These compounds also had some effect on the proportion of the $^{14}$C recovered in the urine.

$N$-Nitrosopyrrolidine inhibits the metabolism of dimethylnitrosamine to formaldehyde by rat liver 10000g supernatant. The form of the dependence of the rate of dimethylnitrosamine metabolism to formaldehyde on inhibitor concentration (shown in Fig. 2) suggests that there is a competitive effect.

These results taken together strongly suggest that there may be a close relationship between the rate-determining step(s) in the metabolism of the two environmentally important carcinogens dimethylnitrosamine and $N$-nitrosopyrrolidine.

**Further Studies on the Metabolism of Dimethylnitrosamine by Rat Liver in vitro**

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The hepatocarcinogen dimethylnitrosamine is metabolized by rat hepatic preparations to methanol and formaldehyde (Lake et al., 1975a, 1976a). It has been suggested that the hepatic metabolism of this nitrosamine is catalysed by enzymes of the microsomal cytochrome P-450-dependent mixed-function oxidase complex (Argus et al., 1976). However, in previous investigations we found a number of important differences between the properties of the enzyme system(s) responsible for dimethylnitrosamine degradation and those of typical mixed-function oxidase enzymes (Lake et al., 1975a,b, 1976a,b). As the metabolism of dimethylnitrosamine in vivo is markedly decreased by either monoamine oxidase (EC 1.4.3.4) substrates or inhibitors (Lake et al., 1976b), we have suggested that hepatic dimethylnitrosamine degradation may proceed through an $N$-oxide intermediate catalysed by a microsomal amine oxidase enzyme. In the present study we have further examined this hypothesis.

Male Sprague-Dawley rats (80–150g) were used in these experiments; they were allowed free access to laboratory diet and water. Livers were homogenized (0.25g fresh wt. of tissue/ml) in 0.154M-KCl/50mm-Tris/HCl buffer, pH 7.4, and centrifuged at 10000g for 20min to obtain the postmitochondrial supernatant fractions. The metabolism of dimethylnitrosamine, ethylmorphine, benzphetamine and aminopyrine was monitored by measuring the production of formaldehyde (Nash, 1953) as previously described (Lake et al., 1976b). Aniline 4-hydroxylase activity was determined by the method of Nakanishi et al. (1971) and kynuramine oxidase by the fluorimetric procedure of Krajl (1965).

The addition of the type-B monoamine oxidase substrate benzylamine (Neff & Yang, 1974; Houslay et al., 1976) produced a marked concentration-dependent inhibition of hepatic dimethylnitrosamine metabolism (Fig. 1). In contrast, the oxidation of the three cytochrome P-450-dependent $N$-demethylase substrates ethylmorphine, benzphetamine and aminopyrine was unaffected by benzylamine concentrations up to 250$\mu$M, whereas the activity of aniline 4-hydroxylase was inhibited by benzylamine concentrations greater than 25$\mu$M (Fig. 1). An inhibition of aniline metabolism is to be expected owing to the close structural similarity of the inhibitor and substrate. However, the $I_{50}$ value (dose giving 50% inhibition) of benzylamine...
The activities of dimethylnitrosamine demethylase (○), aniline 4-hydroxylase (●), ethylmorphine N-demethylase (▲), benzphetamine N-demethylase (□) and aminopyrine N-demethylase (■) are expressed as percentages of control activities (no inhibitor) in the presence of 5–250μM-benzylamine.

Inhibition of aniline 4-hydroxylase was calculated to be 450μM, whereas the I_{50} value for dimethylnitrosamine degradation was only 10μM.

Although most of the monoamine oxidase activity of rat liver is located in the mitochondrial fraction (Neff & Yang, 1974), a significant amount of enzyme activity has been detected in the microsomal fraction (De Champlain et al., 1969; Erwin & Deitrich, 1971). In our studies we have found that the monoamine oxidase substrate kynuramine (Weissbach et al., 1960) is oxidized by both rat hepatic postmitochondrial supernatant and washed microsomal fractions. Thus if an amine oxidase enzyme bearing some resemblance to monoamine oxidase is involved in hepatic dimethylnitrosamine degradation, then benzylamine would be expected to inhibit microsomal kynuramine oxidation. Indeed, benzylamine was found to be a potent competitive inhibitor (K_c 60μM) of kynuramine oxidase (Fig. 2). Further, when hepatic 10000g_{av} supernatant fractions were stored as described by Lake et al. (1976a), kynuramine oxidase was found to be essentially stable to storage for at least 10 days. This property is in keeping with those of the enzymes responsible for hepatic dimethylnitrosamine metabolism (Lake et al., 1976a).

In conclusion, the results of the present studies indicate that hepatic dimethylnitrosamine degradation is markedly affected by the monoamine oxidase substrate benzylamine. As a number of cytochrome P-450-dependent oxidations were relatively unaffected, the results suggest that dimethylnitrosamine may be metabolized in part by enzymes unrelated to the mixed-function oxidase complex. The possible involvement of an amine oxidase enzyme in hepatic dimethylnitrosamine degradation is supported.
Fig. 2. Kinetics of the inhibition of kynuramine oxidase in rat hepatic postmitochondrial supernatant fractions

Kynuramine oxidase activity was determined in 0.1 M-phosphate buffer, pH 7.4, over a substrate concentration range of 0.0067–0.083 mM in the presence of (●) 0, (▲) 0.1 and (■) 0.5 mM-benzylamine.

by the observations that benzylamine inhibits hepatic metabolism of both kynuramine and dimethylnitrosamine and by the stability to storage of microsomal dimethylnitrosamine demethylase and monoamine oxidase. Clearly, it would be of interest to examine the interaction of dimethylnitrosamine and other dialkylnitrosamines with hepatic amine oxidase enzymes.