, sedimentation, and ultraviolet absorption data as a function of time of enzyme action. As can be seen from Figure 3, the reduced viscosity falls to 10 per cent of its initial value in 1 hour, while the sedimentation coefficient hardly changes. At concentrations of 0.35 per cent DNA

the sedimentation coefficient actually increases initially, but this cannot be taken to indicate a coiling of the macromolecule. The sedimentation coefficient of DNA is greatly dependent on concentration, and the value at 0.35 per cent is less than half

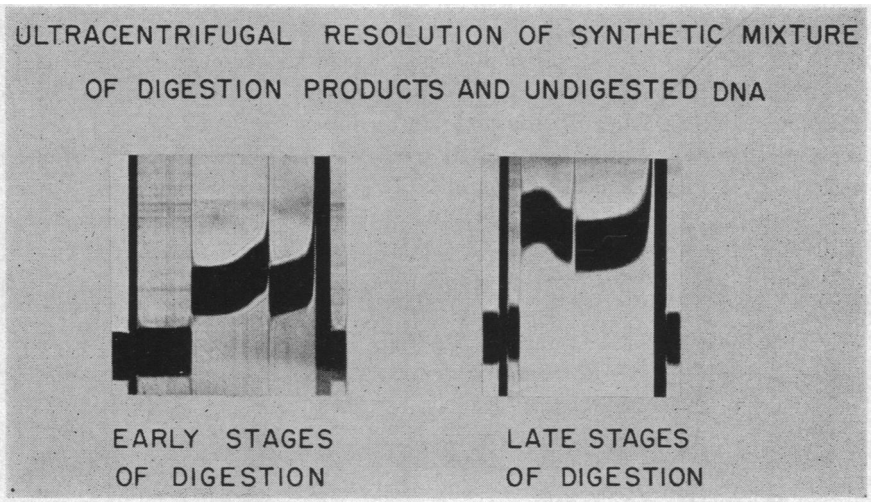

FIG. 2.—Ultracentrifuge patterns of mixtures of partially digested DNA and "native" DNA. The studies were performed immediately after adding the "native" DNA.

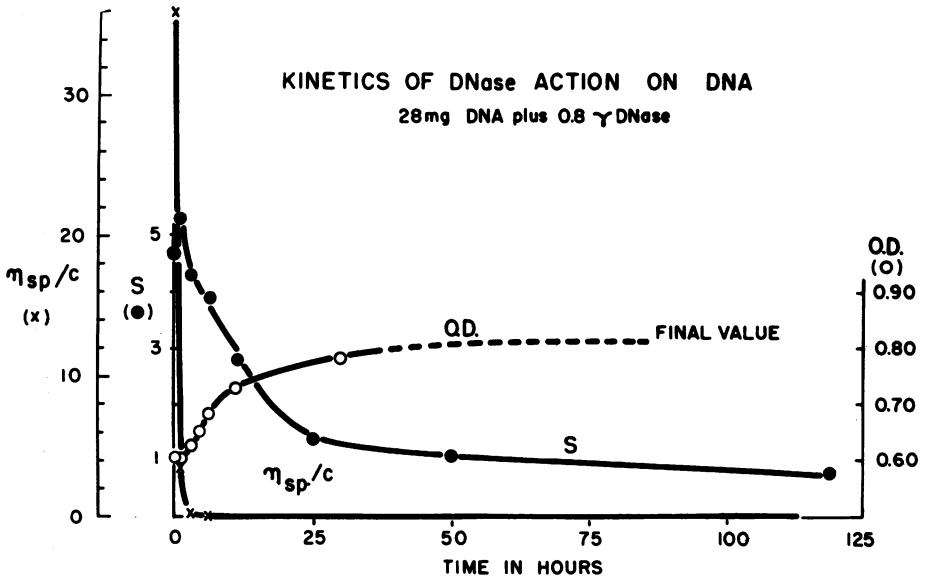

FIG. 3.—Kinetics of digestion of DNA by DNase as followed by viscometry, ×; ultracentrifugation, ●; and ultraviolet spectrophotometry, O.

the infinite dilution value. For the partially degraded material it is likely that the dependence of sedimentation coefficient on concentration would be less than that observed for DNA. Proof of this hypothesis is shown in the second study (Fig. 4), where sedimentation data at two different concentrations were obtained at different

stages of the digestion. At a concentration of 0.13 per cent DNA the sedimentation coefficient increased perceptibly during the first 5 hours, and then there was a decrease with time. The sedimentation coefficient at 0.04 per cent, however, showed a marked decrease even in the very early stages of the reaction. This decrease in sedimentation rate, it should be noted, is only about 20 per cent during the first 5 hours, whereas the reduced viscosity falls 70 per cent during this same time interval. As discussed previously, this behavior is to be expected for elongated molecules undergoing splitting into shorter molecules.

In the study represented by Figure 3 there appeared to be a lag period of about 1 hour before the optical density increased. This induction period is shown more clearly in Figure 4. In the latter study the optical density remained constant for the first 15 hours, whereas the viscosity decreased to about 7 per cent of its initial value. Using a value of 7*S* for the sedimentation coefficient of the digested ma-

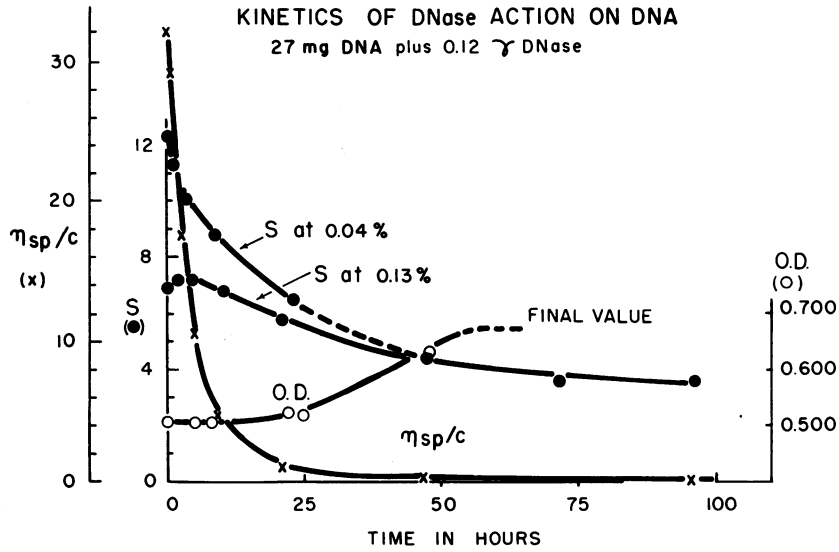

FIG. 4.—Kinetics of digestion of DNA by DNase as followed by viscometry, ×; ultracentrifugation, ●; and ultraviolet spectrophotometry, ○.

terial, we can calculate that the average molecular weight decreases to about 100,000 before an increase in optical density can be detected. The results suggest that the enzymatic attack in the early stages involves a splitting of the long and slightly curved threadlike structure into shorter pieces. This degradation is accomplished presumably by the breaking of phosphodiester bonds in the backbone structure without altering the relationship between the purine and pyrimidine rings which are responsible for the ultraviolet absorption.

Continued enzyme action produces fragments of still smaller molecular weight and causes the increase in optical density at 259 mμ first observed by Kunitz.[24] The molecular weight is about 35,000 when the increase in optical density amounts to 80 per cent of the total increase observed in the complete digestion of DNA. By the time the optical density has attained its final value, the molecular weight has fallen to about 5,000. From these studies we can conclude that the complex intra-

molecular organization, characteristic of DNA, no longer exists when the average molecular length is of the order of one hundred nucleotides. This estimate is, of course, a maximum one, since it is based on a one-strand structure. If there are two strands, then the average length would be only fifty nucleotides. It seems likely that the number of breaks in the polynucleotide strands is large when the average molecular weight of the digestion products is in the neighborhood of 100,000. Cleavage of a few more phosphodiester bonds by the enzyme increases the number or proximity of breaks in the strands to a critical point at which thermal energy could cause the rupture of the hydrogen bonds remaining between breaks, thereby leading to a complete separation of the individual chains. This separation of the chains would be accompanied by an increase in optical density because of the disruption of the intramolecular organization within the DNA molecule. Thus no direct action of the enzyme need be invoked to explain the increase in optical density.

In order to learn more about the beginning stages of the enzymatic digestion, a third study (Fig. 5), was performed at a still lower enzyme concentration. In-

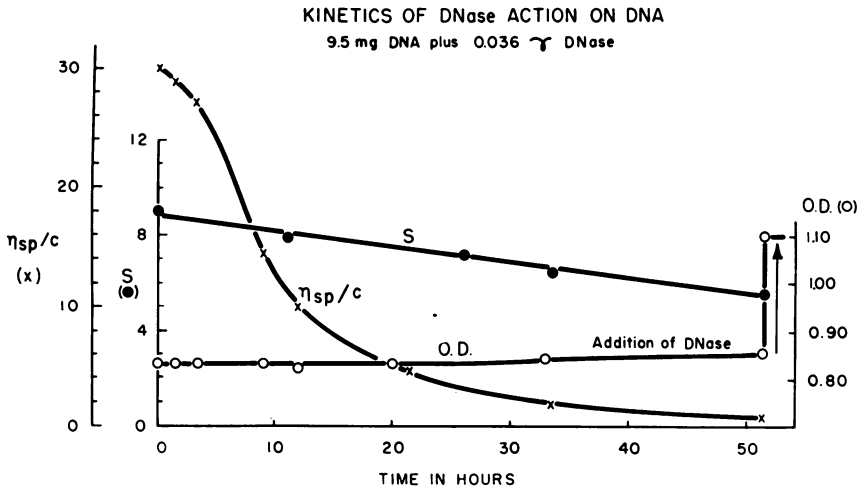

FIG. 5.—Kinetics of digestion of DNA by DNase as followed by viscometry, ×; ultracentrifugation, ●; and ultraviolet spectrophotometry, ○.

itially, the change of viscosity with time was small. After 4 hours the rate of decrease of viscosity with time became greater. Zamenhof *et al.*[36] have observed a lag period for the change in viscosity of DNA solutions subjected to extremely low concentrations of enzyme. During this lag period the transforming activity of the preparation was almost completely destroyed. Further evidence that the enzyme caused structural alterations in the macromolecules during this lag period stems from their studies of the increase in heat sensitivity of the material as a result of brief treatment with enzyme.[41] Although the enzyme action itself caused practically no change in the viscosity of the DNA solutions, these solutions lost their viscosity upon being heated to temperatures at which native DNA is stable. Furthermore, the longer the predigestion of the DNA with DNase, the more readily was the viscosity lost upon subsequent heating. It appeared also that the

activation energy for the process was decreased appreciably. Since it is likely that the enzyme is breaking phosphodiester bonds at a constant rate during the early stages of the digestion process, these results suggest that the initial hydrolytic action of the enzyme is not so efficient in causing a decrease in viscosity as is the subsequent enzymatic action. Such behavior is to be expected for a two-strand or multistrand model in which the hydrogen bonds between the individual strands can hold the structure together even though breaks are being made in the strands.

Models for DNA.—Figure 6 presents some possible models for the native DNA macromolecule. Evidence for the fibrous form in preference to the random coil has been given in detail, and we can thus eliminate model *I*. As indicated earlier, some specific structural feature must be invoked to account for the stiffness and

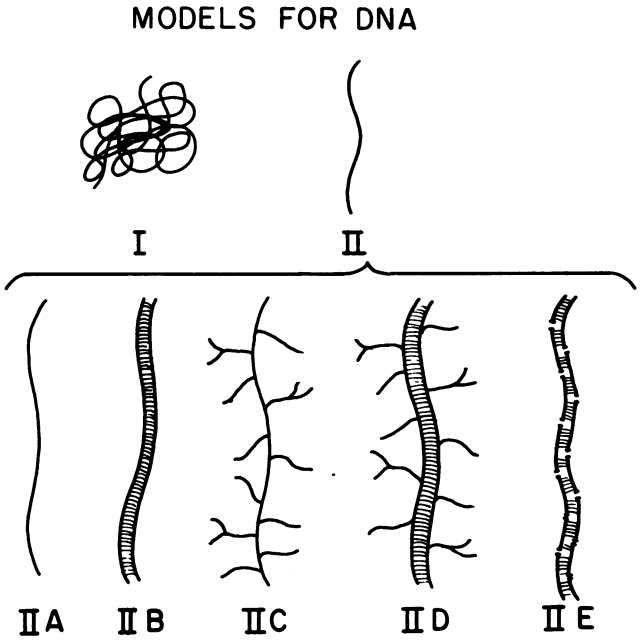

FIG. 6.—Models for the macromolecular structure of DNA.

lack of random coiling of the macromolecule. Rigidity could result either from intrastrand hydrogen bonding such as has been postulated for certain fibrous proteins or from interstrand hydrogen bonding as in the model for DNA suggested by Watson and Crick.[1, 2] Single-strand structures have been eliminated as a possibility because they are incompatible with the data obtained from X-ray diffraction studies and density measurements. We can, therefore, eliminate models *II A* and *II C* from further serious consideration and focus our attention on the remaining models. The two-strand, intertwined, helical model, *II B*, of Watson and Crick or some multistrand model could account for the shape of DNA in solution. The X-ray evidence points strongly toward the two-strand, hydrogen bonded model, which, as Watson and Crick point out, would explain the titration behavior of DNA. Such a model would also account satisfactorily for the increase in optical density observed when the organized structure of DNA is disrupted.

A two-strand model in which the polynucleotide background is continuous throughout each strand is, however, incompatible with the titration and other end-group studies, which suggest that there are three hundred to five hundred terminal groups per molecule of DNA. Further evidence in conflict with a continuous, two-strand model is the relative ease with which the macromolecules can be degraded. The heating experiments, which show a reduction in molecular weight by a factor of one hundred, are of sufficiently brief duration to make it seem unlikely that there is any appreciable hydrolysis of phosphodiester bonds. In preliminary experiments conducted in this laboratory we have failed thus far to detect hydrogen ions which would be liberated if the heating at pH 7.5 actually caused hydrolysis. It should be noted, however, that the experimental demands are quite rigorous since we are dealing with the detection of quantities of the order of one micromole of hydrogen ion. Despite the limitations of both the measurement of the end-groups and the interpretation of the heating experiments, the results of these studies, when taken together, provide sufficient evidence to cast doubt on the adequacy of a model made up of two continuous polynucleotide strands.

A simple modification of the continuous two-strand structure yields a model *II D*, which satisfies the requirements of the end-group studies. If at every fiftieth phosphorus atom there were a triply esterified phosphate leading to the No. 3 position of the deoxyribose moiety of another nucleotide, we would have a series of branches which could terminate in phosphate groups which were only singly esterified and hence showed both primary and secondary ionizations. Such branched structures for DNA have been proposed by many workers.[31, 32, 5] However, it is known that triesterified phosphates are readily hydrolyzed in dilute alkali even at moderate temperatures.[42] Studies by Zamenhof and his collaborators[36] with the DNA preparations possessing transforming activity demonstrate the extraordinary stability of this material to alkali up to pH 10. Even under more alkaline conditions, which would destroy the biological activity, there is no evidence for the formation of low molecular weight material. Frick[25] found, for example, that dialysis of thymus nucleoprotein against 0.1 *M* sodium hydroxide resulted in only a negligible amount of ultraviolet light-absorbing material passing through the membrane. It seems unlikely that the DNA molecules could possess one triesterified phosphate for every fifty phosphorus atoms and still exhibit such stability. If the branch points arose from deoxyribosyl-1-phosphate linkages, the resulting structure would almost certainly show disintegration upon heating due to the lability of such linkages.[43] Such disintegration, however, would cause a lowering of the pH of the solution due to ionization of the monoesterified phosphate groups created by the hydrolysis. As indicated earlier, no liberation of hydrogen ions could be detected. Furthermore, the disintegration by heat of a model like that shown in *II D* might be expected to produce a small but measurable amount of low molecular weight polynucleotides. Using an ultracentrifugal technique capable of detecting such fragments, we have been able to show that the disintegration of DNA by heat does not lead to the formation of such low molecular weight material to the extent of 1.0 per cent of the original weight of the DNA. Further evidence against the existence of a branched structure for DNA comes from studies on the physical-chemical behavior of concentrated solutions of DNA, especially when considered in conjunction with the studies on the DNA which had been subjected to heat. Since poly-

nucleotide chains show a tendency toward aggregation, it is reasonable to assume that branches in the "native" DNA macromolecule would lead to an infinite, three-dimensional network of the type observed when concentrated solutions of DNA are heated to about 85° C. That the three-dimensional network in the heated solutions is the result of cross-linking through secondary forces, such as hydrogen bonds, is shown by the ease with which the gel melts and by its thixotropic behavior when subjected to shearing forces.[37] This behavior is in contrast to that observed for solutions of "native" DNA, which is in accord with that expected of a solution of rigid, elongated particles.[44]

The objections to a branched structure lead to an examination of other possible models, one of which, *II E*, seems to satisfy almost all the physical and chemical properties of DNA. The macromolecule is considered an aggregate of polynucleotides of size consistent with the end-group analysis—i.e., one terminal group for approximately fifty nucleotides. These basic units of average molecular weight 15,000 would then constitute the minimum chemical units. The chains are arranged in a two-strand structure, with the interruptions in the strands being staggered relative to one another. It is of some interest that the various methods of causing the dissociation of DNA into smaller units, without, it is thought, causing the hydrolysis of ester bonds, produce material with molecular weights of that magnitude. Both the mild heating experiments and mild treatment with acid[45] yield such low-molecular-weight material. With such a model, disintegration into small pieces would require rupture of hydrogen bonds only, and the process would be of a co-operative nature leading to a large activation energy. This, too, is in accord with the best available data. Not only can the end-group analyses be accounted for by such a model, but also the heating experiments on both dilute and concentrated solutions can be explained satisfactorily. In dilute solutions heat produces fragments of much smaller molecular weight, whereas in concentrated solutions the probability of recombination of the chains through hydrogen bonds is much higher, and a three-dimensional network results. It is of interest that heat-degraded DNA, but not enzymatically or sonically degraded DNA, shows a tendency toward repolymerization.[33] Partial degradation by the latter two methods probably causes a fission of both strands, leading to shorter, two-strand molecules. With heat, however, there appears to be an unraveling of the strands as a result of the rupture of the hydrogen bonds. The free single chains or those that are partially unraveled from the main structure then tend to repolymerize by hydrogen-bond formation between the purine and pyrimidine rings. Thus the increase in optical density at 260 mμ obtained by heating DNA solutions is less than the full 32 per cent increase obtained by other methods. In support of this explanation is the demonstration that mild heating in the presence of urea, a reagent known to break hydrogen bonds, produces the full increase in optical density. In terms of X-ray diffraction the model depicted by *II E* would appear identical to that proposed by Watson and Crick,[1, 2] since the interruptions in the strands would not affect the X-ray diffraction pattern. There could still be the specificity in pairing of the bases which is an important feature of the model of Watson and Crick. Each chain in the interrupted two-strand model would be subjected to about five turns in the helical structure. Winding of the strands around one another as in the Watson-Crick model would lead to enhanced stability over a model in which the

strands were side by side. This could help explain why DNA is more resistant than proteins to reagents which normally break hydrogen bonds. The tendency of the molecule to expand, as evidenced by the increase in optical density and loss of biological activity when the ionic strength is reduced to very low values,[26, 36] can be satisfactorily explained by the interrupted two-strand model. Reducing the ionic strength permits an increase in the effective repulsive forces due to the high density of negatively charged phosphate groups, and some of the hydrogen bonds are ruptured owing to the expansion of the molecule.

Probably the most crucial test of any proposed model is a kinetic study of the change in molecular weight of DNA as phosphodiester bonds are being broken by DNase. In a single-strand model each attack of the enzyme would be 100 per cent efficient in causing a fission of the macromolecule, as indicated by a decrease in the molecular weight, unless some obscure arrangement of secondary forces holds the pieces together. We would, in general, expect no lag period during the enzymatic hydrolysis of such a structure. In the digestion of a two-strand structure, there are two possibilities for the mode of action of the enzyme. The enzyme may, on the one hand, break neighboring ester bonds in both strands simultaneously. In that case the kinetics of degradation would be identical with those of a single-strand structure. Alternatively, the enzyme may attack only one bond at a time in a random manner, in which case a lag might be expected in the rate of decrease of molecular weight. As shown in the following, the length of this lag period would be an indication of the degree to which the two strands are continuous. In the continuous two-strand model the first attack of DNase on a phosphodiester bond ($C^{3'}$—O—P) cannot cause a decrease in molecular weight unless we make the very unreasonable assumption that all the interchain hydrogen bonds also break as a result of the enzyme action. The probability that the second attack by the enzyme will cause a fission is of the order of 10 in 16,000, since there are roughly 16,000 susceptible ester bonds per molecule of DNA, and fission occurs only when there is hydrolysis of a bond in the second strand in the proximity of that bond already ruptured in the first strand. If the second attack on a molecule of DNA by the enzyme occurs at a bond three or even five phosphorus atoms removed from the one in apposition to the bond ruptured by the first attack, there will be only a few hydrogen bonds holding the pieces together. It seems reasonable that they will break owing to thermal motion, leading to a fission. After the second attack there will be two regions in which subsequent enzyme action can be fruitful, and the efficiency of the third attack will have a probability of 20 in 16,000. The fourth attack will have an efficiency of 30 in 16,000, and so on, until the number of breaks in the strands becomes large enough to require modification of this simple picture. With the interrupted two-strand model containing preformed breaks roughly every fifty nucleotides, the picture is markedly different. The first attack by DNase now has a probability of 10 in 50 of being effective, as contrasted to the inability of the enzyme to cause a fission in its first attack on the continuous two-strand model. In further contrast, the early attacks by the enzyme on the interrupted two-strand model will not cause an appreciable change in the efficiency for subsequent attacks, since a few breaks by the enzyme are negligible compared to the three hundred presumed to exist already. Thus the efficiency at the early stages of DNase action will remain constant for the interrupted two-strand model.

This type of investigation requires the use of sensitive techniques for the detection of the hydrolysis of only a very few phosphodiester bonds. Our preliminary results show that only about five bonds need be broken to produce a significant change in the viscosity; but much more effort will be required before it can be concluded that the results support the interrupted two-strand model. It is hoped that studies of this type will provide definitive evidence in support of some specific model.

It should be pointed out, in summary, that the evidence collected in this communication has its origin in the work of many investigators working with preparations of DNA which no doubt were subjected to different influences during the isolation procedures. Some were more degraded than others, and this is perhaps the reason why the literature contains discordant results. If titration or other end-group studies on preparations of DNA from different sources, and especially those known to possess some biological activity, also showed an appreciable number of terminal groups per molecule, then we would be more confident about proposing that the "native" DNA is an aggregate of smaller units held into a two-strand configuration of the type suggested by Watson and Crick. For the moment, it seems that the bulk of the available data on the physical and chemical behavior of DNA isolated by modern procedures is most nearly satisfied by such an interrupted two-strand model.

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*THE UNCOUPLING OF RESPIRATION AND PHOSPHORYLATION BY THYROID HORMONES**

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The demonstration of the uncoupling of respiration of respiration and phosphorylation by nitrophenols[1] introduced a new approach to the understanding of intercellular energy regulation by exposing relatively easily accessible experimental means of interrupting the link between energy supply and energy utilization. Furthermore, with mitochondria, halophenols such as di- or tribromo or iodophenols were as active as the nitro compounds, as was first observed with arabacia eggs by Krahl and Clowes.[5] This brought particularly to our attention the possibility of defining the activity of the physiological halophenol, the thyroid hormone, as likewise due to a dissociation of respiration and phosphorylation.

Efforts therefore were started with the hope of obtaining uncoupling effects with thyroxine. We have reported relatively briefly on the results of these rather extensive studies,[2, 3, 4] mainly because the picture which developed with these experiments, although quite indicative, remained for some time inconclusive. It is the purpose of this paper to report in greater detail on our results, particularly the more recent ones.

Loomis made the obvious attempt to duplicate the nitro, halophenol effect on mitochondria suspensions with thyroxine, using rabbit kidney preparations. However, as in unpublished and published experiments, by many others, cf. e.g.,[6] no reliable effect could be observed. It is well known, however, that the easily and consistently obtained increase in respiratory rate with tissue slices taken from thyrotoxic animals (Barker[7]) could never be reliably produced by an in vitro addition of thyroxine to normal slices. This indicated that cell membranes were relatively impermeable