The Metabolism of Nicotinamide-Adenine Dinucleotide in Various Classes of Rat Liver Nuclei

By M. E. HAINES, I. R. JOHNSTON, A. P. MATHIAS and D. RIDGE. (*Wellcome Laboratories, Department of Biochemistry, University College London, Gower Street, London W.C.1*)

Rat liver nuclei may be fractionated by zonal centrifugation into various classes (Johnston, Mathias, Pennington & Ridge, 1968). We have investigated the activities of two nuclear enzymes concerned with the metabolism of NAD in the different classes of nuclei. These are (i) ATP-NMN adenyltransferase (NAD pyrophosphorylase, EC 2.7.7.1) and (ii) an enzyme that uses NAD to make a polymer of ADP-ribose, called here poly-ADP-ribose polymerase. NAD pyrophosphorylase was assayed by the method of Kornberg (1950), and poly-ADP-ribose polymerase by the method of Nishizuka, Ueda, Honjo & Hayaishi (1967). The highest specific activities of these two enzymes occur in different classes of nuclei. For NAD pyrophosphorylase the activity between diploid and tetraploid peaks was 40–80 μ moles of NAD/hr./ 10^9 nuclei and in the tetraploid peak 20–40 μ moles of NAD/hr./ 10^9 nuclei. Both in very young and mature rats there exists a correlation between the highest specific activities of NAD pyrophosphorylase and DNA synthesis. In contrast, during the stage characterized by the transition of the parenchymal cells from the diploid to tetraploid state this correlation is less evident. Greenbaum & Pinder (1968) have also observed a correlation between DNA synthesis and NAD pyrophosphorylase activity in lactating mammary gland.

Poly-ADP-ribose polymerase activity is associated with the main diploid and tetraploid peaks and tends to follow the incorporation of [3 H]orotic acid into RNA. For instance in tetraploid nuclei the activity of poly-ADP-ribose polymerase is approx. 1.5 μ moles of ADP-ribose incorporated/hr./ 10^9 nuclei. In assessing the factors that regulate cellular concentrations of NAD, the activity of this enzyme and the fate of its product, poly-ADP-ribose, must be borne in mind.

The specific activities of the two enzymes measured *in vitro* therefore differ by an order of magnitude, although it is claimed that NAD pyrophosphorylase is present *in vivo* in a 100-fold excess (Siebert, 1968).

The maintenance of the concentrations of nicotinamide nucleotides is an important regulatory feature in tissues. In addition there is an inverse correlation between the concentrations of nicotinamide nucleotides and the mitotic index (Morton, 1961). The patterns of activity of the two enzymes

studied by us will be discussed in relation to pathways of NAD metabolism.

This work was supported by the Science Research Council. M.E.H. was in receipt of a Science Research Council post-graduate studentship.

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Subcellular Fractionation of *Saccharomyces carlsbergensis* by Zonal Centrifugation

By T. G. CARTLEDGE, J. K. BURNETT, R. BRIGHTWELL and D. LLOYD. (*Medical Research Council Group for Microbial Structure and Function, Department of Microbiology, University College of South Wales and Monmouthshire, Cardiff*)

Studies were carried out to investigate the distribution of marker enzymes in homogenates prepared from *Saccharomyces carlsbergensis*. Sphaeroplasts were prepared by the two-stage method of Duell, Inoue & Utter (1964) and lysed by using a Teflon hand-operated homogenizer (clearance 0.004 in.). After unbroken cells had been centrifuged off, the homogenate was loaded on to an unbuffered sucrose gradient in a BXIV rotor and centrifuged at 30 000 rev./min. for 2.75 hr. ($\approx 73 800g$ for 60 min. at the sample zone).

The following results were obtained with aerobically grown cells for percentage sedimentabilities and median buoyant densities (g./ml. in parentheses) of membrane-bound activities: protein, 16% (1.18); cytochrome oxidase, 100% (1.18); catalase, 46% (1.17 and 1.20); acid phosphatase, 36% (heterogeneous density distribution from 1.12 to 1.24); esterase (substrate *p*-nitrophenyl acetate), 34.5% (also heterogeneous but major peak at 1.21). NADH- and NADPH-cytochrome *c* oxidoreductases were 72% and 80% sedimentable respectively, and NADH- and NADPH-ferri-cyanide oxidoreductases 74% and 57% sedimentable; all equilibrated at $\rho = 1.18$ –1.19.

The similarity in distributions of acid phosphatase and esterase do not agree with the findings of Matile & Wiemken (1967) with *Saccharomyces cerevisiae*, where it was claimed that esterase but not acid phosphatase occurs in the cell vacuole. Vacuole fractions separated by Ficoll flotation contained a small proportion of both enzymes but

there was no purification over the initial whole homogenate.

Cells grown anaerobically in the presence of 10% (w/v) glucose contained no detectable cytochrome oxidase or catalase. Cells grown anaerobically with 5% (w/v) glucose (and maintained as anaerobic as possible during sphaeroplast production and lysis) did, however, show low activities of both these enzymes. The percentage sedimentability of enzymes in homogenates of these cells was as follows: protein, 7%; acid phosphatase and esterase, not sedimentable; NADH-cytochrome *c* oxidoreductase, 19%; NADPH-cytochrome *c* oxidoreductase, 4%; NADPH-ferri-cyanide reductase, 33%.

Cells grown anaerobically with galactose in the presence of Tween 80 and ergosterol (Wallace, Huang & Linnane, 1968) do possess detectable activities of cytochrome oxidase and catalase and the percentage sedimentability of these enzymes was 87% and 55% respectively. The median density of sedimentable protein (1.19) corresponded with that of NADH- and NADPH-cytochrome *c* oxidoreductases and NADH- and NADPH-ferri-cyanide oxidoreductases; acid phosphatase was 47% sedimentable.

R.B. was I.C.I. Fellow during the course of this work.

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The Subcellular Acquisition of Carbohydrates by Glycoproteins

By N. J. COWAN and G. B. ROBINSON. (*Department of Biochemistry, University of Oxford*)

Several reports have indicated that the microsomal membranes are of primary importance in the addition of carbohydrate to the protein moiety of glycoproteins (Lawford & Schachter, 1966; Molnar, Robinson & Winzler, 1965). We have investigated the subcellular sites of acquisition of carbohydrate by protein in the immunoglobulin A-secreting plasmacytoma, MPC-1, by using a technique of incubation *in vitro*.

Homogenization and fractionation of incubated myeloma tissue on a continuous sucrose gradient gives a number of distinct bands. The specific radioactivity of protein from these bands after incubation of the tissue with [¹⁴C]lysine is largely uniform, whereas there are marked differences between the specific radioactivities of bands obtained after incubation with [¹⁴C]glucosamine. Uptake of both [¹⁴C]lysine and [¹⁴C]glucosamine is linear with time up to 140 min. The radioactive myeloma protein synthesized in these subcellular

fractions was identified by immunoelectrophoresis of sonicated extracts of the fractions, followed by radioautography of the resulting precipitin lines.

In the presence of puromycin at a concentration that inhibits protein synthesis by 98%, [¹⁴C]-glucosamine incorporation into trichloroacetic acid-insoluble glycoprotein is decreased by only 50–60%. Further, there is a decrease of only 20–30% in radioactive glycoprotein appearing in the incubation medium. Thus there must exist in the cell a large pool of partially glycosylated protein that continues to be glycosylated and secreted after protein synthesis has virtually ceased.

Whether ribosomes are involved in the initial attachment of hexosamine to glycoprotein is a matter of contention. Preparations of ribosomes from tissue incubated with [¹⁴C]glucosamine showed a linear increase in specific radioactivity up to 140 min. Tissue preincubated with [¹⁴C]glucosamine for 30 min. and 70 min., and subsequently incubated for a further 10 min. with added puromycin, showed an exactly similar decrease in the specific radioactivity of the ribosomes after the puromycin addition. However, this decrease constituted only 29% and 16% respectively of the total specific radioactivity of the ribosomes. Thus it is suggested that two factors contribute to ribosomal labelling: glucosamine attached to nascent polypeptides, and radioactivity from adsorbed labelled material.

N.J.C. is indebted to the Medical Research Council for a scholarship for training in research methods.

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Studies on Sialoglycoprotein Complexes of Human Erythrocyte Membranes

By R. W. STODDART (*Medical Research Council Molecular Pharmacology Research Unit, Mill Lane, Cambridge*) and S. M. METCALFE (*Department of Biochemistry, Tennis Court Road, Cambridge*)

Proteins and glycoproteins have been prepared by the disruption of erythrocyte membranes in several ways (Rega, Weed, Reed, Berg & Rothstein, 1967; Blumenfeld, 1968; Harris, 1968; Marchesi & Steers, 1968; Rosenberg & Guidotti, 1968; Schneiderman & Junga, 1968). We have modified the techniques of Marchesi & Steers (1968) and Blumenfeld (1968) to enable us to prepare two glycoprotein complexes from human erythrocyte 'ghosts'.

Erythrocyte 'ghosts' (water-washed, 2%, w/w) were treated with mercaptoethanol (50 mM) and