(1969). This gave fractions of differing maturity as judged by the incorporation of [32 P]phosphate into RNA and DNA.

Histone F2c was present in all the fractions obtained in these studies and, when due allowance had been made for non-erythroid-cell contamination no significant change in the proportion of this histone relative to that of the other histones could be established. It was found, however, that the incorporation of [32 P]phosphate into the lysine-rich fraction declined with maturation, confirming the result obtained by Adams, Vidali & Neelin (1970).

These studies indicate that the function of histone F2c, if related to terminal maturation, may depend on its phosphate content. It seems possible, however, that it may be involved at an earlier point in differentiation than has so far been suggested, since it appears to be present at the earliest stages of erythroid development analysed in these experiments.

Adams, G. H. M., Vidali, G. & Neelin, J. M. (1970). *Can. J. Biochem.* **48**, 33.

Dick, C. & Johns, E. W. (1969). *Biochim. biophys. Acta*, **175**, 414.

Lucas, A. M. & Jamroz, C. (1961). *Atlas of Avian Hematology*. Washington, D.C.: U.S. Department of Agriculture.

Mathias, A. P., Ridge, D. & Trezona, N. St G. (1969). *Biochem. J.* **111**, 583.

Neelin, J. M., Callahan, P. X., Lamb, D. C. & Murray, K. (1964). *Can. J. Biochem.* **42**, 1743.

Changes in Rat Liver Non-histone Nuclear Proteins during Nitrosamine Carcinogenesis

By M. GRONOW. (*Department of Experimental Pathology and Cancer Research, University of Leeds, Leeds LS2 3AR, U.K.*)

It has been reported (Harbers, Lederer, Sandritter & Spaar, 1968) that when rats are given diethylnitrosamine in their drinking water at 3 mg/kg per day then liver tumours are produced after 180 days. At this low dose no cirrhotic changes were observed in the liver, but in the nuclei a doubling of heterochromatin content, accompanied by a corresponding decrease in euchromatin, was found to occur after about 40 days of dosage with the carcinogen. As this probably indicates a profound change in the chromatin structure that may lead to neoplasia, investigations were made to ascertain whether any change in the composition of the nuclear proteins occurs at this point. Initial studies had indicated that no change occurred in the composition of the histones after administration of the carcinogen. This communication is concerned with preliminary attempts to investigate the non-histone proteins of the liver cell nucleus.

Various methods have been described for the extraction of the non-histone nuclear proteins. These usually involve several stages, using combinations of dilute buffers, dilute and concentrated sodium chloride solutions, detergent mixtures and phenol (see Hnilica, 1967). A method has been described (Gronow, 1969) by which practically all of the nuclear non-histone protein can be extracted by using 8M-urea-50mM-sodium phosphate buffer, pH 7.6. Proteins in this mixture have been separated into about 22 components by using the technique of isoelectric focusing in polyacrylamide gel (pH 3-10 Ampholine). Separation on the basis of molecular weight by using sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Elgin & Bonner, 1970) gave about 18 bands. When these techniques were applied to rat liver nuclear proteins extracted with other solvents (e.g. dilute and concentrated saline) it was found that each fraction contained, in various amounts, components present in others.

Nuclei prepared by the Chauveau, Moulé & Roueller (1956) technique from the livers of rats given diethylnitrosamine for 50 and 70 days were extracted with 8M-urea-50mM-sodium phosphate buffer, pH 7.6. The Coomassie Blue-stained isoelectric-focusing polyacrylamide gels and sodium dodecyl sulphate-polyacrylamide gels of these extracts looked identical with the controls. However, when the thiol groups of these proteins were labelled with [14 C]-N-ethylmaleimide, an increase in the specific radioactivity of the proteins from the 70-day-treated animals was found. Components focusing on the pH 7-8 region were found to have a marked increase in thiol content.

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Chauveau, J., Moulé, Y. & Roueller, C. (1956). *Exptl Cell Res.* **11**, 317.

Elgin, S. C. R. & Bonner, J. (1970). *Biochemistry, Easton*, **9**, 4440.

Gronow, M. (1969). *Eur. J. Cancer*, **5**, 497.

Harbers, E., Lederer, B., Sandritter, W. & Spaar, U. (1968). *Virchows Arch., Abt. B Zellpath.* **1**, 98.

Hnilica, L. S. (1967). *Prog. nucleic Acid Res. molec. Biol.* **7**, 25.

Lipase Activity against 1,2-Diglyceride in Rat Erythrocyte Membranes

By R. H. MICHELL and R. COLEMAN. (*Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.*)

Erythrocyte membranes prepared from fresh heparinized rat blood were treated with phospholipase C (from *Clostridium welchii*; Sigma Chemical Co., St Louis, Mo., U.S.A.). Lipids were then

extracted and examined (Skipski, Smolowe, Sullivan & Barclay, 1965). Up to 70% of the phospholipids were hydrolysed. Although hydrolysis of diacylglycerophospholipids by phospholipase C releases 1,2-diglyceride, this does not accumulate and a major product appeared to be unesterified fatty acid. This suggested that a lipase active against 1,2-diglyceride might be present in the membranes.

Erythrocytes were therefore incubated with [^3H]palmitate to label their phospholipids (Shohet, Nathan & Karnovsky, 1968), and the transfer of radioactivity from phospholipids to other lipids was followed during phospholipase treatment. Labelling of 1,2-diglyceride was unchanged, and the unesterified fatty acid and monoglyceride fractions together accumulated radioactivity equal to that lost by the phospholipids.

The total lipids of both unlabelled and labelled membranes were isolated, emulsified and treated with phospholipase C. Under these conditions the only major product was 1,2-diglyceride, both by inspection of chromatograms and by transfer of radioactivity from phospholipids to other lipids.

The difference in products seen after treatment of membranes and of emulsified lipids was further confirmed by measuring the acyl ester and unesterified fatty acid content of these preparations after phospholipase treatment. There was a loss of acyl esters and a concomitant increase in unesterified fatty acids only when membrane-bound lipid was hydrolysed.

The observed hydrolysis of 1,2-diglyceride appeared to be a property of the membranes rather than of a contaminant in the phospholipase C preparations. It was, however, still possible that the commercial phospholipase C preparations contained a lipase that would act only on membrane-bound diglyceride. The products formed during treatment of two other membrane preparations, human erythrocyte membranes from cells stored in acid-citrate-glucose medium and rat cerebral-cortical myelin (fraction $\text{ML}_{0.8}$ described by Rodrigues de Lores Arnaiz, Alberici & De Robertis, 1965) were therefore examined. In each case the only major product that appeared was 1,2-diglyceride.

These results indicate that the membranes of rat erythrocytes contain a lipase that is highly active against 1,2-diglyceride at pH 7.4 with 0.75 mM- Ca^{2+} . Further characterization of this activity will initially be aimed at defining conditions under which externally added substrates are hydrolysed.

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Rodrigues de Lores Arnaiz, G., Alberici, M. & De Robertis, E. (1965). *J. Neurochem.* **14**, 215.

Shohet, S. B., Nathan, D. & Karnovsky, M. L. (1968). *J. clin. Invest.* **47**, 1096.

Skipski, V. P., Smolowe, A. F., Sullivan, R. C. & Barclay, M. (1965). *Biochim. biophys. Acta*, **106**, 386.

Cytoplasmic Redox Control of Protein Synthesis

By R. D. NOLAN* and M. B. HOAGLAND† (introduced by H. N. MUNRO). (*Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03755, U.S.A.*)

Partial hepatectomy initiates biochemical events causing a greatly increased rate of protein synthesis (Bucher, 1963). The purpose of this investigation was to derive a system *in vitro* that would accurately reflect the known changes in protein-synthetic activity *in vivo*.

A technique (Scornik, Hoagland, Pfefferkorn & Bishop, 1967) for removing lysosomes from rough endoplasmic reticulum (microsomal fraction) has been used here to obtain microsomal preparations from normal and regenerating rat livers. By using a low concentration (0.01 mM) of GTP and transfer factors and measuring the initial rate of amino acid transfer from aminoacyl-tRNA to peptide it was shown that the microsomal preparation from normal liver has only 50% the rate of transfer carried out by the preparation from regenerating liver. Raising of the GTP concentration to 0.4 mM eliminates these differences, largely by stimulating the microsomal preparation from normal liver, there being relatively little effect on the preparation from regenerating liver, GSH and other thiols also have a relatively larger stimulatory effect on the former microsomal preparation than on the latter.

A marked decrease in the initial rate can be produced by adding GSSG, and again the inhibitory effect is more dramatic on the microsomal preparation from normal liver than on that from regenerating liver, thus increasing the difference between the activities of the two preparations. Rat livers were briefly perfused with the oxidizing agent $\text{NNN}'\text{N}'$ -tetramethylazofornamide (diamide) (O'Brien, Weitzman & Morris, 1970). The microsomal preparation derived from livers of animals treated with this drug had a lower initial rate of synthesis, greater stimulation by thiols and markedly diminished sensitivity to inhibition by GSSG compared with the control preparation.

* Present address: Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02172, U.S.A.

† Present address: Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Mass. 01545, U.S.A.