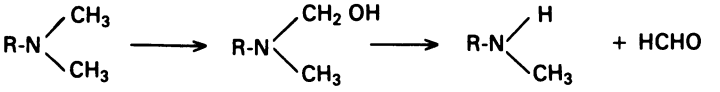

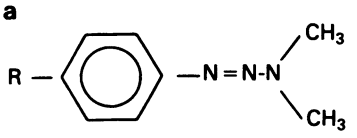

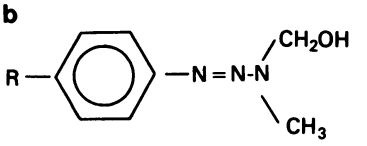

which may be intermediates of dimethyltriazene N-demethylation, have shown them to be active cytotoxic agents both *in vitro* and *in vivo* (Gescher, Hickman, Simmonds, Stevens & Vaughan, 1978), and we suggested that they may be the species responsible for the selective cytotoxicity of dimethyltriazenes 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

Table 1 Effect of (+)-amphetamine (AMPH, 10 mg/kg) on the inhibition of ^{14}C-serotonin deamination by phenelzine (PHEN, 2 mg/kg) in striatum and rest of the brain

*Drug Treatment**	*MAO activity* *n mol deaminated (mg tissue)$^{-1}$h^{-1} ± s.e. mean (n)* *Striatum*	*Rest of the brain*
None	14.23 ± 1.76 (4)	13.84 ± 1.07 (4)
Phenelzine	3.92 ± 0.50 (5)†	4.54 ± 0.29 (5)†
Amphetamine	14.30 ± 1.02 (4)	14.30 ± 0.75 (4)
Amphetamine + Phenelzine	7.17 ± 0.61 (6)‡	7.13 ± 0.40 (6)‡

*(+)-Amphetamine was given s.c. at zero time, phenelzine s.c. at 1 h and the rats were killed at 25 h. Significance of differences: † from None $P < 0.001$; ‡ from Phenelzine, $P < 0.01$ (Student's t-test).

protection against type A MAO inhibition by phenelzine. These data confirm those reported by Green & El Hait (1978) for whole mouse brain. In other experiments, (−)-amphetamine (10 mg/kg) and cocaine (15 mg/kg) failed to protect. Additionally, with phenylethylamine (a type B substrate), (+)-amphetamine failed to protect against phenelzine. Phenelzine itself, inhibited serotonin deamination to a greater extent than phenylethylamine, suggesting preferential MAO type A inhibition.

The same 'protection experiment' was made but the reserpine-like agent Ro4-1284 (2 mg/kg, i.p.) was given 30 min prior to sacrifice and striatal dopamine measured. Phenelzine alone greatly retarded dopamine depletion. (+)-Amphetamine, prior to phenelzine, restored significantly the effect of Ro4-1284.

We conclude that (+)-amphetamine inhibits MAO type A within striatal dopaminergic neurons. Differences in the ability of (+)- and (−)-amphetamine to lower striatal DOPAC may be related, at least in part, to their relative potencies as MAO inhibitors.

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Inhibition of early embryogenic development in mice by α-difluoromethyl ornithine, an enzyme-activated irreversible inhibitor of L-ornithine decarboxylase

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Decarboxylation of L-ornithine by L-ornithine decarboxylase (ODC; E.C. 4.1.1.17) is the initial and, at least in mammals, rate-limiting step in the biosynthesis of the polyamines, putrescine, spermidine and spermine (Williams-Ashman *et al.*, 1972). Although basal activity of ODC is generally low in most tissues, marked increases are characteristically associated with rapid tissue growth (Jänne, Pösö & Raina, 1978), and particularly with mammalian and non-mammalian embryogenesis (Russell & McVicker, 1972; Manen, Hadfield & Russell, 1977). The development in our laboratories of the irreversible inhibitor of ODC, α-difluoromethylornithine (α-DFMO, RMI 71782, Metcalf, Bey, Danzin, Jung, Casara & Vevert, 1978) provided the opportunity to investigate the functional significance of ODC for early embryogenesis in the mouse.

Proven fertile, CDA, HAM-ICR albino mice of 30–45 g initial body weight were mated, and the day following detection of the vaginal plug was designated day 1 of gestation. α-DFMO was included in the drinking water at a concentration of 2% and the 24 h intake by this means was constant at approximately