temperature of their dissociation. The resulting mass spectrum shows contributions of the free base and the free acid superimposed. Figure 1b is the mass spectrum obtained from lysine monohydrochloride. The peaks in the region of m/e 35 through 39 in Fig. 1b represent hydrochloric acid while the rest of the spectrum is due to free lysine which again illustrates the similarity to the spectrum of its ethyl ester.[1] Histidine dihydrochloride and cysteine hydrochloride also give rise to good spectra.

The simplicity, speed, and sensitivity of the method described make it a valuable tool for the identification and characterization of extremely small amounts of amino acids.

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RECEIVED MAY 25, 1962

NEW COTTON EFFECTS IN POLYPEPTIDES AND PROTEINS[1,2]

Sir:

Recently we have described the presence of a Cotton effect in the optical rotatory dispersion curves of α-helical polypeptides and in both fibrous and globular proteins.[3,4,5] This Cotton effect, having an inflection point at 225 mμ, and a minimum (or trough) at 233 mμ has been shown to be conformation-dependent. The original observations have been confirmed and extended by other workers using other proteins.[6,7]

In this communication we report the finding in the far ultraviolet of (a) a new, large, positive Cotton effect characteristic of helical polypeptides and proteins, and (b) a weak, negative Cotton effect in the random form of polypeptides. The new "helical" Cotton effect has a maximum (or peak) at approximately 198 mμ and an inflection point at about 190 mμ; some data are shown in the figures. Like the 225 mμ Cotton effect, the magnitude of the 190 mμ Cotton effect is conformation-dependent and is shown with the data from the helical and random forms of polyglutamic acid (Fig. 1). The data for the helical form (Fig. 1, curve A) also reveal an inflection point at about 215 mμ. This is the place where the negative 225 mμ Cotton effect would turn down if it were not for the presence of the ascending limb of the positive 190 mμ Cotton effect which evidently dominates the rotation around these wave lengths.

The *helical form* of poly-α,L-glutamic acid *shows a positive 190 mμ Cotton effect* with a peak residue rotation, [R′], of about 80,000; in striking contrast, the *random coil form* of this polypeptide not only *shows* a much lower rotation in this spectral region but also the presence of *a weaker negative Cotton effect* with a trough around 204 mμ, an inflection point at 197 mμ, and a peak around 190 mμ (Fig. 1, curve B). Possibly the 197 mμ Cotton effect is characteristic of a peptide bond from an asymmetric α-amino acid without superimposed conformational effects. Thus it seems likely that the large positive Cotton effect observed in the helical form of the polypeptide contains buried under it the smaller negative Cotton effect seen with the random material.

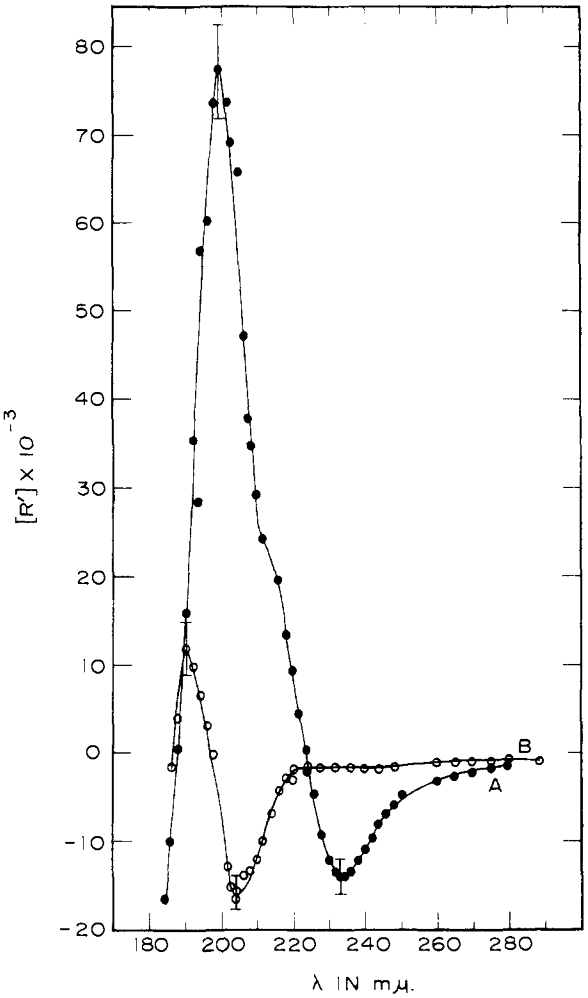

Fig. 1.—Curve A, ●-●-●, the ultraviolet optical rotatory dispersion of the helical form of poly-α,L-glutamic acid in water solution, pH 4.3. A 1-mm. cell was used. Concentrations ranged from 0.0137 to 0.0456%. Curve B, O-O-O, the ultraviolet optical rotatory dispersion of the random coil form of poly-α,L-glutamic acid (sodium salt), pH 7.1 in water solution. A 1-mm cell was used. Concentrations ranged from 0.0176 to 0.400%. The vertical lines at the peaks and troughs indicate the range of experimental uncertainty. All rotatory dispersion measurements were obtained with a newly designed spectropolarimeter (O. C. Rudolph and Sons, Model 220/200/1012/658-313/100) which employs a double prism monochromator. [R′] was calculated using the refractive index of water at 240 mμ.

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[1] This is Polypeptides XXXVIII. For the preceding paper in this series see S. M. Bloom, G. D. Fasman, C. de Lozé and E. R. Blout, *J. Am. Chem. Soc.*, **84**, 458 (1962). Alternate address for E. R. Blout, Chemical Research Laboratories, Polaroid Corporation, Cambridge 39, Massachusetts.

[2] This work was supported in part by U. S. Public Health Service Grant A2558, and in part by the Office of the Surgeon General, Department of the Army.

[3] N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

[4] N. S. Simmons, C. Cohen, A. G. Szent-Gyorgyi, D. B. Wetlaufer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

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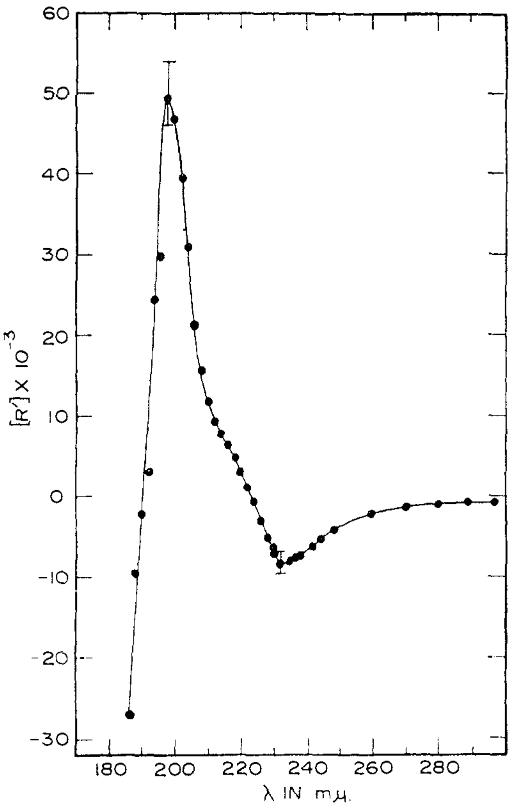

Fig. 2.—The ultraviolet optical rotatory dispersion of native bovine serum albumin in water solution, pH 7.1. A 1-mm. cell was used. Concentrations ranged from 0.0083 to 0.892%. Instrument use was as in Fig. 1.

The optical rotatory dispersion data for bovine serum albumin (Fig. 2), like helical polyglutamic acid, show two Cotton effects. This protein has been estimated to have between 38 and 58% helix by other methods[8]; the observed magnitudes of both the 225 mμ and the 190 mμ Cotton effects indicate a helix content of 55 to 60%. We have also confirmed the presence of the 190 mμ Cotton effect by measurements of other water-soluble synthetic polypeptides capable of undergoing helix → random transformations[9] and of other proteins.

The new 190 mμ Cotton effect is quite evidently related to the strong $\pi - \pi^*(NV_1)$ transition of amides and polypeptides whose absorption maximum lies around 190 mμ.[10,11,12] It has been known for some time that the rotatory strength of optically active absorption bands is not entirely determined by the intensity of absorption.[13] In this connection it is interesting that in the work presented here the ratio of the magnitude of the rotation to the extinction coefficient is larger for the 225 mμ Cotton effect than for the 190 mμ Cotton effect, although the size of the rotations observed with the 190 mμ Cotton effect is severalfold greater. Finally it should be noted that as yet it has not been possible to obtain reliable measurements below 184 mμ but extrapolation of our data indicate that the trough of the 190 mμ Cotton effect should be between 175 and 182 mμ. Further details and results with other materials will be published in due course.

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[8] For original references see a recent review by P. Urnes and P. Doty in "Advances in Protein Chemistry," Vol. 16, Edited by C. B. Anfinsen, N. L. Anson, K. Bailey and J. T. Edsall, Academic Press, New York, N. Y., 1961, p. 401.

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[12] K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci., U. S.*, **47**, 1775 (1961).

[13] See for example W. Kuhn, *Ann. Rev. Phys. Chem.*, **9**, 417 (1958).

THE OPTICAL ACTIVITY OF POLYPEPTIDES IN THE FAR ULTRAVIOLET

Sir:

The optical rotatory dispersion of polypeptides and proteins, which has been usefully related to their conformation,[1] must have its origin in ultraviolet electronic transitions. A complete explanation of the dispersion in relation to conformation requires knowledge of both spectra and optical activity in the far ultraviolet. For the α-helical conformation, spectra[2] reveal two bands (at 191 and 206 mμ) due to exciton splitting of the $\pi \rightarrow \pi^*$ band and apparently a third, at 222 mμ, probably an $n \rightarrow \pi^*$ transition. Rotatory dispersion has, however, been measured to only 212 mμ. This reveals complex dispersion in the visible and, moreover, shows a negative Cotton effect with a trough at 233 and a crossover at 225 mμ.[3,4] Whereas Moffitt's analysis[5] assumes that the complex dispersion arises solely from $\pi \rightarrow \pi^*$ transitions, the location of this Cotton effect has led to the suggestion[2,3] that the $n \rightarrow \pi^*$ transition might be predominantly involved.

For the case of the disordered polypeptide chain, the spectra reveal only the 192 mμ transition and the rotatory dispersion remains simple above 216 mμ.

In order to assess optical activity at shorter wave lengths, we have measured circular dichroism by an adaptation of a Beckman DK-2A spectrophotometer. Circular dichroism possesses the advantage of intrinsic discreteness and can be transformed to rotatory dispersion. Circularly polarized light was produced in the spectrophotometer sample beam by a Rochon prism and quarter-wave plate, both of cultured quartz.[6] Measure-

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[2] W. B. Gratzer, G. Holzwarth and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1785 (1961).

[3] N. S. Simmons, C. Cohen, A. G. Szent-Györgyi, D. B. Wetlaufer and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 4766 (1961).

[4] N. S. Simmons and E. R. Blout, *Biophys. J.*, **1**, 55 (1960).

[5] W. Moffitt, D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci. U. S.*, **43**, 723 (1957), and preceding papers; but see also J. A. Schellman and P. Oriel, *J. Chem. Phys.*, in press. We thank Dr. Schellman for making a preprint of his manuscript available.

[6] With identical reference and sample solutions, a rotational band causes sinusoidal oscillations in the transmission spectrum. Their envelope and phase are directly related to the magnitude and sign of the dichroism, respectively, for the small oscillations observed (0.02 to 3%T), provided adequately narrow slit-widths are maintained.