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OPTICAL ROTATORY PROPERTIES OF POLYNUCLEOTIDES AND NUCLEIC ACIDS

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THE nucleic acids are currently a major focal point of investigation for the molecular biologist. This is because it is now recognized that this group of macromolecules plays a central role in the hereditary process, and in the dissemination of the genetic information within a given generation.

In a search for better ways to reveal some of the more subtle aspects of nucleic acid conformation in solution, a number of laboratories[1-5] have recently turned to the method of optical rotation. It is not difficult to understand this, when one considers the success with which this tool has been employed within the last decade to detect and quantitate α-helical secondary structure in synthetic polypeptides and proteins.[6-8] Indeed, from this work it has become abundantly apparent that optical rotation can reflect regularities in main chain configuration which are not directly related to asymmetric atoms. Now, in the case of nucleic acids, there exist similar possibilities for optical rotatory effects arising from secondary structure; for their covalent nature would seem to require that structural regularity will arise from some form of helical distribution of the polar groups in the backbone, due to the need to maintain maximum charge separation.

It will become apparent from what follows that our understanding of the optical rotatory properties of nucleic acids has not reached the degree of refinement achieved in the case of polypeptides. Indeed, there are wide gaps even at the observational level. Nevertheless, a number of generalities are beginning to emerge, and it is to these positive aspects that I direct my attention.

Centers of optical activity in nucleic acids

Primary structure. A priori, one might expect nucleic acids to show a baseline level of optical activity, merely due to the presence of asymmetric atoms. These will include several carbon atoms in the pentose of each nucleotide (monomer unit), but not the phosphorus atom involved in the internucleotide linkage, since the latter contains two equivalent oxygens. The disposition of atoms in a polynucleotide chain may be seen in Fig. 1, which shows a section of a hypothetical ribonucleic acid (RNA) chain. The backbone of deoxyribonucleic acid (DNA) differs from that shown only in that the pentose C-2, in lacking oxygen, is no longer asymmetric.

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[2] J. R. Fresco, *Trans. N.Y. Acad. Sci.* Ser II, **21**, 653 (1959).
[3] B. H. Levedahl and T. W. James, *Biochem. Biophys. Acta* **17**, 453 (1955).
[4] A. Pour-El and C. A. Dekker, *Abstr. Amer. Chem. Soc. 136th Meeting* p. 7c (1959).
[5] P. Ts'o, *Tetrahedron.* This volume(1961).
[6] P. Doty, *Proceedings of the Fourth International Congress of Biochemistry* Vol. IX, p. 8. Pergamon Press, London (1959).
[7] E. Blout, *Tetrahedron.* **13**, 123 (1961).
[8] J. T. Yang, *Tetrahedron.* **13**, 143 (1961).

The fully randomly coiled polynucleotide chain is, nevertheless, not to be expected to display optical activity predictable simply from that of its pentose constituent. For it has been observed that the base substituent on the pentose while not optically active by itself has a significant effect on the resulting nucleoside.[9,10] Thus, whereas those with purine substituents have $[\alpha]_D$ values somewhat negative, pyrimidine nucleosides

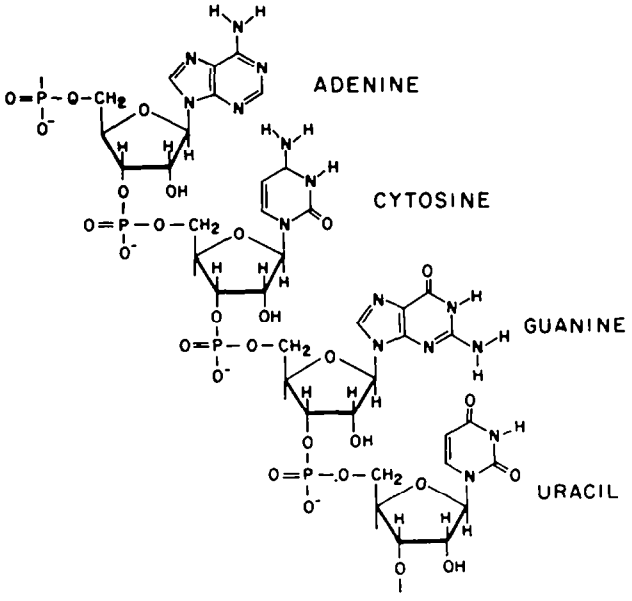

FIG. 1. The chemical structure of ribonucleic acid.

show small positive rotations. In general, also, those nucleosides containing D-2-deoxyribose have an $[\alpha]_D$ about 20° more positive than their D-ribose counterparts.[11] The introduction of a phosphate group to the 3′ or 5′ position of a nucleoside has a small and apparently unsystematic effect on the rotatory power.* From all this, we can predict that for a completely disordered polynucleotide chain, be it of the RNA or DNA type, the $[\alpha]_D$ will be in the region of 0°.

Secondary structure. What, then, are we to expect when the polynucleotide back-bone is brought into some type of ordered helical conformation? A number of multi-stranded *completely* helical polynucleotides are now known. These include DNA and several synthetic polyribonucleotide complexes†, e.g. poly(A + A)[12-14]

* Unfortunately, the data on nucleosides and nucleotides are sparse and sometimes contradictory. These generalities have been made after careful selection of what seemed to the author to be the most reliable data.

† The following abbreviations have been used. A = adenine; G = guanine; U = uracil; C = cytosine; I = inosine, where the base is hypoxanthine. The prefix *poly* is used to indicate a homo or copolymer of nucleotide(s) of the indicated base(s). Polynucleotide complexes are indicated by the prefix *poly* followed in parentheses by the summated homopolymer chain symbols.

[9] P. A. Levene and E. S. London, *J. Biol. Chem.* **83**, 793 (1929).
[10] B. H. Levedahl and T. W. James, *Biochim. Biophys. Acta* **26**, 89 (1957).
[11] J. Baddiley in *The Nucleic Acids* (Edited by Chargaff and Davidson) Vol. I, pp. 156, 178. Academic Press, New York (1955).
[12] J. R. Fresco and P. Doty, *J. Amer. Chem. Soc.* **79**, 3928 (1957).
[13] J. R. Fresco and E. Klemperer, *Ann. N.Y. Acad. Sci.* **81**, 739 (1959).
[14] R. Steiner and R. Beers, *Biochem. Biophys. Acta* **32**, 166 (1959).

poly(A + U),[15-17] poly(A + U + U),[17] poly(I + C),[18] poly(I + I + I).[19] Of these, the most detailed structure analysis is available for DNA,[20,21] whose comformation is schematically presented in Fig. 2. In this complex, as in all the others, the pentose-phosphate backbone is on the periphery of the helix, and the base residues are hydrogen-bonded in pairs or triplets in the interior. In all these cases, then, additional contributions to the optical rotation may appear due to the helical conformation. One of these should arise from the interaction of the polarized light with the optically active centers in the helically wound backbone strands. It is useful, perhaps, to think of the pentose-phosphate backbones as analogous to the α-helix backbone of the polypeptide, recognizing, however, that the polynucleotide rotation arises from D residues, whereas the polypeptide contains L residues. In the polynucleotide, we might expect, another conformational effect to result from the helical array of the base pairs, which can be thought of as a series of discs translating about 36° every 3·4 Å (or some similar repeat distance in the other complexes) along the helix axis. The contribution due to these chromophores could, in fact, be quite large, if as might be supposed, the base pairs involve one or more $n \rightarrow \pi^*$ transitions[22,23] in addition to $\pi \rightarrow \pi^*$ transitions.†

Dependence of specific rotation on conformation

The helix-coil transition. A large body of physico-chemical evidence is now available which shows that the increases in ultraviolet absorption at 260 mμ that accompany the exposure of helical polynucleotides in solution to elevated temperatures provide a direct measure of the denaturation process.[1] Thus, a plot of ultraviolet absorption versus temperature reflects the *melting* of the helical conformation into a disordered random coil.

This ultraviolet absorption change is undoubtedly related to the fact that in organized polynucleotides, the absorption of the constituent nucleotide units is markedly depressed.[13,15,17,24] This hypochromicity appears to be due to the particular mode of stacking of the bases maintained as a result of hydrogen bond interactions. Thus, when thermal disruption of the hydrogen bonds occurs, the absorbing properties of the chromophores revert almost completely to those of the individual monomer units.[25]

In view of this, and in an attempt to assess the contribution of helical conformation to rotatory power, we investigated the changes in specific rotation which could be thermally induced in several helical polynucleotides.[1,2,13] The results for DNA and poly (A + U) are shown in Figs. 3 and 4. For the sake of comparison, the optical

† It is observed generally that $n \rightarrow \pi^*$ transitions contribute more strongly to rotatory power than do $\pi \rightarrow \pi^*$ transitions, although their inherent absorbancy is at least an order of magnitude lower.

[15] R. C. Warner, *J. Biol. Chem.* 229, 711 (1957).
[16] A. Rich and D. R. Davies, *J. Amer. Chem. Soc.* **78**, 3548 (1956).
[17] G. Felsenfeld and A. Rich, *Biochim. Biophys. Acta* **26**, 457 (1957).
[18] D. R. Davies and A. Rich, *J. Amer. Chem. Soc.* **80**, 1003 (1958).
[19] A. Rich, *Biochim. Biophys. Acta* **29**, 502 (1958).
[20a] M. Feughelman, R. Langridge, W. E. Seeds, A. R. Stokes, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, R. K. Barclay and L. D. Hamilton, *Nature, Lond.* **175**, 834 (1955).
[20] F. H. C. Crick and J. D. Watson, *Proc. Roy. Soc.* A **233**, 80 (1954).
[21] R. Langridge, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *J. Biochem. Biophys. Cyt.* **3**, 767 (1957).
[22] H. Murakami, *J. Chem. Phys.* **27**, 1231 (1957).
[23] M. Kasha in *Light and Life* (Edited by W. D. McElroy and B. Glass). Johns Hopkins, Baltimore (1960).
[24] A. Thomas, *Biochim. Biophys. Acta* **14**, 231 (1954).
[25] J. R. Fresco, R. Haselkorn and P. Doty, unpublished.

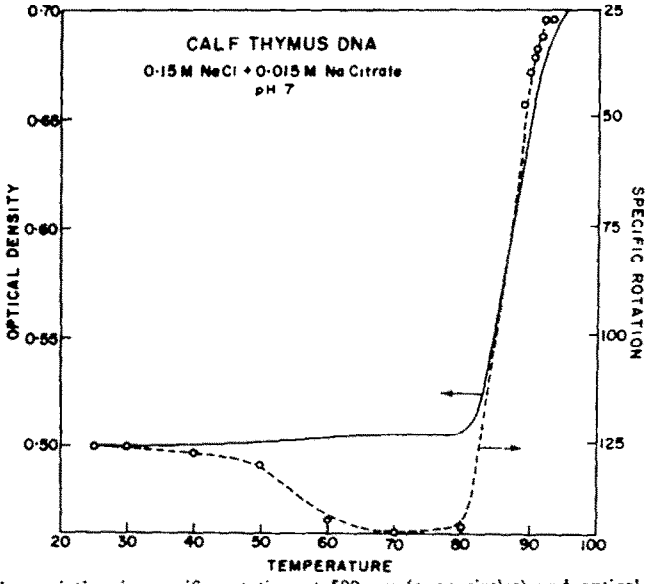

FIG. 3. The variation in specific rotation at 589 mμ (open circles) and optical density at 259 mμ (solid line) with temperature of solutions of calf thymus DNA.[1]

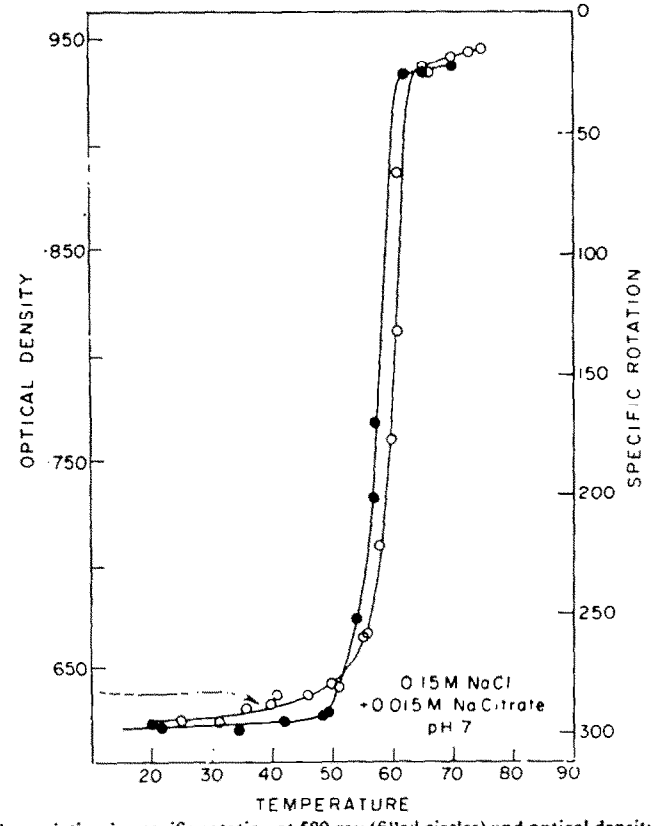

FIG. 4. The variation in specific rotation at 589 mμ (filled circles) and optical density at 260 mμ (open circles) with temperature of solutions of poly (A + U).[1]

density data are also plotted and the two ordinates are normalized. It should be noted that since there are reversible aspects to the transitions, all measurements were made at the ambient temperature. From such data, of which the examples shown are typical, it is clear that optical rotation does depend on conformation.

At a glance, it is evident that the profiles obtained by the two optical methods are essentially parallel. Also, at high temperature, where the polynucleotide chains are disordered coils, the $[\alpha]_D$ approaches 0°, which is consistent with our earlier argument. Finally, it can be seen that the contribution due to helical conformation is a very sizable *positive* one. Since the sign appears to be the same in all the polynucleotides so far

TABLE 1. OPTICAL ROTATION OF POLYRIBONUCLEOTIDES

Polymer	Temperature	$[\alpha]_D$
Poly U—single strand	22	-8
Poly A—single strand	22°	155°
	90°	-5°
8M urea	22°	75°
Poly (A + A)—helix	22°	300°
Poly (A + U)—helix	22	300°
	70°	23°

The solvent is neutral saline solution of 0·2 ionic strength in all cases except for poly (A + A), where the pH is 4·9.

studied, it seems reasonable to conclude that all the helices, like DNA, whose screw sense has been independently established,[20] must be righthanded.

The DNA profile contains two characteristic features which set it apart from those of the polyribonucleotides. One concerns the dip, or increase in positive rotation, in the temperature region between 30 and 80°. It must be emphasized that this is a reproducible observation, and it has also been noted when a similar profile was determined at 289 mμ, in the region of the Cotton effect (see below). This finding suggests that as the temperature is raised, there are subtle changes in the geometry of the molecule, perhaps in the pentose constituent, to which ultraviolet absorption is insensitive. The second point is the much lower magnitude of the positive rotation in native DNA. A simple explanation for this is also not immediately apparent, although it seems possible that 589 mμ, the D line, may represent a position more distant from the critical wave length in the deoxyribose than in the ribose polynucleotides.

Another way to emphasize the effect of helical structure on rotatory power is to compare the specific rotation of a multistranded helix with that of its single-stranded polyribonucleotide components. Some relevant data are presented in Table 1. Consider first the poly (A + A) helix. At room temperature, where this complex is stable, it has an $[\alpha]_D$ of 300°, which is about 145° higher than that of its single-chain component. The poly (A + U) helix presents an even more striking picture. In this instance, the interaction of one strand having an $[\alpha]_D$ of $-8°$ (poly U) with another having an $[\alpha]_D$ of 155° (poly A) results in a helical complex with an $[\alpha]_D$ of 300°. Apparently, when the poly U strand assumes a helical conformation, it contributes with as high a positive rotation as the poly A chain.

Helical content of single-stranded polynucleotides. An obvious extension of the

finding that optical rotatory power reflects helical conformation in multistranded polynucleotide complexes was to exploit this property as a probe for helical order in single-stranded polynucleotides (including RNA).

Prior to this effort it was recognized from an assessment of their ultraviolet hypochromicity that there exists among these single chain polymers a gradation in degree of intramolecular hydrogen bonding. It now became evident that a similar gradation can be made on the basis of degree of positive rotation. Fortunately poly U at room

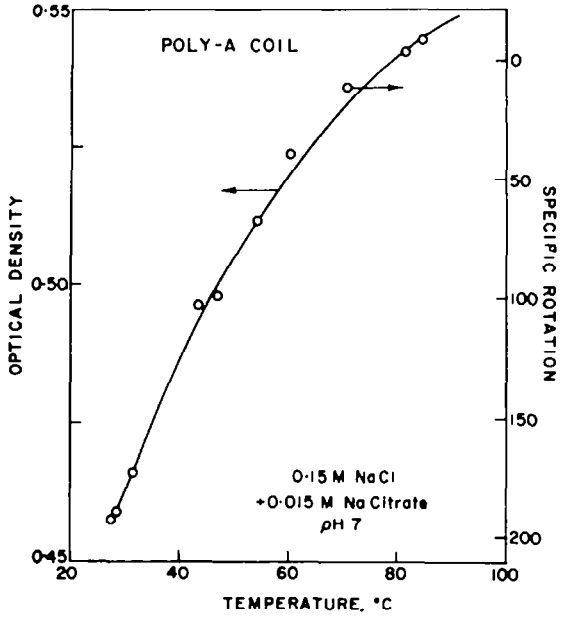

FIG. 5. The variation in specific rotation at 589 mμ (open circles) and optical density at 257 mμ (solid line) with temperature of neutral solutions of poly A single strands.[18]

temperatue provides baseline levels for both these parameters, i.e. it shows almost no ultraviolet hypochromicity, and consistent with this, it has an $[\alpha]_D$ very close to that of its monomer. Poly A, on the other hand, displays considerable hypochromicity, and as shown above, has an $[\alpha]_D$ intermediate between that of the two-stranded helical form, and that of the monomer (whose value is approached when the temperature is raised near 100°). When 8 M urea is added to the solvent, so that a large proportion of the intramolecular hydrogen bonds in poly A are broken at room temperature, there is a marked decrease in the hypochromicity and a concomitant decrease in positive rotation. Indeed, the course of the changes in hypochromicity which can be induced over the accessible temperature range is completely paralleled by decreases in specific rotation (see Fig. 5). Similar observations have been recorded for RNA from different sources (Fig. 6) and for single-stranded denatured DNA (Fig. 7).

From such observations, we are prone to conclude that nearly all the hydrogen bonded bases of single-stranded polynucleotides are contained within helical regions. Naturally, this structural assignment does not rest merely on the qualitative coincidence of the rotatory and absorbance phenomena described above. Equally significant and more revealing are the magnitudes of the rotational effects. For these represent a

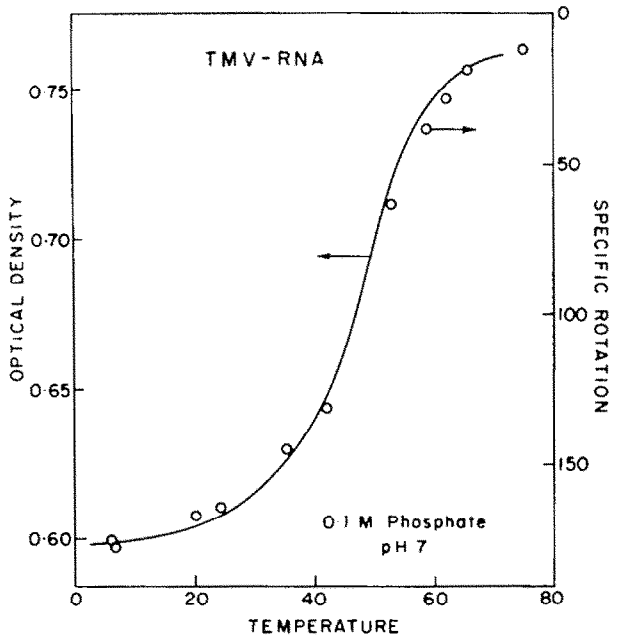

FIG. 6. The variation in specific rotation at 589 mμ (open circles) and optical density at 260 mμ (solid line) with temperature of solutions of Tobacco Mosaic Virus RNA.[1]

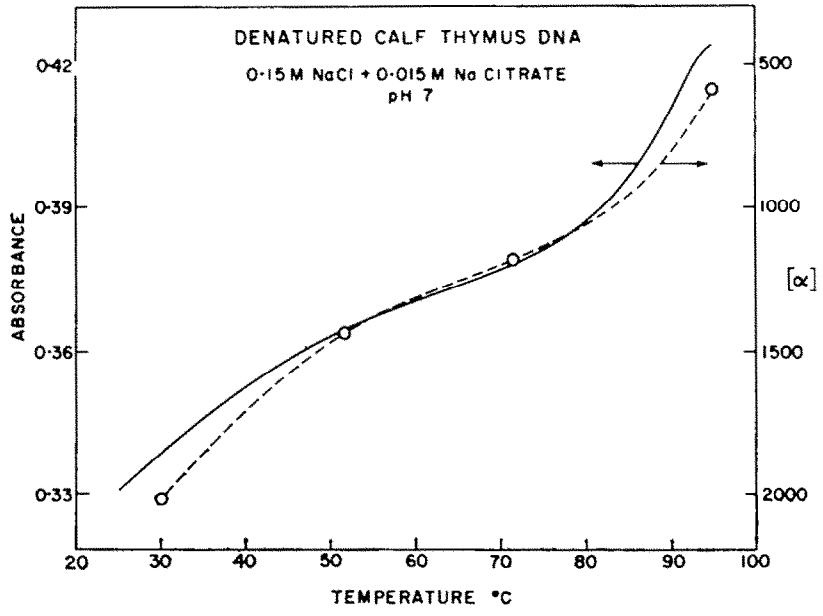

FIG. 7. The variation in specific rotation at 289 mμ (open circles) and optical density at 259 mμ (solid line) with temperature of solutions of heat denatured DNA.[28]

substantial proportion of the changes elicited when a multistranded complete helix undergoes transition to the random coil. In fact, they closely approximate the values predicted from the product of the fraction of hydrogen-bonded bases (adduced from hypochromicity data) and the rotational change expected from the melting of a completely helical polynucleotide.[1]

It must be admitted, nevertheless, that the estimation of helical content of single-stranded polyribonucleotides from specific rotation data depends in some measure on three assumptions: (1) that the nature of the helical regions within the single strand is much like that of the complete helical complexes; (2) that the length of a helical region does not markedly affect rotatory power; and (3) that the types of hydrogen bonded base pairs in the helix are of little importance. There is now being amassed convincing evidence, not appropriately discussed here, that the first assumption is justified at least for RNA and synthetic polyribonucleotide co-polymers.[26] Unfortunately, we can only speculate on the second assumption, since our examination of this matter, using homogeneous oligonucleotides, has just begun. In any helix, an end effect may be expected which will result in a lowering of the average residue rotation. Obviously the significance of this factor diminishes rapidly as chain length increases, so that by the time a ten residue-pair helix is reached the effect is probably quite small. Since the stability-temperature curves of RNA indicate most helical regions are this length or greater,[26] it can be argued that this factor will not markedly distort the calculation of helical content. The validity of the third premise rests on the close similarity of the specific rotational changes which accompany the helix-coil transitions of poly(A – U), poly(A + A) and poly(U + U),[27], i.e. 275, 300 and 290° respectively at 589 mμ. Of course, since the helical regions in RNA probably involve A-U and G-C base pairs, our estimates will have to be considered tentative until data for a poly(G + C) helix becomes available. Nonetheless, it would seem from the examples cited that the scale being used will not be found to be off by more than 10 per cent. With these reservations then, and recognizing that most RNAs in neutral solution of moderate ionic strength display $[\alpha]_D$ values at room temperature in the range of 140—180°, we estimate that these values correspond to helical contents in the neighborhood of 40–60 percent.

Rotatory dispersion and the Cotton effect

The information presented thus far, derived as it is from a single measured parameter, specific rotation at a particular wave length (generally 589 mμ), provides a useful but nonetheless only an empirical elucidation of the dependence of rotatory power of polynucleotides on helical conformation. An extension of such measurements to a broad wave length range in order to delineate the character of the rotatory dispersion naturally suggests itself. Possibly, such data would be amenable to theoretical treatment, from which more refined molecular definition of nucleic acid structure could be achieved. In a more limited sense, rotatory dispersion might be expected to provide a sensitive way of amplifying the conformationally dependent effects revealed by the specific rotation data. For example, it might be found as has been observed in the case of polypeptides,[6-8], that the helical polynucleotides exhibit marked departures from the one-term Drude equation expected for the completely disordered forms. In this event, a more satisfactory means of quantitating helical content of single-stranded

[26] J. R. Fresco, B. M. Alberts and P. Doty, *Nature, Lond.* **188**, 98 (1960).
[27] M. Lipsett, *Proc. Natl. Acad. Sci.* **46**, 445 (1960).

polynucleotides could be anticipated. In connection with this, anomalous dispersion particularly associated with the region of absorption of the purine and pyrimidine bases might be expected, since, as was pointed out earlier, these chromophores are an integral part of the helical structure in nucleic acids. The study of such an anomaly, which would take the form of a Cotton effect, could lead to an exposition of molecular and electronic interactions related to base pairing.

In actual fact the realization of the potentialities inherent in rotatory dispersion data of nucleic acids is only now beginning to be effected. It may nevertheless be profitable to attempt some evaluation of the little amount of information at hand.

Nucleosides. The rotatory dispersion of the individual nucleoside components of DNA has been investigated[10] over the region 320–600 mμ, and found to conform in each case to the Drude equation. It is instructive to compare the λ_c values (λ_c is supposed to indicate the wave length of an optically active transition) reported in that study with the wave lengths of maximum absorption, λ_{max}, of the nucleosides, since in these relatively simple substances one might hope to sort out direct relationships between these two optical properties. The λ_c of adenine, cytosine and thymine deoxynucleosides coincide with their$_{max}$, λ suggesting that these two parameters are the result of a common transition. Consistent with this conclusion, the long wave length absorption bands of these nucleosides, 230–300 mμ, appear to be simple. At first sight the guanine nucleoside presents a somewhat different picture, for it has a λ_c about 20 mμ higher than its λ_{max}. This dilemma is readily interpreted, however, upon consideration of the guanosine absorption spectrum. This spectrum is not simple, having a distinct peak (at pH 7) at 253 mμ, and a prominent shoulder at longer wave lengths. The shoulder is probably due to a second band, about half the intensity of the one at 253 mμ, that has a λ_{max} corresponding very closely to the λ_c observed for guanosine. This shows that these two absorption bands differ very substantially in their optical activity.

It is evident from these observations that over the wave length range examined, the rotatory properties of nucleosides are dominated by the base linked to the pentose even though the base itself is not optically active. In view of this, it can be surmised that nucleosides will be found to display Cotton effects in the region 230–300 mμ.

Polyriboadenylic acid. Thus far, our examination of poly A has been limited to the region 360–600 mμ. Within this region. all the data, regardless of the conformation of the polynucleotide, fit the one-term Drude equation. Nonetheless, the λ_c does vary with the degree of helical content in a regular manner as is evident from the following: in acid solution, where the completely helical form, poly(A + A) exists, $\lambda_c = 290$ mμ; in neutral salt solution of 0·2 ionic strength, where it is estimated that 50 per cent of the residues in the single chain are organized into short helical regions, $\lambda_c = 272$ mμ; and when urea is added to such a neutral solution of poly A so that about half of the helical regions are disrupted, the λ_c drops to 257 mμ, which is close to the λ_c of the monomer.

Apparently, helical secondary structure in poly A causes certain electronic transitions located on the long wave length side of the 230–300 mμ absorption band to be accentuated. These transitions are probably of the $n \rightarrow \pi^*$ type since they are in the weakly absorbing part of the spectrum, yet are highly optically active. In this connection we have observed that the absorption spectrum of poly A (when traced with a continuous recording spectrophotometer) contains some diffuse fine structure on the

long wave length side in the form of shoulders and slightly resolved peaks. This fine structure is proportional to the helical content, disappears when the helix is completely melted out, and is absent in the monomer.

Ribonucleic acid. The rotatory dispersion of RNA has also been shown to follow the Drude equation regardless of the helical content.[30] In this case, however, the λ_c changes from 250 mμ to 310 mμ upon going from the partially helical conformation to the completely random one. The direction of this change is opposite that which has been observed for poly A, and for the moment there is no ready explantion for this.

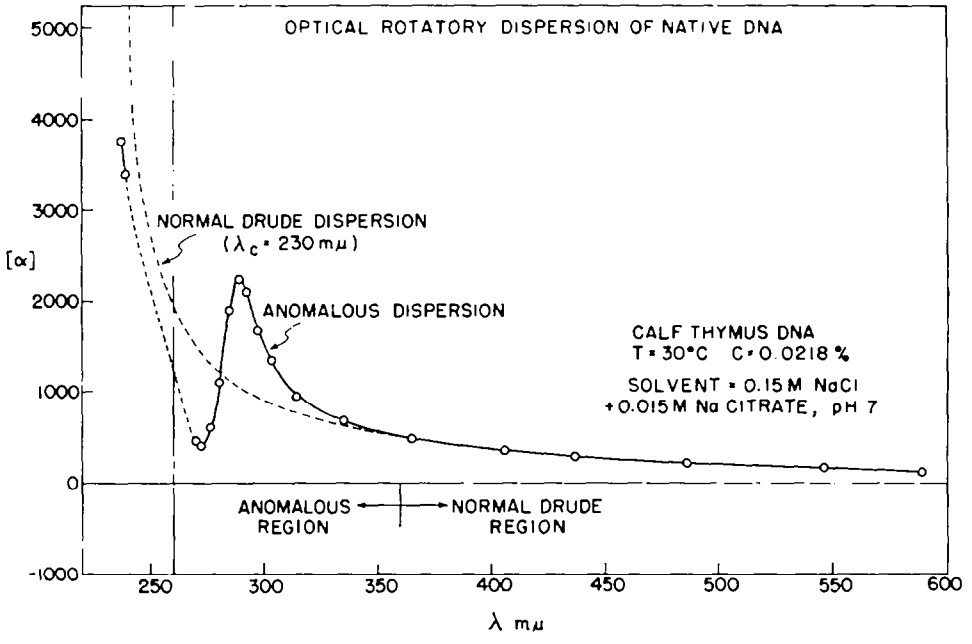

FIG. 8. The variation of specific rotation with wave length of calf thymus DNA.[28]

We can only speculate that in RNA the observed λ_c is a composite of a number of different optically active transitions resulting from a multiplicity of base pair sequences. It can be hoped that these will be resolved when it becomes possible to observe the rotatory dispersion within the region 230–300 mμ.

Deoxyribonucleic acid. Previous to our own recent efforts[2,28] it was reported[3] that the rotatory dispersion of DNA is of the simple Drude type between 320 and 700 mμ. The scatter in the λ_c values and the ill defined nature of the samples used in this work prompted us to reexamine the problem. Our investigations have taken two related directions. One aim was to delineate accurately the rotatory dispersion of native DNA in the readily accessible region above 320 mμ, and having done so, to examine the changes in the dispersion which accompany denaturation. Related to this, we wished to probe as far into the 230–300 mμ region as would be possible with the most intense light sources available in an effort to observe any Cotton effects which might occur there. Some progress can now be reported in both these directions.

A composite plot of our observations is given in Fig. 8. It can be seen that the

[28] J. R. Fresco and R. Gorn, unpublished.

dispersion is of the simple Drude type at longer wave lengths, but becomes anomalous below 360 mμ. Evidently, this anomaly is related to the prominent Cotton effect which becomes well defined below 300 mμ. To more sensitively demonstrate the region of Drude-type dispersion and ascertain the λ_c associated with it, the data are shown plotted in Fig. 9 according to the Yang modification[29] of the Drude plot. From the square-root of the slope of the straight-line portion, a λ_c of 230 was obtained, which is somewhat lower than the value quoted above for RNA.

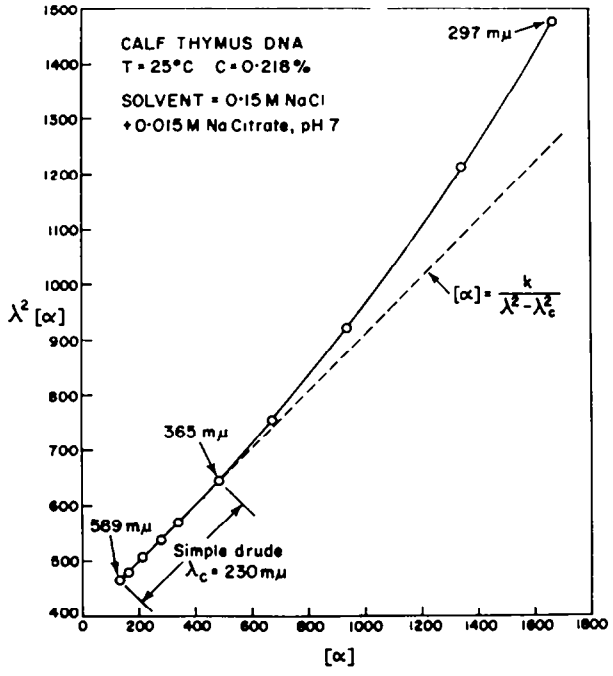

FIG. 9. A Yang-modified Drude plot of the rotatory dispersion of native DNA.[28]

Unfortunately, the measurement of optical rotation of nucleic acids in the region below 300 mμ is attended by considerable difficulty. A very intense light source is required for such work because of the high absorbance of the purine and pyrimidine residues. The measurements on DNA reported for wave lengths below 300 mμ were made using a quartz-jacketed, water cooled, high pressure mercury lamp (AH-6, General Electric). Even then it was not possible to make measurements between 273 and 239 mμ; and in contrast to the excellent reproducibility of measurements made above 300 mμ ($\pm 2\%$) it was not possible to delineate the Cotton effect below 300 mμ to a reliability of better than 20 per cent. The characteristic features of the profile were nevertheless repeatedly observed, and the profile shown represents an averaging of four series of observations. It should be emphasized that the variability was in the magnitude of the rotation and not on the location of the profile on the wave length axis.

An expanded picture of the region of the observed Cotton effect is shown in Fig. 10. The rotation reaches a maximum at 289 mμ and may be approaching a well near

[28] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[29] H. Boedtker, *J. Mol. Biol.* **2**, 171 (1960).

275 mμ, but the data are insufficient to assign a wave length to this Cotton effect. Upon complete denaturation at 95°, a marked change is noted in the region of the Cotton effect. The magnitude of the rotation drops substantially, but the Cotton effect does not seem to disappear; significantly, however, the wave length of maximum rotation shifts to the red by 4 mμ. Upon allowing the solution to cool, so that a substantial reformation of hydrogen-bonded base pairs occurs[1] the Cotton effect is seen to move partway back to its original position.

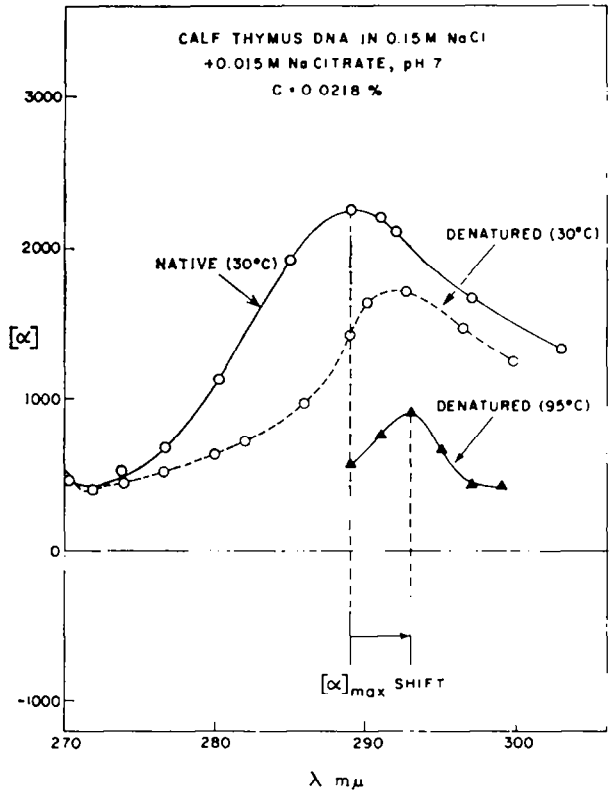

FIG. 10. Alterations in the Cotton effect with conformation of DNA.[18]

Since the observed Cotton effect is markedly displaced and diminished upon denaturation of the DNA, the transition involved is one that is probably accentuated by helical secondary structure. Both the red shift on denaturation and its location in a region where the absorption of DNA is very small suggest that this Cotton effect is due to an $n \rightarrow \pi^*$ transition. Because the Cotton effect is located considerably to the red of the λ_c of DNA we may presume that other Cotton effects exist in the near-ultraviolet region, that some of them are of the $\pi \rightarrow \pi^*$ type, and that these may be of greater magnitude than the one that has been observed.

A fortunate consequence of our observation of a Cotton effect was that it first sensitized us to the existence of minor discontinuities in the absorption spectrum of polynucleotides. As in the case of poly A (cited above) we have observed diffuse fine structure on the long wave length side of the DNA absorption spectrum. This is

evident in Fig. 11, where it can be seen that the prominent shoulder which approximately coincides with the region of the observed Cotton effect disappears upon thermal denaturation of the DNA.

It is apparent from our preliminary exploration of the optical rotatory dispersion of DNA in the region of its near ultraviolet absorption that this region is rich with information which may ultimately prove useful in understanding the molecular structure of nucleic acids.

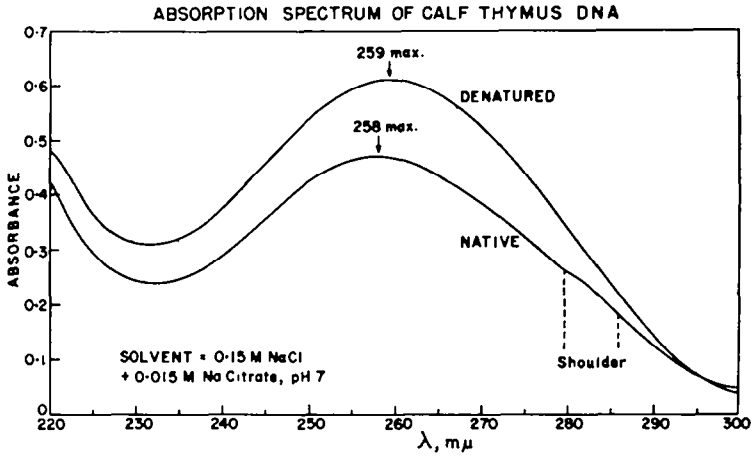

FIG. 11. Ultraviolet absorption spectra of native and heat denatured calf thymus DNA.[25]

Note added in proof: By suitably increasing the photosensitivity of the Rudolph spectropolarimeter we have now been able to extend the dispersion measurements through the near-ultraviolet absorption band of nucleic acids.[31] Native nucleic acids contain several closely spaced Cotton effects which have been sorted out into those due to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The Cotton effect profiles for native RNA and DNA, and their dependence on secondary structure have features of similarity consistent with these two types of nucleic acids having a common element of helical structure. The differential sensitivity to denaturation which the two types of electronic transitions exhibit in both RNA and DNA is indicative of the differential influence of hydrogen bonding between bases and the stacking of base pairs in helical polynucleotides.

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[31] J. R. Fresco, A. M. Lesk, R. Gorn and P. Doty, *J. Amer. Chem. Soc.* In press (1961).