run with unlabelled markers in the two-dimensional thin-layer chromatography system described by Randerath (1963). An extract of the DNP-serine spot was found to have ^{14}C activity, thus showing that serine phosphate was present in the eluate from the paper chromatogram and that the ^{32}P was originally present as serine phosphate.

By using this incubation method *in vitro* some histone fraction f1 was obtained with $34.2\text{ }\mu\text{g.}$ -atoms of P/mg. of protein; this was compared with histone fraction f1 with $22.4\text{ }\mu\text{g.}$ -atoms of P/mg. of protein and differences were found in the priming ability of the histone f1-DNA complexes in the RNA-polymerase system described by Hilton & Stocken (1966). By using ^{14}C -ATP the V_{max} for the material of higher phosphorus content was 900 counts/min. incorporated in 10 min. whereas the

material with the lower phosphorus content incorporated 665 counts/min. in 10 min. This suggests that changes in the level of phosphorylation may be an effective means of modifying the repressing ability of this histone.

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The Role of Thiol Groups in the Modification of the Template Activity of Histone-Deoxyribonucleic Acid Complexes

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Stedman & Stedman (1951) originally proposed that histones might act as repressors of genetic transcription, but ready acceptance of this idea is difficult since there appears to be a lack of specificity between these closely related proteins. More recently, however, systems have been described that suggest that modifications in histones, either by *N*-terminal acetylation (Allfrey, Faulkner & Mirsky, 1964) or by their interaction with polyanions (Frenster, 1965), may convey some of the specificity required of a genetic repressor, and this is further supported by the finding of RNA associated with histones (Huang & Bonner, 1965). The presence of thiol groups in histones was reported by Marsh, Ord & Stocken (1964) and Phillips (1965). Ord & Stocken (1966) have also shown that a 50 mM-HCl extract of diffuse chromatin contains a higher proportion of its sulphur in the thiol form than does a similar extract of the dense chromatin. Evidence is now presented showing that modification of thiol groups alters the degree of repression produced by the arginine-rich histones.

Calf-thymus nucleohistone was prepared by the method of Zubay & Doty (1959) and histone f3 was

extracted by method 2 of Johns (1964). The nucleohistone pellet from 20 g. of calf thymus was blended in 200 ml. of ethanol and after centrifugation the pellet was extracted in a high-speed blender for 3 min. with 40 ml. of acid-ethanol (ethanol-1.25 N-HCl; 4:1, v/v). The extract was dialysed overnight at 2° against 1 l. of ethanol and the precipitated f3 fraction dried *in vacuo*.

Thiol groups were determined by the method of Ellman (1959) in 0.02 M-tris-HCl buffer, pH 8.2, and disulphide was measured as SH after reduction in 4 M-urea with NaBH_4 (5 mg./mg. of protein) (Moore, Cole, Grundlach & Stein, 1958).

In six preparations of fraction f3 the total thiol content ranged between 38 and 51 μmoles of SH group/mg. of protein. Only one preparation contained disulphide groups (4%) and in all cases the thiol groups reacted with *N*-ethylmaleimide without treatment of the protein with urea.

To ensure that no disulphide groups were present reduction was carried out by treating the protein in 0.02 M-tris-HCl buffer, pH 8.2, with 0.3 M- β -mercaptoethanol for 30 min. at 37° or 2 hr. at room temperature. The β -mercaptoethanol was removed

by gel filtration on a column of Sephadex G-25 (fine grade) and elution with 0.02M-tris-HCl buffer, pH 8.2.

Oxidation of the thiol groups in histone f3 was achieved by dialysing a solution of the protein against aerated 0.02M-tris-HCl buffer, pH 8.2, at room temperature (20°) for up to 2 days. The slightly opalescent protein solution was clarified by centrifugation at 70000g for 20min. The sedimented material accounted for 1% of the protein. Oxidized histone f3 contained 0.6mμmoles of SH group/mg. of protein, and all the thiol groups could be regenerated by treatment with 0.3M-β-mercapto-ethanol.

Thiol groups were blocked with chloromercuribenzenes-*p*-sulphonate or bis-3,6-(nitratomercurimethyl)dioxan (Eldjarn & Jellum, 1963). The reactions were carried out in 0.02M-tris-HCl buffer, pH 8.2. A tenfold excess of the monofunctional mercurial was employed, which produced 100% blocking, and a stoichiometric amount of bis-3,6-(nitratomercurimethyl)dioxan blocked 90% of the thiol groups. Excess of both reagents was removed by gel filtration through Sephadex G-25 (fine grade).

The effect of histone f3 and its derivatives on DNA-dependent synthesis of RNA was assessed in the following system. The incubation mixture was made up at 0°: 0–20μg. of calf-thymus DNA in 0–0.4ml. of 1.5mM-trisodium citrate–15mM-NaCl and 0–40μg. of histone in 0–0.1ml. of 0.02M-tris-HCl buffer, pH 8.2, were mixed in a final volume of 0.15ml. of 0.02M-tris-HCl buffer, pH 8.2. To each tube was added 0.09ml. of a mixture containing 1μmole of MgCl₂, 0.25μmole of MnCl₂, 1μmole each of GTP, CTP and UTP, 0.5μmole of

[8-¹⁴C]ATP (1.0mc/m-mole), 10μmoles of tris-HCl buffer, pH 8.0, and 12μmoles of KCl. The mixtures were incubated at 37° for 10min. before initiating the reaction by the addition of 0.01ml. of RNA polymerase (EC 2.7.7.6) (purified to Stage 3 of Chamberlain & Berg, 1962) and were incubated for a further 10min. at 37°. The reaction was terminated by rapidly cooling the mixture to 0° and adding 0.2ml. of 4M-NaCl. The tubes were gently agitated until the histone-DNA aggregate dissolved. Then 0.3ml. of 10% (w/v) trichloroacetic acid was added, and after standing for at least 5hr. at 2° the precipitates were collected and washed on Oxoid membrane filters and counted in a gas-flow counter. The incubation mixture contains 0.05μmole of SH groups added as GSH, a component of the medium in which the enzyme is stored.

The disulphide form of histone f3 is more effective than the thiol form in decreasing the template activity of DNA in the RNA-polymerase system (Table 1). The results were obtained by means of Lineweaver-Burk plots, although this method of analysis is not strictly permissible since the binding between the enzyme and DNA and between histone and DNA is irreversible under the conditions of the assay (Wood & Berg, 1964; Marushige & Bonner, 1966).

It was possible that the histone thiol groups were involved in the enzymic complex since RNA polymerase requires the presence of GSH for stability during storage and is inhibited by chloromercuribenzenes-*p*-sulphonate. To test this possibility the thiol groups of fully reduced histone f3 were blocked with chloromercuribenzenes-*p*-sulphonate and the priming ability of the complexes

Table 1. *Effect of derivatives of histone fraction f3 on the template activity of DNA in RNA synthesis*

Experimental details are given in the text.

Expt. no.	Histone	Histone/DNA ratio	K_m (μg. of DNA)	$V_{\max}$. (counts/min. of [¹⁴ C]AMP incorporated/10 min.)
1	—	—	5.5	4250
	f3SH	1.67	5.5	2750
	f3S-Sf3	1.67	5.5	1820
2	—	—	5.0	3300
	f3S·R	1.50	5.0	1300
	(f3S) ₂ R'	1.50	5.0	1000

oxidized histone f3-DNA, reduced histone f3-DNA and mercurial-blocked histone f3-DNA were compared. When 18.3 μ g. of histone was combined with 10 μ g. of DNA, oxidized histone f3-DNA, reduced histone f3-DNA and mercurial-blocked histone f3-DNA complexes showed 5, 27 and 28% respectively of the priming ability of 10 μ g. of DNA alone.

The fact that monofunctional blocking of the thiol groups of reduced histone f3 does not affect the template activity of the histone f3-DNA complex suggests that any importance of histone thiol groups in relation to the control of synthesis of RNA lies in their ability to form intermolecular disulphide bridges. To obtain some support for this the thiol groups of histone f3 were blocked with the difunctional mercurial bis-3,6-(nitratomercurimethyl)dioxan and the priming ability of the difunctionally blocked histone f3-DNA complex compared with that of the chloromercuribenzenep-sulphonate-blocked histone f3-DNA complex (Table 1). The results are consistent with the thesis that the formation of intermolecular crossbridges between thiol groups modifies the ability of histone f3 to decrease the template activity of DNA.

The present results suggest further ways in which the effectiveness of DNA as a template for RNA synthesis can be modified. Littau, Burdick, Allfrey & Mirsky (1965) showed that the removal of histone f1 from nuclei transformed dense into diffuse chromatin. It has already been pointed out

there is a difference in the thiol content of the two chromatins, and in view of the difference in activity of diffuse and dense chromatin as templates of RNA synthesis it could be that modifications of an already bound histone could also alter its metabolic potential.

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The Stereochemistry of Phytol Biosynthesis in the Leaves of *Ficus elasticus* and *Aesculus hippocastanum*

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The biosynthesis of polyisoprenoid compounds involves the isomerization of isopentenyl pyrophosphate to 3,3-dimethylallyl pyrophosphate and the condensation of isopentenyl pyrophosphate with either dimethylallyl pyrophosphate or its higher isoprenologues. In each of these steps the formation of a carbon-to-carbon double bond is preceded by the expulsion of a proton from what was originally C-4 of mevalonic acid.

The stereochemistry of the elimination of this hydrogen atom has been studied with [2-¹⁴C-(4*R*)-

4-³H₁]mevalonate and [2-¹⁴C-(4*S*)-4-³H₁]mevalonate as substrates for polyisoprenoid-synthetase systems. Cornforth, Cornforth, Donninger & Popják (1966) have shown that in the conversion of these substrates into *trans-trans*-farnesyl pyrophosphate and all-*trans*-squalene by a rat-liver preparation all the 4*S*-hydrogen is lost and all the 4*R*-hydrogen is retained. The biosynthesis of squalene and ubiquinone by the mould *Aspergillus fumigatus* (Stone & Hemming, 1965) and of squalene and phytoene by carrot-root preparations (Goodwin