Metabolic Activation of Halothane and Its Covalent Binding to Liver Endoplasmic Proteins in vitro

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Summary. 1. In suspensions of rabbit liver microsomes reduced by dithionite or NADPH, halothane produces a difference absorption spectrum with a maximum at 473 nm and a minimum at 408 nm. The “optical affinity” ($K_a$) was in the region of $3 - 6 \times 10^{-6}$ M for dithionite reduced microsomes. The maximal absorption with dithionite reduced microsomes for halothane (473–550 nm) was 0.017–0.019 and for $\text{CCl}_4$ (454–500 nm) 0.04–0.05 per nmol of cytochrome P-450. The appearance of the difference absorption with halothane is faster than that with $\text{CCl}_4$.

2. During anaerobic incubation with NADPH-reduced liver microsomes from phenobarbital pretreated rabbits, $^{14}$C-labelled halothane (1 mM) was covalently bound to microsomal proteins at a rate of 2 nmol/mg protein in 30 min (CCl$_4$: 11 nmol/mg protein in 30 min). Reduction by dithionite was ineffective. The binding of halothane was 60% inhibited in a gas phase of air, 75% by CO, 55% in the presence of 1 mM metyrapone, 50% by $\text{CCl}_4$, but only 20% by red. glutathione. The binding of the radioactivity from labelled halothane and $\text{CCl}_4$ to proteins of isolated rabbit lung and kidney microsomes was approximately proportional to the concentrations of cytochrome P-450 in the organ fractions.

3. Like $\text{CCl}_4$, halothane (1 mM) inhibited several microsomal drug oxidation reactions.

4. Irreversible binding of halothane or its metabolite(s) to endoplasmic proteins might be connected with halothane liver damage.

Key words: Halothane — Carbon Tetrachloride — Microsomal Metabolism — Cytochrome P-450 — Covalent Protein Binding.

There is no firm evidence linking the administration of halothane to hepatic necrosis, but the data on repeated halothane anaesthesia imply an increased hazard. Relatively rare occasions of liver damage resulting from halothane anesthesia have been attributed to a direct hepatotoxic action, to a facilitation of virus hepatitis or the development of hypersensitivity after repeated halothane exposure. In the latter case, halothane or a metabolite of it must be conjugated to liver macromolecules. The growing literature in this field has recently been reviewed (Carney and Van Dyke, 1972; Cohen, 1971; Dykes et al., 1972; Van Dyke, 1972).

Halothane was originally introduced as a biologically stable, halogenated anaesthetic. Nevertheless, after halothane narcosis Stier (1964 a and b)
reported on the urinary excretion of bromide in rats and man and of trifluoroacetic acid in rabbits. Van Dyke et al. (1964) and Van Dyke and Chenoweth (1965) observed inorganic chloride and CO₂ as products of halothane metabolism in vivo and in vitro. This metabolic route is catalyzed in the liver microsomal fraction.

It was stated (Stier, 1968; Van Dyke, 1972) that the known products of halothane metabolism are not toxic, at least at the concentrations reached in the body. But, if highly reactive, transient intermediates in the metabolic degradation of halothane are formed, any acceleration of drug metabolism by enzyme induction or by individual factors might result in an increased toxicity. This has been shown to be so for CCl₄ (Garner and McLean, 1969; Reiner et al., 1972) and for halothane (Van Dyke, 1966; Stenger and Johnson, 1972).

New perspectives in the metabolism of halogenated alkanes were opened by the observation of haloalkane binding to reduced microsomal cytochrome P-450, producing a difference absorption with the peak at 454 nm (Reiner and Uehleke, 1971). Halothane effects a change of difference light absorption in suspensions of liver microsomes with a peak at 473 nm.

During dehalogenation of CCl₄, CHCl₃ and others at the microsomal cytochrome P-450 reduced with NADPH (Reiner et al., 1972) these haloalkanes are firmly bound to microsomal proteins and lipids (Uehleke et al., 1973). After intravenous injection of halothane-2-¹⁴C into the mouse Cohen (1969) has observed an accumulation of labelled nonvolatile metabolites in the liver. We have, therefore, compared the binding of halothane to microsomal cytochrome P-450 and the covalent binding of ¹⁴C-labelled halothane to liver microsomal proteins under various experimental conditions. The binding of halothane to kidney and lung microsomes was also investigated.

**Materials and Methods**

The labelled halothane (1,1,1-trifluoro-2-bromo-2-chloroethane-1-C¹⁴, with a specific activity of 2 mCi/mmol) and ¹⁴CCl₄ (7 mCi/mmol) were purchased from New England Nuclear, Boston. They were diluted with unlabelled halothane or CCl₄ to obtain a final activity of 1 μCi/μmol. The radiochemical purity of halothane was given as about 99%, the only demonstrated impurity was CF₃CH₂Br (less than 1%).

Liver microsomes of rabbits (Thüringer Gemsen, Institute bred) were prepared as described by Uehleke et al. (1970). The animals were pretreated with 0.1% phenobarbital sodium in the drinking water. The isolation of rabbit kidney microsomal fraction was described by Uehleke and Greim (1968) and lung microsome were prepared according to Uehleke (1969). The microsomal fractions were resuspended and sedimented by centrifugation twice.

The irreversible binding of halothane was followed in incubation mixtures which contained per ml: 2 mg microsomal protein, 8 μmol glucose-6-phosphate,