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PRELIMINARY COMMUNICATION

CHEMICAL REACTIVITY, METABOLIC OXIRANE FORMATION AND BIOLOGICAL REACTIVITY OF CHLORINATED ETHYLENES IN THE ISOLATED PERFUSED RAT LIVER PREPARATION

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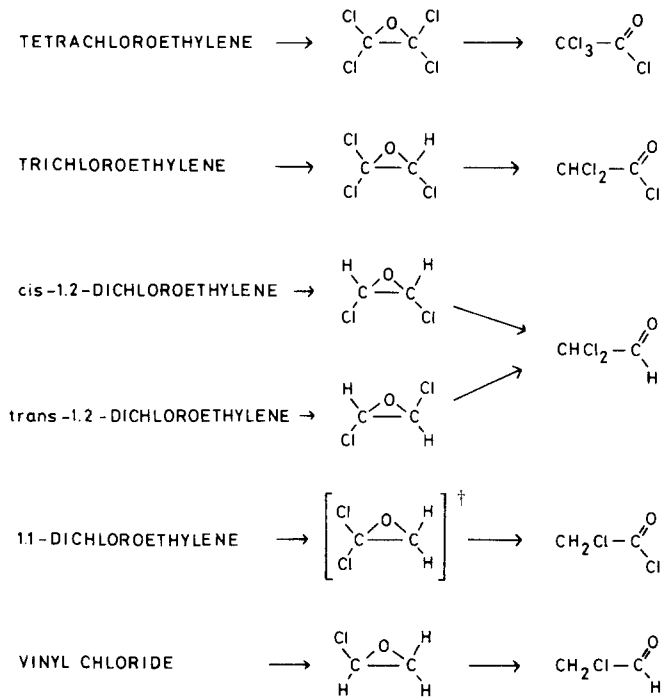
In olefinic compounds an increase in substitution with chlorine atoms causes enhanced thermal and chemical stability. Some stable unsaturated chlorocarbons have been described for which the corresponding hydrocarbons without chlorine are only short-lived or were unknown till recently (1-3). This stability rule applies also to the chlorinated ethylenes and we became interested in the relationship between their chemical reactivity and their metabolic behaviour in biological systems. We therefore studied the uptake and metabolism of all six of the chlorinated ethylenes (ClE) in relation to their acute liver toxicity.

Livers of female Wistar rats (170-230 g) were placed in the apparatus described by Miller and Hems (4,5). The chlorinated ethylenes were added as vapors to the carbogen - a CO_2 (5%) and O_2 (95%) mixture used for oxygenating blood in the perfusion - at constant rates which allowed for steady state conditions of substrate uptake and conversion. Samples (1 ml) of the perfusate before and after passage through the liver and of liver tissue homogenized in n-hexane at the end of the experiment were analysed for metabolites and liver enzyme activities. The perfusate or homogenate (0.5 ml) was shaken with 1 ml of either n-hexane, n-heptane, cyclohexane, or benzene for direct GC analyses of the solvent(s). The following metabolites were determined as described previously: trichloroacetic acid (6), dichloroacetic acid (7), chloral (8), glucuronides (8,9), free alcohols as the difference between total and glucuronidated forms; gaseous chlorinated ethylenes (8) in the carbogen.

Chlorinated oxiranes are the expected intermediates of the oxidative metabolism of chlorinated ethylenes (10-13), and we have synthesized most of them by chlorine catalysed photo-oxidation (14,15). They are fairly unstable (e.g.the half-life of trichloroethylene oxirane in nonpolar solvents at 60° C is 25 min (14)) and rearrange thermally with chlorine migration forming acyl chlorides and

di- and mono-chloroacetaldehyde (16,17), thus:



Only some of the above products of oxirane rearrangements are, however, encountered as metabolites in vivo. The results of our studies with the isolated perfused liver are as follows:

Tetrachloroethylene (fig.1a). Prehepatic perfusate saturation is reached after 30 min. Unlike other chlorinated ethylenes, the posthepatic concentration approaches, after 60 min, the prehepatic level and in some experiments an intersection of the curves was observed.

Trichloroacetic acid was found to be the only metabolite, 10-15% ‡ being

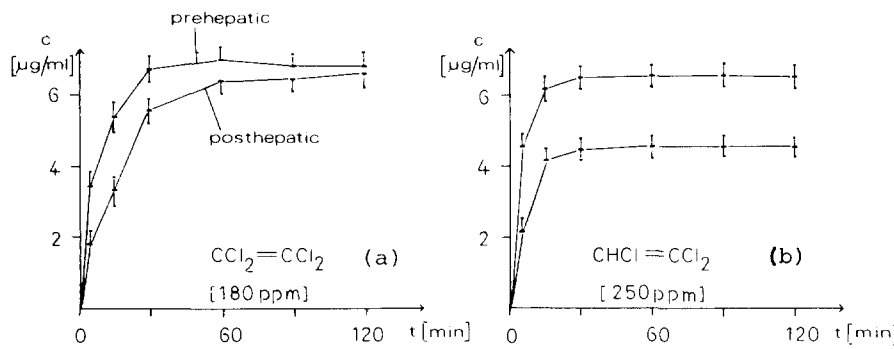
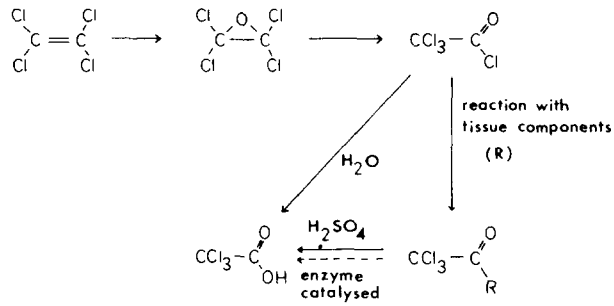


Fig.1. Pre- and post-hepatic concentrations of tetra(a)- and trichloroethylene (b) in the isolated rat liver preparation.

† Attempts to prepare and isolate this compound were unsuccessful.

‡ All values are given as percent of the total uptake by liver tissue.

in the circulating perfusate, and 3-5% bound to liver tissue and extractable only after acid hydrolysis (20% sulfuric acid at 80° C for 6 hr). This finding is interpreted on the basis of the following mechanism:



The acyl chloride is in part hydrolyzed directly to TCA, but some reacts with functional groups (e.g.OH,SH,NH_2). This (new) type of covalent binding probably undergoes hydrolytic, may be enzyme catalysed, cleavage and is presently under investigation. A similar mechanism has recently been discussed in connection with halothane metabolism (18). This acylation process may interfere with essential biological structures or biochemical mechanisms and may be one reason for the intersection of the pre- and post-hepatic concentration curves in fig.1a.

Trichloroethylene (Tri). The pattern of steady state uptake and conversion which is representative of all other ClEs (di- and mono-) is given in fig.1b. The proportion of uptake is independent of the Tri concentrations used (2-8 µg/ml). The metabolites of trichloroethylene identified were chloral, trichloroacetic acid und trichloroethanol. In vitro, trichloroethylene oxirane undergoes a rapid and quantitative conversion to dichloroacetyl chloride (14). In H_2O and in methanol, the oxirane forms respectively, dichloroacetic acid and its methyl

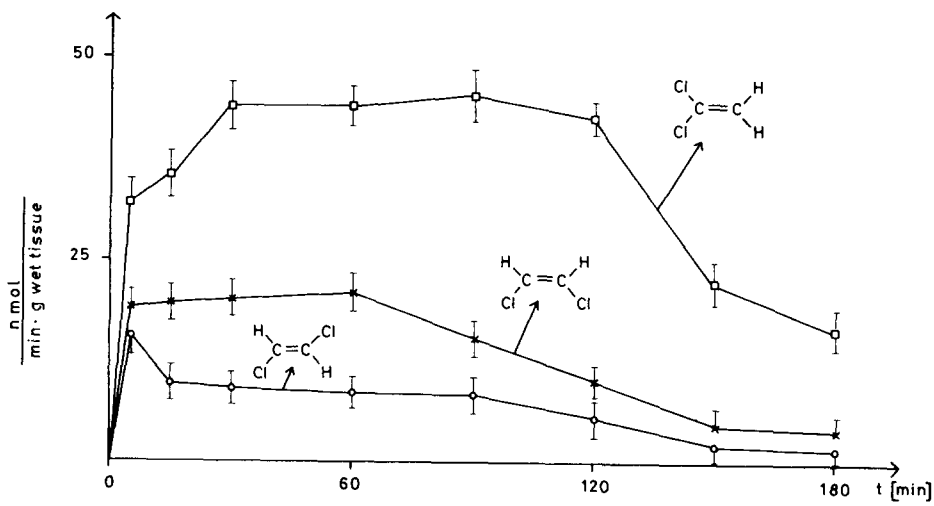
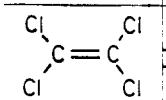
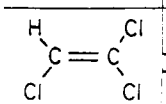
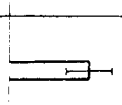
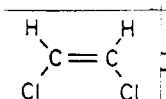
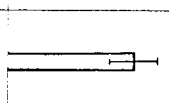
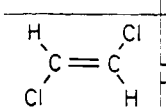
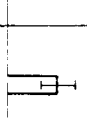
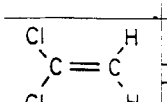
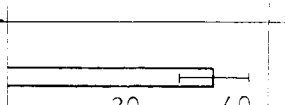


Fig.2. Uptake of dichloroethylenes under identical prehepatic concentrations (55.0 ± 2.5 nmol/ml).

Table 1. Comparative metabolism of chlorinated ethylenes in the isolated perfused rat liver under equimolar substrate concentrations (55.0 ± 2.5 nmol/ml). Each value represents the mean of 3 to 5 experiments.

Compound (conc.gas-phase ppm)	Uptake[a] (nmol/ml)	Solubility[b] in liver tissue (%)	Metabolites[b] (%)	Enzyme activities (perfusate) lactate/pyruvate			GPT (mU/ml·g liver^{-1})				GOT (mU/ml·g liver^{-1})			
				0'	60'	180'	0'	60'	120'	180'	0'	60'	120'	180'
		41 ± 5	CCl_3COOH (perfusate) 10-15	7.3 ±	7.4 ±	7.9 ±	1.9 ±	2.2 ±	2.2 ±	2.4 ±	1.8 ±	1.9 ±	2.2 ±	2.2 ±
			CCl_3COOH (bound in tissue) 3-5	2.2	2.2	2.4	0.6	0.8	0.8	0.8	0.6	0.8	0.8	0.8
		4 ± 1	CCl_3CHO 2-4 CCl_3COOH 15-20	7.9 ±	7.9 ±	8.0 ±	2.0 ±	2.1 ±	2.1 ±	2.1 ±	1.9 ±	1.9 ±	2.2 ±	2.3 ±
			CCl_3CH_2OH[c] 65-75	2.5	2.5	2.5	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9
		3 ± 1	$CHCl_2COOH$ 1-3 $CHCl_2CH_2OH$ 8-10	8.0 ±	7.1 ±	17.4 ±	1.8 ±	2.0 ±	7.7 ±	23.0 ±	1.8 ±	2.0 ±	5.2 ±	14.0 ±
				2.5	2.2	4.0	0.8	0.8	3.0	4.5	0.8	0.8	2.0	3.5
		6 ± 2	$CHCl_2COOH$ $CHCl_2CH_2OH$ 0.5-1	7.4 ±	6.7 ±	12.3 ±	1.4 ±	1.5 ±	1.5 ±	3.6 ±	1.9 ±	2.4 ±	3.1 ±	5.4 ±
				2.2	2.1	3.0	0.4	0.6	0.6	1.2	0.8	0.8	1.0	1.4
		1 ± 0.5	d)	7.4 ±	6.6 ±	11.7 ±	1.2 ±	1.4 ±	1.4 ±	4.0 ±	1.2 ±	1.4 ±	2.3 ±	4.6 ±
				2.3	2.2	2.9	0.3	0.3	0.3	0.8	0.3	0.3	0.8	1.0

a) uptake (at 60 min), conditions: perfusate flow 1.0 ± 0.05 ml/min·g liver^{-1}.

b) all values as percent of total uptake.

c) total = free (5%) and conjugated (95%) forms.

d) not determined.

ester (19). Opening of the oxirane-ring to chloral only occurs in the presence of Lewis acids (20). In vivo, however, the formation of dichloroacetyl chloride does not occur, since we were unable to demonstrate detectable (GC-MS) formation of dichloroacetic acid. In fact, chloral is the only observed primary intermediate. It originates from the oxirane by an unknown interaction with the biological environment which prevents the "chemical" chlorine migration and ring-opening to dichloroacetyl chloride.

Dichloroethylenes (DCE). All the DCEs in prehepatic perfusate concentrations identical to those used with Tetra and Tri (fig.2) cause damage to the liver : After 60 min with cis-1,2-DCE, 90 with trans-1,2-DCE, and 120 min with 1,1-DCE, the drop in the conversion rate is accompanied by a concomitant rise in the activities of certain liver enzymes (see table 1). The initial decrease of trans-1,2-DCE-uptake is due to an inhibition of mixed function oxidases (21). The amount of metabolites originating from the oxirane rearrangement does not correlate to the rate of uptake (table 1). This points to widely varying proportions of other metabolic pathways (e.g. enzymatic cleavage of the oxirane rings, peroxidation) in this series of chlorinated ethylenes.

A rough correlation exists between the number of chlorine substituents and the rate of conversion, the order being Per> Tri> cis-1,2-DCE> 1,1-DCE (except trans-1,2-DCE, see above) thus supporting the rule of chemical reactivity as outlined earlier. The reverse is true for solubility in liver tissue (see table 1). Limitations to the practical significance of this rule result from the increasing vapor pressure of the chlorinated ethylenes. Thus, to obtain 55.0 nmol/ml in the perfusate requires 1800 ppm of 1,1-DCE, but 3000 ppm of vinyl chloride. These conditions render metabolic studies with vinyl chloride more difficult, and relevant experiments with this compound will therefore be reported in a separate paper.

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