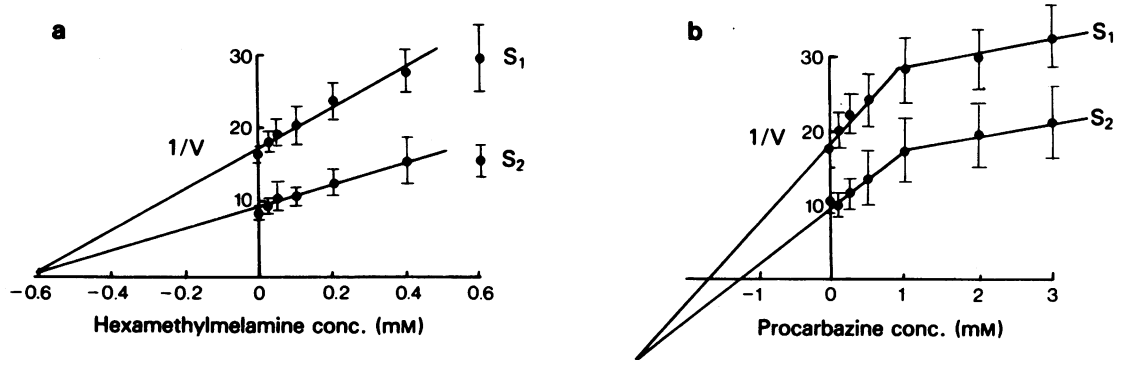

Figure 1 Inhibition of p-nitroanisole O-demethylase by (a) hexamethylmelamine and (b) procarbazine. S1 and S2 represent respectively p-nitroanisole substrate concentrations of 0.03 mM and 0.1 mM. Velocities (V) are given as nmoles of p-nitrophenol formed per 80 mg of liver during a 30 min incubation period. Each point shows the mean of at least four experiments.

of administration of seven antineoplastic agents given in doses which cause inhibition of tumour growth in mice were investigated. The drugs were administered i.p. to CBA lac mice (3 weeks old) as a single dose of 40 mg/kg (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, carmustine, chlorozotocine) or five daily doses of 40 mg/kg (procarbazine, hexamethylmelamine, dacarbazine, p-carbmethoxyphenyldimethyltriazene). Two days later hepatic N-demethylase activity was measured as an index of metabolic drug oxidation using a crude 9000 *g* liver preparation. The experiments indicated that the drugs exert only weak to negligible inhibition of drug metabolism at these dose levels.

In order to investigate interference by the cytotoxic drugs with oxidative drug metabolism at the enzymic site their ability to inhibit p-nitroanisole-O-demethylation *in vitro* was measured. The nature of the inhibition was characterised by plotting the reciprocal of the rate of O-demethylation against inhibitor concentration according to Dixon (1952) (Figure 1). The resulting inhibition profiles showed that high concentrations (>0.1 mM) of most drugs or their metabolites are required to exhibit appreciable inhibition. It is therefore possible that inhibitory drug metabolism interactions involving these drugs are not of major importance in the treatment of malignancies with drug combinations.

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In vitro biotransformation of carbinolamine metabolites of cytotoxic dimethyltriazenes

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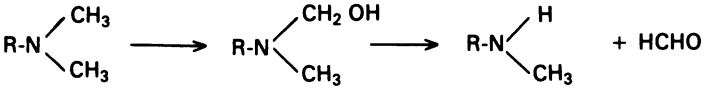
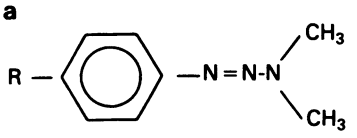
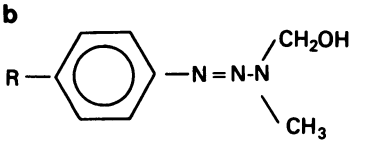
N-demethylation reactions are major metabolic pathways of most tertiary amine xenobiotics with N-dimethyl moieties. The products of the reactions, the secondary amine and formaldehyde, are thought to be formed by the non-enzymatic breakdown of an intermediate carbinolamine (Figure 1). Carbinolamines are in most cases highly transient species and have been rarely isolated because of their chemical instability (Testa & Jenner, 1976).

Antineoplastic dimethyltriazenes (Figure 1*a*) belong to a group of cytotoxic agents for which it has been ascertained that an N-methyl group is crucial for activity (Connors, Goddard, Merai, Ross & Wilman, 1976). Dimethyltriazenes are not cytotoxic *in vitro* and it is generally considered that they are metabolically N-demethylated to form active cytotoxic agents *in vivo* (Connors, Goddard, Merai, Ross & Wilman, 1976). We have recently reported the chemical synthesis of the carbinolamine triazenes (Figure 1*b*),







which may be intermediates of dimethyltriazene N-demethylation, have shown them to be active cytotoxic agents both *in vitro* and *in vivo* (Gescher, Hickman, Simmonds, Stevens & Vaughan, 1978), and we suggested that they may be the species responsible for the selective cytotoxicity of dimethyltriazenes observed *in vivo* (Hickman, 1978). Such a suggestion implies that the triazene carbinolamines have a degree of stability which allows them to leave their site of formation, presumably the liver, before decomposition to the monomethyltriazene and formaldehyde, and to reach distant tumour cells. In order to test this hypothesis we have studied the rate of oxidation by liver aldehyde dehydrogenases of both formaldehyde and a triazene carbinolamine ((Figure 1*b* R = CO_2Me). Whole homogenate equivalent to 50 mg of liver was used from CBA lac mice (3 weeks old) and incubated with 0.1 mM substrate and 1 mM NAD at 37°C. Both the triazene carbinolamine and formaldehyde react in the same way with Nash reagent (Nash, 1953) to give a coloured product, a reaction which allows the quantitation of both agents and so a measurement of their disappearance by oxidation. After 4 min incubation time 70.3 ± 4.9% were left of the formaldehyde, after 8 min only 57.4 ± 3.6%. Of the triazene carbinolamine 84.8 ± 4.7% were unchanged after 4 min and 77.3 ± 3.3% after 8 minutes. Evidently these carbinolamines are not as rapidly oxidised by liver aldehyde dehydrogenases as formaldehyde and so given sufficient chemical stability should be able to reach the site of tumour growth unchanged.

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Monoamine oxidase inhibition by (+)-amphetamine *in vivo*

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In vitro, (+)-amphetamine is a reversible inhibitor of monoamine oxidase (MAO) with a preference for MAO type A (Mantle, Tipton & Garrett, 1976; Miller & Clarke, 1978). Recent studies by Braestrup (1977) and Green & El Hait (1978) suggest that MAO inhibition may occur *in vivo*. The present study was undertaken to examine this possibility further.

Dose-response studies in rats with (+)- and (−)-amphetamine showed the (+)-form to be 4 to 5 times more potent at lowering striatal 3,4-dihydroxyphenylacetic acid (DOPAC). A similar potency ratio was found *in vitro* for MAO type A inhibition in striatal homogenates. Since both isomers were less active but approximately equi-potent as inhibitors of MAO type B, type A MAO inhibition by (+)-amphetamine is suggested *in vivo*. To gain further evidence, experiments were made to determine whether (+)-amphetamine would protect against irreversible MAO inhibition by phenelzine (see Table 1). Under the assay conditions, serotonin was deaminated by MAO type A only. Thus, (+)-amphetamine produced a significant