Activation of the antineoplastic drug isophosphamide by rat liver microsomes

Isophosphamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-1, 2, 3-oxazaphosphorine-2-oxide; NSC 109724; Z-4942 Asta Werke) a new antineoplastic-analogue of cyclophosphamide (2-[bis (2-chloroethyl)amino]tetrahydro-2*H*-1, 3, 2-oxazaphosphorine-2-oxide; Cytoxan; Endoxan) has shown antineoplastic activity in animal tumours (Brock, 1967) including tumors resistant to cyclophosphamide and appears in initial clinical trials to be superior to it in the treatment of small cell carcinoma of the lung in man (Scheef 1971). Cyclophosphamide is inactive *in vitro* and is activated to alkylating materials *in vivo* by the NADPH dependent mixed function oxidases of the liver microsomal fraction (Brock & Hohorst 1963). Isophosphamide is currently in clinical trial in this unit; we have, therefore, investigated the microsomal activation of this drug to compare it with that of its analogue.

Subcellular fractions from the livers of 150g male Sprague-Dawley rats were prepared according to Creaven, Parke & Williams (1965). Both drugs (3 mM final concentration) were each incubated for 15 min in air with shaking with a fortified microsomal suspension (Sladek, 1971). Alkylating activity was assayed essentially as described by Friedman & Boger (1961) and is expressed as μmol of mechlorethamine equivalents (45 μmol of mechlorethamine has an absorbance of 0·40).

Maximal activation of isophosphamide occurs with the microsomal fraction. Activities of other fractions as a percent of that of the microsomal fraction are: 1000 $\times$ *g* supernatant 92%, 10 000 $\times$ *g* supernatant 74%, 105 000 $\times$ *g* supernatant 15%. No activation of either drug occurs in the absence of NADPH or an NADPH generating system. Inhibition of the activation of isophosphamide by other substrates of the microsomal oxidase system is 0 and 4% by 4 and 6 mM aniline, 20 and 30% by 0·5 and 2·5 mM ethylmorphine, and 22% by 3 mM 2,2-diethylaminoethyl-diphenylvalerate (SKF 525-A) respectively. V_{max} for the activation of the two drugs is the same for both (5·4 μmol/g liver per h); K_m is 19·4 mM and 4·0 mM respectively (determined with the aid of a continuous simple linear regression program on a Olivetti Underwood Programma 101; r = 0·98 (isophosphamide) and 0·92 (cyclophosphamide).

Like cyclophosphamide, isophosphamide is activated by a system having the characteristics of the microsomal mixed fuction oxidase system and is inhibited by substrates which show type I binding to microsomal haemoprotein. The lower affinity of isophosphamide for the activating enzyme is correlated with a higher toxic dose of it compared with cyclophosphamide in initial clinical trials (Scheef 1971).

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Nuclear magnetic resonance studies of interactions between oxyethylene-oxypropylene polymer, macrogol and phenol

The interactions occurring between phenol and cetomacrogol have been examined using nuclear magnetic resonance (nmr) spectroscopy. The changes of chemical shift of the various protons have been interpreted in terms of the changes of environment which occur during solubilization (Jacobs, Anderson & Watson, 1971b). The investigation has been extended to include the interactions of phenol with an oxyethylene polymer macrogol ("Carbowax" 4000, Union Carbide) and an oxyethylene-oxypropylene block polymer POEPOP ("Pluronic F68", Wyandotte Chemicals). The phenol used was reagent grade; water was freshly distilled from an all-glass still. Spectra were obtained and treated as described previously (Jacobs, Anderson & Watson, 1971b).

Macrogol. Aqueous systems containing phenol and macrogol are clear if the ratio of macrogol to phenol is sufficiently high, but are turbid if this ratio is low. The turbidity is due to the separation of a phenol–macrogol-rich phase. The two phases occurring in the system containing 3% phenol and 6% macrogol were separated and the composition of the phenol–macrogol-rich phase determined from the integrated nmr spectrum; the molar ratio of phenol : ethylene oxide units : water was about 3 : 8 : 10·5.

The nmr signals from the phenol ring protons shift upfield with increasing macrogol concentration, but the magnitude of these shifts are small compared to those previously observed in corresponding cetomacrogol–phenol and sodium dodecyl sulphate–phenol systems (Table 1). The smaller shift is consistent with the absence of micelles from solutions of macrogol in water, so that most of the phenol molecules remain in an essentially aqueous environment; the small upfield shift would then be explained as interaction of some fraction of the phenol molecules with the macrogol chains.

Addition of phenol to aqueous macrogol also causes the signal from the macrogol

Table 1. *Changes of chemical shift of ring protons of phenol (2%) in the presence of other agents with respect to signals of 2% phenol in water (all systems clear).*

Concentration of agent		Upfield shift (Hz)
Macrogol	10%	0·5 (*ortho*, *meta* and *para* protons)
	15%	0·8 (*ortho*, *meta* and *para* protons)
	20%	1·0 (*ortho*, *meta* and *para* protons)
Cetomacrogol[1]	10%	3·7 (*ortho* protons)
		8·0 (*meta* and *para* protons)
Sodium dodecyl sulphate[2]	10%	3·3 (*ortho* protons)
		7·4 (*para* protons)
		9·0 (*meta* protons)
	20%	3·5 (*ortho* protons)
		7·6 (*para* protons)
		9·2 (*meta* protons)

[1] From Jacobs & others (1971b).
[2] From Jacobs & others (1971a).