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

By A. WISEMAN, H. O. HANLEY and N. J. WILLIAMS.
(Department of Biochemistry, University of Surrey,
Guildford, Surrey, U.K.)

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.

Bernhard, S. A. & Rossi, G. L. (1968). In *Structural Chemistry and Molecular Biology*, p. 88. Ed. by Rich, A. & Davidson, N. San Francisco and London: Freeman.

Grisolia, S. & Joyce, B. K. (1959). *Biochem. biophys. Res. Commun.* 1, 280.

Joly, M. (1965). *A Physico-Chemical Approach to the Denaturation of Protein*, p. 32. New York and London: Academic Press.

Yonetani, T. & Theorell, H. (1962). *Archs Biochem. Biophys.* 99, 433.

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

By S. MAGOUR* and J. G. NIEVEL. (British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, U.K.)

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

* Present address; Institute of Neuropsychopharmacology, Free University, Berlin, W. Germany.

overall toxicity of repeated doses of 40, 30 and 20 mg of diethylnitrosamine/kg, as manifested by faster growth rate and increased surviving time.

In conclusion, the experimental findings suggest that the metabolism of diethylnitrosamine can be stimulated by various inducers of drug-metabolizing enzymes and that diethylnitrosamine inhibits *de novo* synthesis of de-ethylating enzymes and also impairs the function of the preformed enzymes. The induced metabolism of diethylnitrosamine and protein synthesis by phenobarbitone decreased the subacute toxicity. This protection against diethylnitrosamine toxicity *in vivo* by phenobarbitone is probably due to the formation of non-toxic metabolites; however, some additional mechanisms may also participate in this effect.

Druckrey, H., Preussmann, R., Ivankovic, S. & Schmähl, D. (1967). *Z. Krebsforsch.* **69**, 103.

Magee, P. N. & Barnes, J. M. (1956). *Br. J. Cancer*, **10**, 114.
Mirvish, S. S. (1969). *Jerusalem Symp. Quantum Chemistry and Biochemistry*, vol. 1, p. 305.

Stotz, E. (1943). *J. biol. Chem.* **148**, 585.

Interaction of Nitrite with Mammalian Mitochondrial Cytochromes

By I. H. BURGER and C. L. WALTERS (*British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey, U.K.*) and D. V. PARKE. (*Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K.*)

The oxidation of nitrite by oxygen-carrying haem pigments to their ferric forms was reported by Lemberg & Legge (1949), and was subsequently applied by Chance (1952) to the spectrophotometric differentiation of cytochrome *b* and oxymyoglobin in the Soret region. Colpa-Boonstra & Minnaert (1959) showed that a compound was formed of ferrocytochrome *a*₃ and nitric oxide, and Walters & Taylor (1964) demonstrated the formation of nitric oxide by the reduction of nitrite in whole skeletal muscle. Nitrite is used widely in the preservation of food, the permitted effective concentration being approx. 200 p.p.m. as sodium nitrite (2.9 mM) in the product. The present investigations are on the action of nitrite on the cytochromes of pig skeletal-muscle mitochondria.

The introduction of sodium nitrite (0–10 mM) and a small quantity of mitochondrial suspension (12 ng of protein) to ferrocytochrome *c* (4 μM) at pH 6 at 37°C results in oxidation of the cytochrome to the ferric form. As the nitrite concentration is increased (from 0 to 3 mM) the rate of oxidation of ferrocytochrome *c* decreases to a minimum, but at higher concentrations of nitrite (3–10 mM) increases to reach approximately the original value obtained in the absence of nitrite. In the presence of potas-

sium cyanide (20 μM) the rate of ferrocytochrome *c* oxidation is directly proportional to the nitrite concentration, as was observed in the direct action of nitrite on ferrocytochrome *c* in the absence of mitochondria (cytochrome oxidase).

Nitrite can act as an inefficient terminal electron acceptor to mitochondrial electron transport, it can also oxidize ferrocytochrome *c* directly and it may also exercise a very weak competitive inhibition on cytochrome oxidase in the presence of O₂. It is suggested that these three distinct actions of nitrite may be responsible for the unusual inhibitory effects that were observed in the oxidation of ferrocytochrome *c* in the presence of cytochrome oxidase. At nitrite concentrations below 3 mM, in the presence of mitochondria, the oxidation of ferrocytochrome *c* is mainly enzymic (via cytochrome oxidase). Within this range the oxidation of ferrocytochrome *c* is increasingly inhibited as the nitrite concentration is raised. Above 3 mM-nitrite the oxidation of ferrocytochrome *c* proceeds principally via the direct action of nitrite. However, some evidence has been obtained of increasing enzymic oxidation of ferrocytochrome *c* (cyanide-sensitive) at nitrite concentrations above 3 mM, and the implications of these observations will be discussed. It is noteworthy that the concentration of nitrite normally used in food preservation produces minimal oxidation of the components of the respiratory chain under the conditions *in vitro*.

We thank Dr A. W. Holmes, Director of the British Food Manufacturing Industries Research Association, for his continued interest in this problem, and the Nuffield Foundation for their financial support.

Chance, B. (1952). *J. biol. Chem.* **197**, 557.

Colpa-Boonstra, J. P. & Minnaert, K. (1959). *Biochim. biophys. Acta*, **33**, 527.

Lemberg, R. & Legge, J. W. (1949). *Hematin Compounds and Bile Pigments*, p. 389. New York: Interscience Publishers Inc.

Walters, C. L. & Taylor, A. McM. (1964). *Biochim. biophys. Acta*, **86**, 448.

Induction of a New Hepatic Microsomal Haemoprotein by Safrole and Isosafrole

By D. V. PARKE and H. RAHMAN. (*Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K.*)

Safrole (4-allyl-1,2-methylenedioxybenzene), an anutrient present in many essential oils and natural flavours, and its isomer isosafrole (1,2-methylenedioxy-4-propenylbenzene) have been shown to produce, in the rat, hypertrophy of the liver accompanied by marked increases in a number of hepatic microsomal enzymes, including biphenyl