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.

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Interaction of Nitrite with Mammalian Mitochondrial Cytochromes

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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*.

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Induction of a New Hepatic Microsomal Haemoprotein by Safrole and Isosafrole

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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