sap, whereas it stimulated when ascites ribosomes were used in the presence of their own sap. When reticulocyte sap was incubated with ascites cell ribosomes EMC-RNA had no significant effect.

One interpretation of these results is that ascites-cell sap contains a factor absent from reticulocyte-cell sap which is necessary for the translation of the added EMC-RNA. It seems that reticulocyte ribosomes are able to combine with added RNA in such a way that incorporation on the endogenous message is decreased.

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Variations in the Microstructure of Histones during Early Cleavage Stages in *Echinus*

By MARGERY G. ORD and L. A. STOCKEN (*Department of Biochemistry, University of Oxford*)

Earlier work on nuclear proteins from sea-urchins indicated that in the mature unfertilized egg the thiol group(s) on the histone(s) was present in the disulphide form and that an increase occurred in the proportion of thiol after fertilization, reaching a peak just before cleavage (Ord & Stocken, 1968). These findings have now been confirmed and extended by using *Echinus esculentus* and histone extracted from washed nuclei in ethanol-1.25M-HCl (4:1, v/v) (Johns, 1964). Fertilization and subsequent incubation of the eggs were performed at 8°; the first furrow appeared at 180min. after fertilization. The eggs were collected at 10-20min. intervals from fertilization to the second cleavage. The packed eggs were stored at -15° for about a fortnight before extracting the histones. The acid-ethanol extracts were precipitated immediately with 5 vol. of acetone-HCl and were dialysed against 50mM-HCl and water at 2° before analysis. Only traces of lysine-rich histones are present in *Echinus* in early cleavage stages; the material analysed contained components migrating on electrophoresis on polyacrylamide gel in positions similar to that of the arginine-rich histone F3 and of the F2A group. Neither phosphate nor thiol is present in the latter histones so that the SH+S·S and phosphate contents are predominantly accounted for by histone F3 (Ord & Stocken, 1967).

The total SH+S·S content of the histones analysed did not vary (69.3 ± 13.0 nmol/mg. of protein), but the proportion of thiol increased from less than 10 to more than 50% at 140, 180 and 275min. after fertilization. From work on thymi-

dine uptake into *Strongylocentrotus purpuratus* at 15° (Hinegardner, Rao & Feldman, 1964), these times probably correspond to periods of DNA synthesis. As reported earlier there is also a sharp peak in the percentage of SH 30min. after fertilization that is not found in subsequent cycles. The phosphate content of the proteins (53.6 ± 5.9 ng.atoms of P/mg. of protein) remained constant for 10-90min. after fertilization; preliminary results indicate it may then vary reciprocally to the proportion of thiol.

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Histone Phosphorylation and Cell Division

By W. S. STEVELY and L. A. STOCKEN (*Department of Biochemistry, University of Oxford*)

An approximate temporal correlation between the phosphorylation of histone F1 and DNA synthesis in regenerating rat liver has been reported by Ord & Stocken (1967). More detailed work has now shown that the increase in phosphorylation of histone F1 precedes the incorporation of ^{32}P into DNA by approx. 1hr.

Partially hepatectomized and control sham-operated animals were injected with $100\mu\text{C}$ of [^{32}P]phosphate/100g. body wt. intramuscularly 30min. before death. Groups of three or four rats were killed at $\frac{1}{2}$ hr. intervals over the period 15-19hr. after hepatectomy. Histone F1 was obtained from washed nuclei by extraction with perchloric acid (Johns, 1964) and its phosphate content determined by the method of Bartlett (1959). ^{32}P incorporation into DNA was measured by the method of DeLuca, Rossiter & Strickland (1953). Tissue phosphate soluble in 20% trichloroacetic acid was used as a reference standard for determining relative specific radioactivities.

A further indication of a link between DNA synthesis and phosphorylation of histone F1 comes from the finding that 1000 rads total body γ -irradiation at 12hr. after partial hepatectomy prevents the phosphorylation of histone F1 in a manner similar to the inhibition of DNA synthesis (Holmes & Mee, 1955).

Unilaterally nephrectomized rats have also been found to give increased phosphorylation of histone F1 24hr. after the operation when DNA synthesis is known to be taking place. 1000 rads total body

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