ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS*

BY V. G. ALLFREY, R. PAULKNER, AND A. E. MIRSKY

THE ROCKEFELLER INSTITUTE

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The experiments to be described are concerned with the role of histones in nuclear function, with particular emphasis on biosynthetic reactions which modify the structure of histones by the introduction of acetyl and methyl groups. These reactions have been studied in isolated calf thymus nuclei in vitro, using acetate-2-C\textsuperscript{14} and methionine-methyl-C\textsuperscript{14} (see ref. 1) as precursors, comparing their incorporation with that of C\textsuperscript{14}-lysine and other amino acids, and testing the effects of Puromycin on the synthesis of different histone fractions. Evidence will be presented to show that, in the cell nucleus, the acetylation and methylation of the histones occur very probably after completion of the polypeptide chain.

Such modifications of histone structure, acetylation in particular, may affect the capacity of the histones to inhibit ribonucleic acid synthesis in vivo. This view is supported by the finding that, when isolated arginine-rich histones are subjected to a limited acetylation, they lose much of their effectiveness as inhibitors of RNA synthesis by the DNA-dependent RNA polymerases of calf thymus nuclei or Escherichia coli. Yet, such modified histones are still strongly basic proteins which retain an affinity for DNA comparable to that of the parent histone from which they were derived.

The findings introduce the possibility that histone effects on nuclear RNA metabolism may involve more than a simple inhibition of RNA synthesis, and that more subtle mechanisms may exist which permit both inhibition and reactivation of RNA production at different loci along the chromosome.

Interest in the histones as regulators of chromosomal activity has heightened considerably in the past few years, as increasing experimental evidence has accumulated to support the view that the role of histones is to inhibit chromosomal
function in differentiated cells in higher animals and plants. The inhibitory role of histones was originally surmised by Stedman and Stedman in 1951, but biochemical evidence that histones do block DNA function is of recent origin. Of particular interest are the observations (a) that histones inhibit RNA synthesis in isolated thymus nuclei; (b) that removal of the histones from the nucleus results in an increased rate of "messenger" RNA synthesis; and (c) that DNA-histone complexes are unable to serve as "primers" for RNA synthesis in vitro in the presence of a DNA-dependent RNA polymerase from pea-seedling nuclei. The inhibition of RNA polymerases by histones has been observed in mammalian and bacterial systems as well, although it should be pointed out that nonspecific polycations, such as polylysine, will also inhibit RNA polymerase activity, and that histones can inhibit a wide range of other enzymatic reactions not directly involved in RNA synthesis.

It has seemed likely that the suppression of RNA synthesis by histones is due in large part to complex formation between the added histones and the DNA needed as a "primer" in the RNA polymerase reaction. Indeed, the correlation between DNA binding and the inhibition of RNA synthesis is evident in the experiments of Huang and Bonner, who showed that histones which protected DNA from thermal denaturation also blocked its "primer" action.

Something more, however, than simply the combination of histone with DNA should be considered. The experiments to be reported below indicate that combination without inhibition is possible, and they suggest that specific (and presumably reversible) changes in histone structure, which do not prevent binding of histone to DNA, may permit DNA to serve as a template in the RNA polymerase reaction. This raises the possibility that relatively minor modifications of histone structure, taking place on the intact protein molecule, offer a means of switching-on or -off RNA synthesis at different loci along the chromosome.

**Histone Synthesis in Isolated Cell Nuclei.**—Amino acid incorporation into histones: It has been shown previously that nuclei isolated from calf thymus tissue in isotonic sucrose solutions can incorporate amino acids into their proteins. The histones become labeled in the process, although they are not as rapidly synthesized as are other protein fractions of the nucleus (see Fig. 1A). There are good reasons to believe that the synthesis or "turnover" of histones is intranuclear and that it can proceed without a concomitant synthesis of DNA, because thymus lymphocytes are not dividing rapidly, and because histone "turnover" has been observed in non-dividing tissues of adult animals. Amino acid incorporation into the histones, or into other proteins of isolated thymus nuclei, is an energy-dependent process. It is inhibited by agents which block nuclear ATP synthesis but it is not affected by CO, Ca++ ions, or methylene blue, all of which block mitrochondrial ATP synthesis without affecting nuclear ATP levels. The synthesis of the histones, like that of other nuclear proteins, appears to require a prior and continuing synthesis of RNA. This is one reason for the lag phase in amino acid uptake (Fig. 1A). Agents which block nuclear RNA synthesis, such as actinomycin D or 5,6-dichloro-β-D-ribofuranosyl benzimidazole, have no immediate effect on histone synthesis, but the rate of histone labeling begins to fall off 30–60 min after the cessation of "messenger" RNA synthesis. The synthesis of thymus "messenger" RNA and its isolation are described elsewhere.
**Inhibition of histone synthesis by Puromycin:** The incorporation of amino acids into the histones is inhibited by the addition of Puromycin to the incubation medium. This antibiotic, widely used as an inhibitor of protein synthesis since its introduction for this purpose by Yarmolinsky and de la Haba,22 effectively stops nuclear protein synthesis at concentrations of 100 \(\mu g/mL\). Since its action involves an interference with transfer-RNA function22 and leads to a release of unfinished peptides from ribosomal sites of synthesis,24, 46 it can be concluded that histones, like other nuclear proteins, are probably made on nuclear ribosomes.25—27

When the syntheses of different types of histones are compared (Table 1), some are found to be less susceptible to Puromycin inhibition than others. The uptake of amino acids into the lysine-rich histones, in particular, seems more resistant to this level of the antibiotic than is amino acid uptake into the arginine-rich fraction (Table 1), but the inhibition is still definite.

**TABLE 1**

| Protein tested | Puromycin concentration, \(\mu g/mL\) | Specific Activity of Proteins after 60' Incubation with | | |
|---|---|---|---|
| | | Lysoine-1-\(^{14}C\) | Arginine-G-\(^{14}C\) | Alanine-1-\(^{14}C\) |
| Total nuclear protein | 0 | 275 | 90.8 | 50.8 | 86.3 | 21.2 | 86.6 |
| 100 | | | | | | | |
| Total histones | 0 | 95.1 | 95.1 | 95.1 | 95.1 | 95.1 | 95.1 |
| 100 | 36.7 | 61.4 | 61.4 | 61.4 | 61.4 | 61.4 |
| Lysine-rich histones* | 0 | 142 | 61.4 | 61.4 | 61.4 | 61.4 |
| 100 | 75.5 | 53.1 | 53.1 | 53.1 | 53.1 |
| Lysine-rich histones† | 0 | 136 | 61.4 | 61.4 | 61.4 | 61.4 |
| Intermediate lys-rich histones | 0 | 42.6 | 61.4 | 61.4 | 61.4 | 61.4 |
| 100 | 10.9 | 74.4 | 74.4 | 74.4 | 74.4 |
| Arginine-rich histones* | 0 | 44.5 | 61.4 | 61.4 | 61.4 |
| 100 | 11.0 | 75.3 | 75.3 | 75.3 | 75.3 |
| Arginine-rich histones† | 0 | 156.3 | 61.4 | 61.4 | 61.4 |
| 100 | 27.4 | 82.5 | 82.5 | 82.5 |

* Histones prepared by chromatography on carboxymethylcellulose columns.27
† Histones prepared by direct extraction in 5% perchloric acid.31
‡ Histones extracted in 80% ethanol-1.25 N HCl30

**Modification of Histone Structure.—Acetylation of histones in isolated nuclei:** The presence of acetyl end-groups in thymus histones was detected originally by Phillips,30 who recently observed that 54–58 per cent of the total N-terminal groups are acetylated.31 Histones differ in this respect; the lysine-rich histones are highly acetylated (73–80% N-terminal acetyl groups) as compared with 36–48 per cent N-terminal acetyl groups in the arginine-rich fraction.31

We have observed that when isolated nuclei are incubated in the presence of sodium acetate-2-\(^{14}C\) (under the conditions described in Materials and Methods), there is a rapid incorporation of isotope into the histones. The time course of uptake into histones and other proteins is shown in Figure 1B. It is of interest that acetate-\(^{14}C\) uptake into the histones proceeds without a lag phase and tapers off quickly, while the uptake of lysine-\(^{14}C\) into the basic proteins shows the typical lag and proceeds longer (Fig. 1A). In this respect, acetate uptake into histones resembles the uptake of uridine and other precursors into nuclear RNA.32, 33

Evidence that the acetate taken up is incorporated as acetyl groups attached to histones is given in Materials and Methods.
Comparison of acetate-C\textsuperscript{14} uptakes into different histone fractions: When the acetylabeled histones are separated by chromatography on carboxymethylcellulose columns,\textsuperscript{28} it is observed that different types of histone differ in their rates of C\textsuperscript{14}-acetate uptake. The specific activity of the arginine-rich fraction is particularly high (Fig. 2). This is a surprising finding considering the relatively low total acetate content of this fraction as compared with that of lysine-rich histones.\textsuperscript{31} Because of the extensive acetate uptake into the arginine-rich histones, this fraction was selected for studies of the effects of acetylation on histone inhibition of the RNA polymerase reaction (see below).

Failure of Puromycin to inhibit acetate uptake into histones: Although Puromycin effectively inhibits the uptake of arginine-G-C\textsuperscript{14}, lysine-1-C\textsuperscript{14}, and other amino acids into histones (Table 1), it has no effect on the uptake of acetate-2-C\textsuperscript{14} into the basic proteins of the nucleus. The differences between acetylation and synthesis are clear-cut when one compares the effects of Puromycin on lysine-1-C\textsuperscript{14} and acetate-2-C\textsuperscript{14} uptakes (Table 2). The table lists the specific activities of the

**TABLE 2**

**COMPARATIVE EFFECTS OF PUROMYCIN ON ACETYLATION OF HISTONES AND AMINO ACID INCORPORATION INTO NUCLEAR PROTEINS**

<table>
<thead>
<tr>
<th>Conditions of experiment</th>
<th>Isotopic precursor</th>
<th>Total activity (cpm/mg)</th>
<th>Soluble histones (cpm/mg)</th>
<th>Histones (cpm/mg)</th>
<th>Residue (cpm/mg)</th>
<th>Activity recovered as C\textsuperscript{14}-acetate from histones (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Lysine-1-C\textsuperscript{14}</td>
<td>487</td>
<td>1203</td>
<td>215</td>
<td>611</td>
<td>—</td>
</tr>
<tr>
<td>Nuclei + 100 \mu g Puromycin</td>
<td>&quot;</td>
<td>65.1</td>
<td>102</td>
<td>57.9</td>
<td>38.4</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>Acetate-2-C\textsuperscript{14}</td>
<td>1229</td>
<td>1343</td>
<td>2100</td>
<td>849</td>
<td>2724</td>
</tr>
<tr>
<td>Nuclei + 100 \mu g Puromycin</td>
<td>&quot;</td>
<td>857</td>
<td>253</td>
<td>2076</td>
<td>381</td>
<td>2688</td>
</tr>
</tbody>
</table>

\* Proteins plus ribosomes extractable in 0.01 M "tris" buffer (pH 7.6).
\+ Histone specific activity after extraction with 10% TCA at 90° for 15 min.
\† Acetate recovered by steam distillation of histone hydrolysate (see text). More than 70% of total counts in histones after acetate-labeling experiments are recoverable as acetyl-C\textsuperscript{14}.
histones in cpm/mg and also gives the total counts recovered as acetate-C\(^{14}\) from the basic protein hydrolyzates.

The failure of Puromycin to inhibit the acetylation reaction indicates that histones are very probably modified by acetylation after completion of the polypeptide chain. It is therefore unlikely that acetate is activated and transferred by sRNA's as are the amino acids. This conclusion is in accord with recent work on the acetylation of tobacco mosaic virus protein, in which no evidence for acetyl-amino acid intermediates could be obtained.\(^{38}\)

**Methylation of histones in isolated nuclei:** The presence of epsilon-N-methyl-lysine in calf thymus histones was recently detected by K. Murray,\(^1\) who also observed that the group donor for histone methylation was methionine.\(^1\)

We have studied the incorporation of C\(^{14}\)-methyl groups into the histones of isolated thymus nuclei and tested the effects of Puromycin on this process. The results are presented in Table 3. It is clear that concentrations of Puromycin which inhibit synthesis of the nonhistone proteins by over 90 per cent, and which lowered lysine-C\(^{14}\) uptake into the histones by 73 per cent, had no effect on methylation of the histones. Thus, it is very probable that methylation of the histones, like that of amino acid-transfer RNA's\(^{37, 38}\) proceeds after the synthesis of the primary structure of the molecule has been completed.

**TABLE 3**

**Comparative Effects of Puromycin on Methylation of Histones and Amino Acid Incorporation into Nuclear Proteins**

<table>
<thead>
<tr>
<th>Conditions of experiment</th>
<th>Isotopic precursor</th>
<th>Specific Activity of Nuclear Proteins</th>
<th>Activity recovered as methyl-lysine in histones(\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total protein (cpm/mg)</td>
<td>Histones (cpm/mg)</td>
</tr>
<tr>
<td>Control</td>
<td>Lysine-1-C(^{14}) (\ast)</td>
<td>457</td>
<td>215</td>
</tr>
<tr>
<td>Nuclei + 100 (\mu)g Puromycin</td>
<td></td>
<td>65.1</td>
<td>57.9</td>
</tr>
<tr>
<td>Control</td>
<td>Methionine-M- (\ast)</td>
<td>725</td>
<td>198</td>
</tr>
<tr>
<td>Nuclei + 100 (\mu)g Puromycin</td>
<td>Methionine-M-C(^{14})</td>
<td>318</td>
<td>120</td>
</tr>
</tbody>
</table>

\(\ast\) Proteins plus ribosomes extractable in 0.01 \(M\) tris buffer (pH 7.6).

\(\dagger\) Methyl-lysine separated from methionine on Dowex-50, eluted with the basic amino acids, and counted by scintillation.

**Effects of Acetylated Histones on RNA Synthesis.—**Is there a connection between acetylation of the histones and their role in the regulation of RNA synthesis?

Evidence that histones can inhibit RNA synthesis in the nucleus was presented in 1961,\(^4\) and it was later shown that selective removal of the histones leads to increased rates of "messenger" RNA synthesis.\(^6\) These findings, together with work on isolated enzyme systems,\(^7\) have led to the view that histone-DNA complexes are unable to serve as "primers" for RNA synthesis.\(^7\)

This view does not allow any obvious or easy mechanism for reversing the histone inhibition, except by removal of the histones. However, the experiments now to be described indicate that acetylation of the histones can lower their effectiveness as inhibitors of the RNA polymerase reaction, and suggest a dynamic and reversible mechanism for "activation" as well as "repression" of RNA synthesis.

**Acetylation of isolated histones:** To test the effects of acetylation on the inhibitory action of histones, we selected the arginine-rich histone fraction and employed limited acetylation by chemical methods (see Materials and Methods).
Effects of acetylated histones on RNA polymerase reactions: Calf thymus nuclei are a good source of an “aggregate-enzyme” preparation which can incorporate nucleoside triphosphates into RNA.\(^5\)\(^,\)\(^41\) The reaction can be followed by measuring the incorporation of ATP-8-C\(^{14}\) or UTP-C\(^{14}\) into an acid-insoluble, RNAase-sensitive product.\(^5\)\(^,\)\(^42\) The formation of RNA is blocked by DNAase treatment, by actinomycin D, and by the addition of thymus histones.\(^5\)\(^,\)\(^42\)

The effects of adding arginine-rich histones on RNA synthesis by the “aggregate enzyme” are shown in Table 4. Inhibition, though variable from one experiment to another, usually ranges from 60 to 75 per cent. On the other hand, when histones of varying degrees of acetylation are added to this system, the inhibition is markedly reduced. Even small amounts of acetylation, which hardly affect the electrophoretic mobility of the protein (Table 4) or its ability to combine with DNA (Fig. 3), can lower the inhibition from 66 to 21 per cent of the control value. More highly acetylated histones, although still basic, hardly inhibit at all (Table 4).

TABLE 4

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Histone mobility* (mm/hr)</th>
<th>(^{14})C-ATP uptake into RNA(^t) (cpm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control—no histone added</td>
<td>-</td>
<td>68.6</td>
<td>-</td>
</tr>
<tr>
<td>Arginine-rich histone (control for acetylation)</td>
<td>-</td>
<td>22.2</td>
<td>67.6</td>
</tr>
<tr>
<td>Arginine-rich histone (untreated)</td>
<td>63</td>
<td>27.6</td>
<td>60</td>
</tr>
<tr>
<td>Acetylated histone I(\dagger)</td>
<td>59</td>
<td>49.8</td>
<td>27.4</td>
</tr>
<tr>
<td>Acetylated histone II</td>
<td>46</td>
<td>60.5</td>
<td>11.8</td>
</tr>
<tr>
<td>Acetylated histone III</td>
<td>40</td>
<td>68.7</td>
<td>0</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control—no histone added</td>
<td>-</td>
<td>550</td>
<td>-</td>
</tr>
<tr>
<td>Arginine-rich histone (control for acetylation)</td>
<td>-</td>
<td>317</td>
<td>42.4</td>
</tr>
<tr>
<td>Arginine-rich histone (untreated)</td>
<td>63</td>
<td>288</td>
<td>47.6</td>
</tr>
<tr>
<td>Acetylated histone II</td>
<td>46</td>
<td>429</td>
<td>22.0</td>
</tr>
</tbody>
</table>

* Electrophoretic mobility on cellulose polyacetate (Sepharose II); 0.1 M acetic (pH 4.0); 6 v/cm.
\(\dagger\) Average of 5 separate experiments for thymus RNA polymerase, and of 2 experiments for E. coli polymerase.

\(\dagger\) Acetylated histones numbered in order of increasing acetylation, using 0.032, 0.32, and 3.2 meq acetic anhydride per 100 mg arginine-rich histone.

Similar results have been obtained with RNA polymerases from *Escherichia coli* and *Azotobacter vinelandii*. (We are greatly indebted to Dr. Edward Reich of The Rockefeller Institute for the gift of the *E. coli* enzyme, and to Dr. J. S. Krakow of New York University for supplying the *Azotobacter* enzyme.) The *E. coli* results are included in Table 4; as in the case of the thymus enzyme, the acetylated histones did not inhibit RNA synthesis as effectively as did the parent proteins.

The failure of acetylated histones to block the RNA polymerase reaction is not due to their inability to combine with DNA. On the contrary, these proteins very effectively protect DNA from thermal denaturation. In Figure 3 the melting curves are shown for DNA, for the DNA-arginine-rich histone complex (66% inhibition of RNA synthesis), and for the DNA-acetylated histone complex (21% inhibition of RNA synthesis). It should be noted that the denaturation studies were carried out at the same pH value (7.4) as were the RNA polymerase assays.

Acetylated histones are also able to combine effectively with nuclei from which the histones have been previously extracted with ethanolic HCl.\(^43\)

The results make it clear that complexes of DNA with acetylated histones can be formed, but, under these conditions, “primer” activity for RNA synthesis is
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Fig. 3.—Thermal denaturation curves for calf thymus DNA and for complexes of DNA with the arginine-rich histone fraction and with a weakly acetylated arg-rich histone (0.032 meq acetic anhydride per 100 mg histone). DNA and histone were dissolved in 1 M NaCl at 20 μg/ml and dialyzed against 5 × 10⁻⁴ M "tris" (HCl) buffer (pH 7.4). Optical density at 260 μg is plotted versus temperature, and Tₘ's are indicated by the dark lines crossing the curves.

in acetate labeling experiments was 2.5 μc/ml sodium acetate-2-C¹⁴ (Sp. Act. 20.5 mc/mM); in methylation studies 1.2 μc of L-methionine-methyl-C¹⁴ (Sp. Act. 140 mc/mM) was added per ml.

Mixed histones were extracted in 0.2 N HCl from nuclei previously washed with 0.01 M "tris" buffer (pH 7.6) and with 80% ethanol. The lysine- and arginine-rich fractions were prepared according to Johns and Butler²³ or by chromatography.²⁸ Soluble DNA-histone complexes were prepared by mixing in 1 M NaCl and dialysis versus 5 × 10⁻⁴ M "tris" buffer (pH 7.4).

The thymus RNA polymerase was prepared and assayed by modifications of the methods of Biswas and Abrams.⁴¹ The E. coli enzyme was assayed according to Chamberlin and Berg.⁴⁴ Evidence that the acetate taken up is incorporated as acetyl groups attached to histones has been obtained in several ways: (a) purification of the histones by chromatography shows that the label is eluted with the basic proteins (Fig. 2); (b) acetylated histones can be separated by electrophoresis on cellulose polyacetate strips;⁴⁴ (c) acetate-C¹⁴ is not removed when the histones are treated with organic solvents (to remove lipids); and (d) more than 75% of the acetate-C¹⁴ remains after the histones are treated with hot 16% trichloroacetic acid (TCA) at 90° for 15 min (to remove polysaccharides and nucleic acids).

When TCA-treated histones are hydrolyzed to their constituent amino acids (using 6 N H₂PO₄ at 110° for 2 hr), the C¹⁴-acetyl is released as C¹⁴-acetic acid. This can be separated from the amino acids by steam distillation, and trapped in dilute alkali. Scintillation counting of the distillate allows a measure of the C¹⁴-acetyl content of the histone proteins. These counts are plotted in Figure 1B, curve 3. Two thirds of the total counts are recoverable as acetate-C¹⁴.

**Uptake of L-methionine-methyl-C¹⁴ into histone:** Nuclei were incubated in the presence of L-methionine-methyl-C¹⁴ with or without the addition of Puromycin-HCl at a final concentration of 100 μg/ml. After 60 min at 37°, the histones were prepared for counting. Although histones do not contain more than traces of methionine, some C¹⁴-methionine does appear in acid extracts of thymus nuclei, presumably as a component of nonhistone proteins that are present in small amounts. To remove this methionine contamination and simplify interpretation of the data, the histone fraction was hydrolyzed to its component amino acids (using 6 N HCl at 110° for 2 hr). The amino acids were adsorbed on Dowex-50 columns and eluted by stepwise elution with HCl, following the procedure of Stein and Moore.²⁶ This method gave a clear separation of a methionine marker from the basic amino acids of the digest. The lysine peaks and not lost. It may be suggested that specificity in DNA-histone binding, alterable by acetylation of the histone, can influence the rate of RNA synthesis. This would allow a means of switching-on or -off RNA synthesis at different times, and at different loci of the chromosomes. Although the evidence supporting this view is, at best, tentative (because the acetylated histones tested were acetylated after isolation), it should be recalled that histone acetylation does occur in the nucleus and that its time course resembles that for RNA synthesis.

**Materials and Methods.—**Calf thymus nuclei were isolated as described earlier,¹⁴ ₁⁵ suspended at a concentration of 40 mg/ml in a buffered sucrose medium containing added glucose, NaCl, and MgCl₂, and the isotopic precursor.¹⁴ ₁⁵ The isotope concentration in acetate labeling experiments was 2.5 μc/ml sodium acetate-2-C¹⁴ (Sp. Act. 20.5 mc/mM); in methylation studies 1.2 μc of L-methionine-methyl-C¹⁴ (Sp. Act. 140 mc/mM) was added per ml.
adjacent fractions were collected, concentrated to a known volume, and counted in a scintillation counter for determination of their C\textsuperscript{14}-methyl contents.

Acetylation technique: The technique employed was based on Reid's work on the acetylation of growth hormone,\textsuperscript{19} in which conditions were selected to give maximum acetylation of the \(\alpha\)-amino groups and minimum acetylation of the \(\epsilon\)-amino groups of lysine. In our experiments, 100-mg portions of arginine-rich histones (prepared by the ethanolic-HCl extraction procedure of Johns and Butler\textsuperscript{30}) were dissolved in 2.0 ml H\textsubscript{2}O, and 20 ml of half-saturated sodium acetate solution (pH 8.1) was added. Very small amounts of acetic anhydride were then added, ranging from 0.032 to 3.2 meq, and the mixture was stirred in the cold for 4 hr. The histones were dialyzed extensively against cold distilled water and then lyophilized. Control preparations were made in which no acetic anhydride was added to the reaction mixture. The products were strongly basic proteins with high electrophoretic mobilities, and all retained a capacity to combine with DNA.

We are greatly indebted to Mrs. Sarah Reynolds and to Mr. Barry L. Horowitz for their skillful and conscientious technical assistance.

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AN ANALYSIS OF THE OPTICAL ROTATORY DISPERSION OF POLYPEPTIDES AND PROTEINS, II*

BY E. SHECHTER AND E. R. BLOUT

DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL

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In a previous communication\(^1\) we reported a new analysis of visible and near-ultraviolet rotatory dispersion data of aqueous solutions of polypeptides and proteins having \(\alpha\)-helical or random conformations, or mixtures of both. The rotations were described by an expression designated a modified two-term Drude equation:\(^4\)

\[
[R'] = \frac{A_{(\alpha,p)}(193)\lambda^2_{193}}{\lambda^2 - \lambda^2_{193}} + \frac{A_{(\alpha,p)225}\lambda^2_{225}}{\lambda^2 - \lambda^2_{225}}
\]

A linear relation was obtained by plotting \(A_{(\alpha,p)}(193)\) versus \(A_{(\alpha,p)225}\),

\[
A_{(\alpha,p)225} = -0.55A_{(\alpha,p)}(193) - 430,
\]

and it was found that the helix content could be expressed in terms of either one of the two parameters.

As a result of this analysis it was concluded that the rotatory dispersion parameters of polypeptides and proteins (in aqueous solutions) existing in \(\alpha\)-helical or random conformations, or mixtures of the two, fit equation (2). A failure to fit this equation was taken as an indication of the presence of other structures.

In this communication we extend this analysis to the optical rotatory dispersions of polypeptides and proteins in a variety of organic solvents, and compare the results so obtained with the ones obtained in aqueous solutions.

Analysis of Some Optical Rotatory Dispersion Data of Synthetic Polypeptides and Proteins in Organic Solvents.—During the past ten years there have been a number of investigations into the effect of various organic solvents on the optical rotation and rotatory dispersion of several synthetic polypeptides and some proteins. Much of this work arose from the fact that many high-molecular-weight polypeptides are soluble only in organic solvents. As a result of these investigations it was found that the type of organic solvent had a pronounced effect on the conformation of the solute. It is now recognized, for example, that highly polar...