Decreased Thermal Stability of Alcohol Dehydrogenase in the Presence of Oxidized Nicotinamide-Adenine Dinucleotide or Oxidized Nicotinamide-Adenine Dinucleotide Phosphate

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Substrates have often been observed to protect enzymes against denaturation by heat and other agents (Joly, 1965; Bernhard & Rossi, 1968). On the other hand, yeast alcohol dehydrogenase activity can be decreased by preincubation at 43°C with NAD⁺ (Grisolia & Joyce, 1959). Liver alcohol dehydrogenase, however, when heated at 75°C (at pH 7.0) with NAD⁺ at low concentration (0.04 mM) shows a small stabilization effect compared with no addition of NAD⁺ (Yonetani & Theorell, 1962).

We find 35–30% recovery of yeast alcohol dehydrogenase activity after preincubation in 10 mM-sodium pyrophosphate buffer, pH 8.8, at 43°C for 20 min, within the range 0–1.5 mM-NAD⁺ or -NADP⁺. This recovery decreased rapidly with increasing concentration of NAD⁺ or NADP⁺ above 1.5 mM, reaching zero at 3.5 mM-NAD⁺ or -NADP⁺. With liver alcohol dehydrogenase 35–30% recovery was obtained after preincubation at 43°C for 50 min within the range 0–2.0 mM-NAD⁺. The recovery reached zero at 4.3 mM-NAD⁺ or 3.3 mM-NADP⁺. No stabilization was observed at 0.04 mM-NAD⁺ under our conditions of preincubation.

Cysteine or bovine serum albumin (most effective at 0.8 and 0.03 mg/ml respectively) included in the preincubation medium had a fairly constant protective action throughout the concentration range of NAD⁺ used, in addition to a small activator effect in the enzyme assay itself. By using 3.5 mM-NAD⁺ with yeast enzyme (normally zero recovery here), 13% recovery was obtained with cysteine and 6% recovery with bovine serum albumin. For liver alcohol dehydrogenase (with 4.3 mM-NAD⁺) recoveries were 7% with cysteine and 14% with bovine serum albumin.

Only the liver enzyme can use NADP⁺ as cofactor, yet both of these enzyme preparations are inactivated at high concentrations of NADP⁺. Accelerated thermal denaturation of these enzymes by NAD⁺ or NADP⁺ could be due to promotion of oxidation of essential thiol groups or conformational change, including dissociation into subunits. Our findings with stabilizers suggest that both of these changes may occur.

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Effect of Inducers of Drug-Metabolizing Enzymes on Diethylnitrosamine Metabolism and Toxicity

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Nitrosamines are strongly hepatotoxic compounds and after chronic administration produce tumours in liver and other organs (Magee & Barnes, 1956; Druckrey, Preussmann, Ivankovic & Schmäh, 1967). The formation of toxic metabolites *in vivo* was assumed to be responsible for the toxic effect of several dialkyl derivatives. However, it has been suggested by Mirvish (1969) that the metabolic activation of diethylnitrosamine as directed by alkylation of macromolecules is not carried out by an inducible drug-metabolizing enzyme system. We investigated the induction of diethylnitrosamine metabolism by studying de-ethylation *in vitro* by liver microsomal fraction and its relation to the overall toxicity.

Female Wistar rats were injected intraperitoneally with 80 mg of phenobarbitone/kg and 20 mg of 3-methylcholanthrene/kg and orally with 200 mg of butylated hydroxytoluene/kg and 160 mg of DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane]/kg. The metabolism of diethylnitrosamine by liver microsomal fraction was found to be stimulated by 533, 190, 269 and 305% respectively as detected by measuring the formation of acetaldehyde (Stotz, 1943) *in vitro*. The relationship between metabolism and toxicity of diethylnitrosamine was examined by injecting female Wistar rats intraperitoneally with increasing doses of 20, 30, 40, 60 or 100 mg of diethylnitrosamine/kg alone and simultaneously with 80 mg of phenobarbitone/kg. Diethylnitrosamine decreased the drug-metabolizing enzyme activity and protein content of liver microsomal fraction. This effect was antagonized by phenobarbitone treatment. However, diethylnitrosamine decreased the inducing effect of phenobarbitone on de-ethylating enzyme activity more than that on microsomal protein. Simultaneous phenobarbitone treatment lowered the

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