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

hydroxylases, nitro reductase, glucuronyltransferase and cytochrome *P*-450 (Parke & Rahman, 1970). Induction of these enzymes by pretreatment of rats with other anutrients, such as phenobarbitone, 3-methylcholanthrene or β -ionone (Parke & Rahman, 1969), gives rise also to an increase of microsomal cytochrome *b*₅. Induction by pretreatment with safrole and isosafrole (125mg/day per kg) gives rise to an increase of cytochrome *b*₅ and also to the appearance of a new redox difference absorption spectrum, which progressively increases over 3 days of continued treatment with these anutrients, and may represent the biosynthesis of a new hepatic microsomal haemoprotein. Whereas the redox difference absorption spectrum of cytochrome *b*₅ (NADH) has a maximum at 425nm and that of cytochrome *P*-450 (NADPH) occurs at 445nm, the absorption maximum for the new haemoprotein occurs at 455nm and is manifest after reduction with NADH or NADPH. This new absorption peak is not therefore attributable to the increase of cytochrome *P*-450.

The new redox difference absorption spectrum does not appear when the safrole pretreatment of rats is accompanied by simultaneous administration of thioacetamide to inhibit nuclear-directed protein synthesis (Adams & Busch, 1963), or of 3-amino-1,2,4-triazole to inhibit the synthesis of haem (Baron & Tephly, 1969). Solubilization of the hepatic microsomal pellet from safrole-treated rats, by treatment with 0.5% deoxycholate, results in the disappearance of both cytochrome *b*₅ (425nm) and the new haemoprotein (455nm) from the 100000g deposit. The cytochrome *b*₅ absorption maximum (425nm) reappears quantitatively in the 100000g supernatant fraction, whereas the absorption maximum at 455nm, although present, is much diminished. Addition of safrole or isosafrole to the 100000g microsomal pellet obtained from liver homogenates from normal rats does not result in the appearance of the absorption maximum of this new haemoprotein, nor to any new absorption maximum other than that due to cytochrome *b*₅. From these observations it is considered that the new redox difference absorption spectrum may be attributed to the formation of a new microsomal haemoprotein, and is not merely an adduct of cytochrome *b*₅ with safrole, isosafrole or any of their metabolites.

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The Deacetylation of *N*-Arylacetamides *in vitro*

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The administration of certain aromatic amines or their acetylated derivatives to experimental animals leads to the development of cancer (Clayson, 1962). However, as dogs fed on diets containing aromatic amines also develop tumours, although being unable to acetylate the toxicant (Poirier, Miller & Miller, 1963), it is possible that deacetylation may be a prerequisite for the initiation of the carcinogenic process resulting from exposure to *N*-arylacetamides.

Further, whereas 2-naphthylamine and 4-aminobiphenyl are carcinogenic, 1-naphthylamine and 2-aminobiphenyl are inactive (Weisburger & Weisburger, 1966). The carcinogenic compounds show considerable variation in their ability to produce tumours in different species. As the metabolism of an acetamido compound may differ from that of the parent amine (Booth & Boyland, 1964) the deacetylase activity of various species was examined *in vitro*.

Deacetylase activity was measured by determining the formation of aromatic amines from the corresponding acetamido compound by a g.l.c. technique (Gorrod, 1968). A deacetylase activity active towards 2- and 4-acetamidobiphenyl, 1- and 2-acetamidonaphthalene and 2-acetamidofluorene was found localized in the hepatic microsomal fraction of various species examined; optimum activity occurred between pH 8 and 9. The deacetylase activity was strongly inhibited by sodium fluoride (1mM) but was not inhibited by SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) (2mM).

Hydrolysis of acetamido compounds was observed with hepatic microsomal preparations obtained from rat, cat, dog, mouse, hamster, cavy and human; 1-acetamidonaphthalene and 2-acetamidobiphenyl were the most rapidly hydrolysed substrates, whereas 2-acetamidofluorene was the poorest. The carcinogenic substrates were generally hydrolysed to their greatest extent in the species in which they were able to produce tumours, although only rarely did the rate of hydrolysis exceed that of the non-carcinogenic counterpart. The ease of hydrolysis of 2-acetamidobiphenyl is in accord with the failure of Gorrod & Carey (1970) to detect this compound as a metabolite of 2-aminobiphenyl.

N-(1-Naphthylthio)acetamide was completely resistant to this deacetylase activity in either rat or cavy preparations, which were easily able to hydrolyse the oxygen analogue.

The results reported do not support the idea of a direct relationship between deacetylation and