Rotary dispersion and the native state of DNA

The rotary dispersion (optical rotation as a function of wavelength) of desoxyribose nucleic acid (DNA) has been determined between 320 mμ and 700 mμ at intervals of 20 mμ using a Rudolph spectral polarimeter. The dispersion curves appear to be of the pseudo-simple type and the data can be readily fit to a one term Drude equation of the type $[\alpha] = K_m/(\lambda^2 - \lambda_0^2)$ where $[\alpha]$ is the specific rotation, K_m is the rotation constant, λ_0 is the wavelength corresponding to a characteristic frequency of the vibration, and λ is the wavelength at which the rotation is measured[1]. The use of the Drude equation permits the calculation of λ_0 and K_m. The plot of $1/[\alpha]$ *vs.* λ^2 gives a straight line whose intercept is λ_0^2. The present plots were first evaluated by eye and then fitted by an unweighted method of least squares if no consistent deviations were seen. In all the plots, straight lines of good fit were obtained between 320 and approximately 600 mμ. In the 700 mμ region considerable scatter was often encountered. Most of this can be attributed to the loss in resolution[2]. It was necessary to use large slit widths to obtain the light necessary to penetrate these samples. All samples were run at 22° C. The constants obtained for DNA before and after a variety of treatments appear to be of considerable significance both with respect to the native state and to its macromolecular structure.

The optical parameters of DNA evaluated by this type of measurement are consistent and at first appearance relatively simple. With the reservation that further work will complicate the present picture, we can state that native DNA shows an optically active absorption band between 170 to 200 mμ in the Schumann region. This absorption band is apparently not influenced by the presence of salt since it is the same for preparations with zero salt concentration and for preparations in 0.2 *N* NaCl.

After acid treatment at pH 2 or 3 and subsequent return to neutrality, the characteristic wavelength of DNA moves into the ultraviolet between 250 and 270 mμ. Heating to 86° C for several hours gives somewhat similar end results. Either the time course of these two reactions are quite different or the mechanisms of denaturation are different even though the end results are consistent and generally agree to within ± 5 mμ. The characteristic wavelength seems to change rapidly from 180 to 260 mμ upon acid treatment. Upon heat treatment a different course is followed (see Table I). Native calf thymus DNA at a concentration of 0.14% has a λ_0 of 170 to 180 mμ. After heat treatment at 85° C for four hours the λ_0 was found to be 137 mμ. On standing at 3° C for 20 hours the value changed to 250 to 260 mμ and remained at this value even after further acid and heat treatment. The time course of this reaction has not been followed rigorously but this work is under way at present.

This delayed change following heat treatment is suggestive of the after-effects observed by TAYLOR, GREENSTEIN AND HOLLAENDER[3] following X-irradiation and may be of significance with respect to the radiation sensitivity of the molecule. An event confined to some portion of the polymer may key the spontaneous dissociation of the entire unit if given sufficient time.

TABLE I

THE EFFECT OF TREATMENT ON ROTARY DISPERSION OF DNA

*Thymus DNA**	*Treatment*	λ_0 Å
Simple extraction	None	1850
	pH2, 10 min	2530
Extracted and purified	None	1740
	pH2, 5 hours	2490
Extracted and purified	None	1740
	Heated 85° C, 4 hours	1370
	Heated 85° C, 4 hours ands tored at 3° C for 20 hours	2500
	Above sample treated pH 3.3 for 3 hours	2450
	Heat-treated acid-treated sample after 24 hours standing 3° C	2520
Extracted and purified	None	2130
	pH 3.3, 20 hours	2660
	Storage at 3° C for 24 hours	2610

* We wish to thank Dr. NORMAN SIMMONS for the calf thymus DNA and Dr. GERTRUDE BLUMENTHAL for the salmon sperm DNA.

For any given preparation the characteristic wavelength was found to be in good agreement on duplicate measurements. However, the rotational constants found for salmon sperm DNA were different from those found for calf thymus DNA. The change in the characteristic wavelength following denaturation by heat or acid moves in the same direction for both substances. This may be a difference due to the methods of preparation rather than a true species difference.

It is tempting to ask if the optically active absorbing groups in the region of 260 mμ are the same absorbing groups commonly found for the nucleic acids; namely, the purines and pyrimidines. If, indeed, these bands arise from the same chromophoric centers, it would lead one to conclude

that the optical activity at 260 mμ was induced by denaturation. The native state might thus be a configuration in which the chromophores participate in dipole-dipole interaction, hydrogen bridging, or vicinal effects which give rise to the optically active groups absorbing in the 180 mμ region. A primary shift in the effective electronic perturbated states[4] would result in the 260 mμ activity once the monomers were set free from the restraints exercised by the native configurations. The labile bonds between the purines and pyrimidines in complementary polymer chains may be the reason for this shift[5,6,7]. Old data on the optical activity of the purine and pyrimidine nucleosides suggest that their activity is of opposite sign[8]. Associations of these two monomer types would give rise to an activity highly sensitive to rearrangements. Undoubtedly the optical behavior of this molecule is very complex but the above argument finds a counterpart in the recent observations of LINDERSTØRM-LANG AND SCHELLMAN on the optical behavior of proteins[9] and is supported by our own measurements on adenosine which shows a λ_0 of 265 mμ.

The preliminary work reported in this note is being expanded in our laboratory to include detailed experiments on the effect of pH and ionic strength on the rotary dispersion of DNA and its component parts. The effect of enzyme depolymerization is at present being investigated by this method. Because of the similarities between DNA and RNA we have begun similar measurements on the RNA molecule and its component parts.

T. W. JAMES
Department of Zoology, University of California, Los Angeles, Calif. (U.S.A.) B. H. LEVEDAHL*

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Preparation and properties of 3-phenyl-2-thiohydantoins of serine, threonine and cystine

In the reaction proposed by EDMAN[1] for the stepwise degradation of peptides, 3-phenyl-2-thiohydantoins (PTH) are formed from the reaction of phenyl*iso*thiocyanate with the N-terminal amino acid residues. While the preparation of the PTH's of some of the amino acids has been achieved by EDMAN[2], the synthesis of the PTH's of serine, threonine and cystine heretofore have not been reported*. In this note, the preparation of these phenylthiohydantoins will be described, and their molar extinction coefficients and absorption maxima will be presented. The absorption characteristics of the PTH's of other natural amino acids have also been determined.

In the course of the investigation, it was observed that when the PTH of threonine (I) was dissolved in 1.0 *N* NaOH, the colorless solution became pink and then changed to yellow within approximately 20 minutes. On acidification of the solution with glacial acetic acid, an amorphous precipitate formed. A crystalline product, which elementary analysis indicates is probably the PTH of *A*-threonine (II), was obtained from ethanol; the final proof for the inferred structure remains to be established.

* Since the completion of this investigation by the late Dr. LEVY, a report by V. M. INGRAM (*J. Chem. Soc.* 3717 (1953)) of the synthesis of the PTH's of serine and threonine has appeared. It is felt that since the present report describes a simplified procedure, publication of the details is justified. It should be noted that the values reported in both instances for the m.p. of serine are in agreement (176–178° given by INGRAM), whereas there is a considerable divergence between the value reported for threonine by the British author (194°) and that reported here (infra). [C. H. LI.]