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Isolation, characterization and quantitation of chloral hydrate as a transient metabolite of trichloroethylene in man using electron capture gas chromatography and mass fragmentography

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A method for the quantitative determination of plasma concentrations of chloral hydrate by electron capture gas chromatography is described. Characterization by multiple ion monitoring confirms, for the first time, that chloral hydrate exists as a transient metabolite of trichloroethylene in man.

The major metabolites of trichloroethylene in man are trichloroacetic acid, trichloroethanol and its corresponding glucuronide (Powell, 1945; Soucek & Vlachova, 1954). Butler (1948, 1949) isolated these same compounds from the urine of dogs following the administration of chloral hydrate, and postulated that this latter compound might be an intermediate in trichloroethylene metabolism (Fig. 1); he could not confirm this hypothesis. The conversion of trichloroethylene to a non-volatile polyhalogenated compound identical to chloral hydrate was shown to take place *in vitro* by incubation with rat liver microsomes, the reaction requiring NADPH and oxygen (Leibman, 1965; Byington & Leibman, 1965). This result has recently been confirmed by Ikeda & Imamura (1973). Concurrent with the present research Kimmerle & Eben (1973a,b) have detected chloral hydrate *in vivo* as a metabolite of trichloroethylene following repeated exposures of rats to concentrations between 50 and 3160 ppm of the anaesthetic. However, they failed to find chloral hydrate in the plasma of humans following repeated exposure to trichloroethylene at the 50 ppm level. The paper presents the isolation, confirmation and quantitation of chloral hydrate in the plasma of human subjects following trichloroethylene anaesthesia.

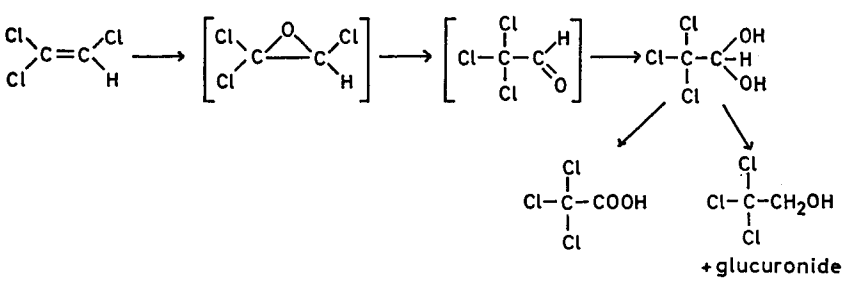

FIG. 1. Metabolic pathway of trichloroethylene.

MATERIALS AND METHODS

Analar diethyl ether (BDH Ltd., Poole, Dorset) was redistilled before use. Dimethyldichlorosilane, chloral hydrate, chlorbutol were obtained from BDH Ltd. Free fatty acid phase (FFAP), Carbowax 20M and Poropak Q-S were obtained from Phase Separations, Flintshire.

Gas-liquid chromatography

Gas-liquid chromatography (g.l.c.) was carried out using a Pye series 104, Model 84 instrument equipped with a ^{63}Ni-electron capture detector operated in the pulsed mode. Three g.l.c. systems were used for analytical purposes. System A:—A coiled glass column (275 cm long × 0·4 cm i.d.) was packed with 20% Carbowax 20M coated onto acid-washed, silanized Diatomite C mesh 100–120 (Pye Unicam, Cambridge). The oven temperature was 150° and the nitrogen flow rate was 40 ml min^{-1}. System B:—A coiled glass column (275 cm long × 0·4 cm i.d.) was packed with 15% FFAP coated onto acid-washed silanized Diatomite C mesh 100–120. The oven temperature was 150° and the nitrogen flow rate was 40 ml min^{-1}. System C:—A coiled glass column (214 cm long × 0·4 cm i.d.) was packed with Poropak Q-S mesh 100–120. The oven temperature was 245° and the nitrogen flow rate 25 ml min^{-1}.

Gas chromatography—mass spectrometry

Gas chromatography–mass spectrometry (g.c.–m.s.) studies were carried out using a Perkin-Elmer 270 combined gas chromatograph–mass spectrometer (Perkin–Elmer, Beaconsfield, Bucks), operated with an electron energy of 70 eV and an accelerating voltage of 2 kV. Authentic standards (10 μg) were injected into a coiled glass column (183 cm long × 0·4 cm i.d.) packed with 20% Carbowax 20M coated onto acid-washed, silanized Diatomite C mesh 100–120. The helium carrier gas flow rate was 10 ml min^{-1} and the column and injector temperatures were maintained at 135° and 175° respectively.

Gas chromatography–mass fragmentography (g.c.–m.f.)

Mass fragmentography studies were performed using a Vg Micromass 12B combined gas chromatography–mass spectrometer. Separations were made using the column in system B under g.l.c. The flow rate of helium carrier gas was 30 ml min^{-1} and the column maintained at 150°. A four-channel peak selector with sample hold unit was used to record continuously the intensity of the ions at *m/e* 111, 113, 146 and 148, generated by the mass spectrometer.

Extraction of plasma

Heparinized blood (1 ml) was placed in a stoppered tube together with 5 μl of internal standard chlorbutol (stock ethanol solution of 0·54 mg ml^{-1}) and throughly mixed using a vortex stirrer. The mixture was shaken with ether (10 ml), of which 1 μl was injected on column linked to the ECD. For g.c.–m.f. studies, heparinized blood (20 ml) was taken from 15 patients approximately 10 min after being anaesthetized with trichloroethylene. The blood was exhaustively extracted with redistilled ether, and dried over anhydrous sodium sulphate. Ether extracts ($\sim$ 2 litres) were

pooled and concentrated to small volume, affording 0·5 g of extractable material. A control experiment utilizing whole blood (400 ml) to which chloral hydrate (20 μg) had been added, afforded a similar ether extract.

RESULTS AND DISCUSSION

Trichloroethylene was administered to patients undergoing surgery. Samples of venous blood were drawn into 2 ml heparinized syringes allowing duplication of results. Chlorbutol was added as an internal standard, and each sample extracted with ether. The ether solution (1 μl) was injected into a Carbowax 20M column operated as in System A. Fig. 2 represents the typical electron capture gas chromatographic elution pattern obtained. The chlorbutol has a retention time intermediate

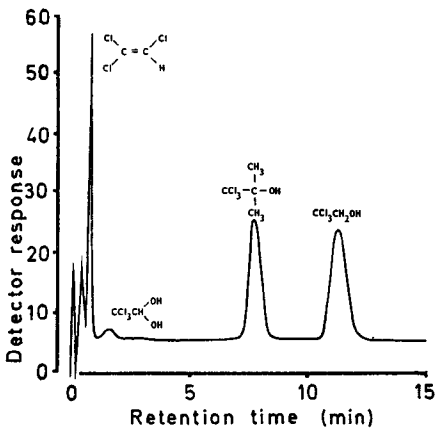

FIG. 2. Electron capture gas chromatographic elution pattern of chlorinated compounds in human blood. Chlorbutol (2·7 μg) was added to heparinized blood (1 ml), and the mixture extracted with ether (10 ml), of which 1 μl was injected into the gas chromatograph.

between that of trichloroethylene and that of the metabolite trichloroethanol. Trichloroethanol has been observed in the plasma of patients within 1 min of administration of the anaesthetic (Cole & Parkhouse, unpublished observations). After approximately 5 min of anaesthesia, a small peak was observed with a retention time slightly longer than that of trichloroethylene. Injection of authentic chloral hydrate and chloral gave peaks with identical retention data to that of the unknown compound in Fig. 2. Corroborative evidence was sought to identify the latter using other g.l.c. systems. The retention times (relative to chlorbutol = 1) of chloral hydrate using g.l.c. systems A, B and C, were 0·20, 0·24 and 0·22 respectively. Similar results were obtained for chloral and the unknown material. System C is only recommended for qualitative work. Using System B and chlorbutol as an internal standard, a plot of the chloral hydrate/chlorbutol peak height ratio versus chloral hydrate concentration gave a linear detector response for chloral hydrate from 150–1000 pg injected. This graph was used for the quantitative estimation of plasma concentrations of chloral hydrate.

Following induction of anaesthesia and administration of trichloroethylene blood samples were taken at 5 min intervals. After return to consciousness samples were taken at 30 min intervals or whenever convenient. The samples were extracted, as described in the experimental section and assayed by gas chromatography. Fig. 3

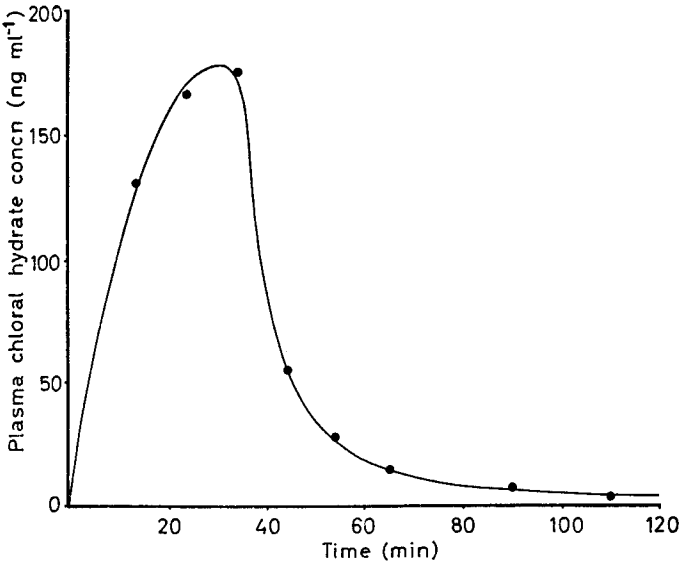

FIG. 3. Rapid decay curve of the plasma concentration of chloral hydrate of a patient who had received trichloroethylene (1%) for 10 min.

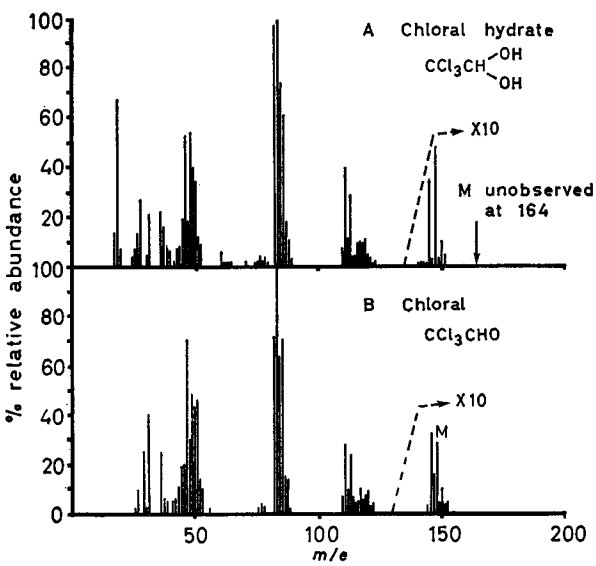

FIG. 4. Normalized mass spectra of chloral hydrate (top) and chloral (bottom).

represents the plasma concentrations of chloral hydrate measured in a patient receiving short duration (10 min) trichloroethylene administration. The rapid decay of plasma levels of chloral hydrate is in agreement with the work of Marshall & Owens (1954) and Kaplan, Forney & others (1967) where plasma levels could not be detected, in some cases, 5 minutes after administration of one gram of the pure hypnotic. Supporting evidence for the existence of the trichloro-metabolite was obtained by g.c.–m.f. Fig. 4 represents the normalized spectra (g.c.–m.s.) of chloral hydrate (top)

and chloral (bottom). A molecular ion (*m/e* 164) was not observed for chloral hydrate; the compound underwent dehydration to give a mass spectrum substantially similar to that of chloral, (molecular ions at *m/e* 146, 148, 150 and 152). This is to be expected since chloral hydrate undergoes decomposition to chloral when heated at 98°, and all g.c.–m.s. conditions used were in excess of this temperature. One of the main fragmentations of chloral is the loss of a chlorine atom giving rise to ions at *m/e* 111, 113 and 115. Mass fragmentography of authentic chloral hydrate and plasma extracts was carried out by continuously recording the prominent ions at *m/e* 111, 113, 146 and 148. Peaks representative of these chloral type ions were obtained at precisely the same retention time (3¼ min) after injection of (*a*) a standard solution of chloral hydrate, (*b*) an extract of plasma to which chloral hydrate had been added, and (*c*) a plasma extract of patients who had undergone trichloroethylene anaesthesia. In all these cases similar peak height ratio were obtained for the ions investigated. These results confirm the presence of chloral within the mass spectrometer. However, since chloral reacts avidly with water it must exist under biological conditions as chloral hydrate. These facts together with the electron capture results confirm for the first time that chloral hydrate exists as a transient metabolite of trichloroethylene in man.

Acknowledgements

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