γ -irradiation again stops phosphorylation of histone F1 together with DNA synthesis. Histone F1 phosphate and ^{32}P incorporation into DNA were determined as for the hepatectomy experiments.

The extent of phosphorylation has been measured in various tissues from rats, calves, lambs and gerbils, and in general the mitotic index parallels the extent of phosphorylation.

The level of phosphorylation of histone F1 has also been found to be greater in diffuse (active) chromatin (90 ng.atoms of P/mg. of protein) than in dense (repressed) chromatin (10 ng.atoms of P/mg. of protein) from rat thymus. It is also higher in rat ascites tumour 5 days after inoculation (87 ng.atoms of P/mg. of protein) than 8 days (52 ng.atoms of P/mg. of protein) when the peak of exponential growth has been passed.

These results are of interest in relation to the control of information transfer and will be discussed in relation to the observation that phosphorylation of histone F1 decreases its capacity to depress DNA-dependent RNA synthesis (Stevely & Stocken, 1966).

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Nuclear Protein Thiol in Cultured Mammalian Cells

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It has been suggested that oxidation and reduction of thiol and disulphide groups might be one of the many possible ways in which histone molecules can be modified to alter their ability to repress transcription and/or replication of the cell genome (Hilton & Stocken, 1966; Ord & Stocken, 1966). Experiments were therefore performed in which basic proteins were extracted from the nuclei of cultured mammalian cells with 0.25 M-HCl and tested for their reactivity towards ^{14}C -labelled *N*-ethylmaleimide at pH 7.5. Long-term cultured lines of Chinese-hamster cells were used. Nuclei were prepared from cells in exponential and plateau-phase growth. From 5 to 25 μmoles of *N*-ethylmaleimide were found to react/mg. of protein in the extract, and the highest reactivities

were found in extracts from nuclei of populations of cells that were in exponential growth and therefore richest in cells synthesizing DNA, as estimated by [^3H]thymidine radioautography. This finding is consistent with hypotheses that include oxidation of thiol groups of histones as a possible means of controlling activities associated with cell multiplication.

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The Solubilization and Partial Fractionation of the Thiol Components of Isolated Nuclei

By M. GRONOW (Department of Biochemistry, University of Oxford)

It has been suggested (Ord & Stocken, 1968) that the oxidation of nuclear thiol groups is an important factor in the primary radiation lesion in animal cells.

The thiol-containing proteins of the nucleus are comprised of the acidic proteins and the histone F3. By extraction of rat liver nuclei with 8 M-urea in 50 mM-phosphate buffer, pH 7.6, 88% of the thiol material and 70% of the total nuclear protein were solubilized (Gronow, 1968) and no DNA could be detected. Treatment of the residue with 250 mM-HCl, to extract the histones, increased the recovery of soluble protein to 98% and of the thiol material to 94%.

This method has also been applied to the ascitic form of hepatoma 223 with essentially the same results. The cells were disrupted by hypo-osmotic shock in 5 mM- Ca^{2+} -0.1 mM- Zn^{2+} followed by homogenization in an all-glass ball-type homogenizer with a 0.001 in. clearance (Dounce, Witter, Monty, Pate & Cottone, 1955). Nuclei were obtained by centrifugation through 2.3 M-sucrose-5 mM- Ca^{2+} -0.1 M- Zn^{2+} (Chauveau, Moulé & Rouiller, 1956). They were found, by examination in the phase and electron microscopes, to be free from cytoplasmic contamination. The average DNA content was 6.7 ± 0.2 pg. per nucleus and the protein to DNA ratio was 8:1.

Attempts have been made to separate the urea-soluble acidic protein fraction from these tumour nuclei on DEAE-cellulose. Metal ions produced aggregation that could be avoided by addition of EDTA. The urea-soluble proteins were labelled with [^{14}C]N-ethylmaleimide and put through a column of Sephadex G-100. Elution with 8 M-urea-5 mM-EDTA-5 mM-tris buffer, pH 8.0, gave a single protein peak (eluted in the void volume) that