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

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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.

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Subcellular Fractionation of *Saccharomyces carlsbergensis* by Zonal Centrifugation

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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