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).

W.S.S. is grateful to the Science Research Council for a Research Studentship.

Bartlett, G. R. (1959). *J. biol. Chem.* **234**, 466.

DeLuca, H. A., Rossiter, R. J. & Strickland, K. P. (1953). *Biochem. J.* **55**, 193.

Holmes, B. E. & Mee, L. K. (1955). In *Radiobiology Symposium*, 1954, p. 220. Ed. by Bacq, Z. M. & Alexander, P. New York: Academic Press Inc.

Johns, E. W. (1964). *Biochem. J.* **92**, 55.

Ord, M. G. & Stocken, L. A. (1967). *Biochem. J.* **103**, 5P.

Stevely, W. S. & Stocken, L. A. (1966). *Biochem. J.* **100**, 20c.

Nuclear Protein Thiol in Cultured Mammalian Cells

By P. TODD (introduced by MARGERY G. ORD)
(Department of Biochemistry, University of Oxford)

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.

I thank the Eleanor Roosevelt Foundation and the International Union Against Cancer for a Research Fellowship.

Hilton, J. & Stocken, L. A. (1966). *Biochem. J.* **100**, 21c.

Ord, M. G. & Stocken, L. A. (1966). *Biochem. J.* **98**, 888.

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

contained 69% of the applied thiol. Some RNA and a discrete peak of low-molecular-weight thiol compounds were retained in the gel. The thiol-containing protein peak was further fractionated on microgranular DEAE-cellulose giving seven components. About 40% passed through the column unabsorbed and, apart from 8M-urea, this protein could only be dissolved in 1M-acetic acid. When the latter solution was passed through a CM-cellulose column, quantitative absorption occurred. The main bulk of the thiol material could be eluted with 20mM-HCl, giving a fraction containing 78nmol of SH/mg. of protein. Analysis gave a ratio of Asp + Glu : Lys + Arg + His of 2:1.

Chauveau, J., Moulé, J. & Rouiller, O. (1956). *Exp. Cell Res.* **11**, 317.

Dounce, A. L., Witter, R. F., Monty, K. J., Pate, S. & Cottone, M. A. (1955). *J. biophys. biochem. Cytol.* **1**, 139.

Gronow, M. (1968). *Arch. Biochem. Biophys.* (submitted for publication).

Ord, M. G. & Stocken, L. A. (1968). *Proc. Roy. Soc. Edinb. B*, **70**, 117.

Effects of Trypan Blue on Rat Liver Heterolysosomes

By M. DAVIES and J. B. LLOYD (*Tenovus Institute for Cancer Research, Cardiff and University College of South Wales and Monmouthshire, Cardiff*)

The effect of intralysosomal trypan blue on the digestive capacity of rat liver heterolysosomes has been studied, the experimental methods being based on those described by Mego, Bertini & McQueen (1967). Bovine serum albumin was labelled with ^{125}I by the method of McConaghey & Dixon (1966) and denatured as described by Mego *et al.* (1967). Male Wistar rats (275–310g.) were injected intravenously with 1.4mg. of protein/kg. body wt. and killed, and their livers were perfused with cold 0.9% NaCl. After homogenization in cold 0.25M-sucrose a pellet sedimenting between 11000g-min. and 330000g-min. was prepared by centrifugation. This pellet was suspended in tris-acetic acid buffer, pH 7.4, containing 0.25M-sucrose and incubated at 37°, and samples were withdrawn at intervals up to 2hr. Each sample was assayed for trichloroacetic acid-soluble radioactivity (a measure of the extent of proteolysis) and for non-sedimentable trichloroacetic acid-insoluble radioactivity (a measure of free albumin and hence of heterolysosome rupture).

Acid-soluble radioactivity increased from 10% to 36–41% of the total radioactivity over 2hr.; no increase was observed when 0.2% of Triton X-100 was included in the incubate, indicating that proteolysis occurs within osmotically active particles. Free albumin increased from 4% to 25–35%

in the first 30min. and remained at this value for the duration of the incubation. Inclusion of 0.2% of Triton X-100 in the incubate caused the immediate release of 80–90% of the albumin.

Rats that had received trypan blue (75mg./kg.) subcutaneously 24hr. before injection with albumin consistently showed a lower rate of protein breakdown than controls, trichloroacetic acid-soluble radioactivity reaching only 20% after 2hr. This decreased rate of proteolysis was accompanied by an increased release of free albumin, which reached 50% of the total radioactivity within 30min. This effect of intralysosomal trypan blue on heterolysosome stability probably explains the lower rate of protein breakdown observed in incubates of particles from dye-treated rats. However, since trypan blue inhibits lysosomal enzymes (Lloyd, Beck, Griffiths & Parry, 1968), intralysosomal enzyme inhibition may also contribute to the decreased rate of protein breakdown.

We thank Tenovus for generous financial support.

Lloyd, J. B., Beck, F., Griffiths, A. & Parry, L. M. (1968). In *Interaction of Drugs and Subcellular Components in Animal Cells*, p. 171. Ed. by Campbell, P. N. London: J. and A. Churchill Ltd.

McConaghey, P. & Dixon, J. (1966). *Int. Arch. Allergy appl. Immun.* **29**, 185.

Mego, J. L., Bertini, F. & McQueen, J. D. (1967). *J. Cell Biol.* **32**, 699.

Pyruvate Carboxylase in Lactating Rat and Rabbit Mammary Gland

By BILQUIS GUL and R. DILS (*Department of Medical Biochemistry and Pharmacology, University of Birmingham*)

Though the role of pyruvate carboxylase (EC 6.4.1.1) in gluconeogenesis is well established, it has been postulated (Wise & Ball, 1964; Ballard & Hanson, 1967) that in adipose and liver the enzyme has a role in lipogenesis. Lactating mammary gland has a high lipogenic activity (Fritz, 1961; Masoro, 1962), yet the presence of pyruvate carboxylase has not been reported in this tissue. We have therefore investigated some properties of this enzyme in lactating rat and rabbit mammary gland.

Preliminary experiments indicated that pyruvate carboxylase was present in both the mitochondrial and particle-free fractions from homogenates of rat mammary gland, but confined to the mitochondrial fraction of homogenate of rabbit mammary gland. Optimum conditions for the assay of this enzyme in these fractions have been determined. The cofactor requirements do not differ significantly