The Chemistry and Toxicology of Dichlorohexafluorobutene

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A study of the chemistry, uptake and distribution, and toxicology of dichlorohexafluorobutene (DCHFB) was undertaken in the experimental animal with additional studies of uptake being carried out in man. DCHFB is a trace contaminant in halothane manufactured by high thermal synthesis and can develop significant increases in concentration during the clinical administration of halothane. Toxicity studies in the rat, dog, and monkey show variable pathologic change with primary involvement of the central nervous system, lungs, and liver. In the former species the LD_{50} is only five to ten fold that concentration of DCHFB vaporized during the clinical administration of halothane. Studies in man demonstrate alveolar uptake and indicate its presumed metabolism. Studies in the monkey incriminate the liver as a site of metabolism. Recommendations for its removal are made.

IN 1945 HENNE [1] first described the synthesis of 2,3-dichloro-1,1,1,4,4,4-hexafluorobutene-2 through directed chlorination of 1,1,1-trifluorobutene-2 followed by treatment with alkali and subsequent fluorination. Additional reports appeared in the chemical literature,[2-5] and a brief toxicological study was carried out by Lu *et al.*,[6] but little interest was evidenced in the compound until its isolation as an impurity of anesthetic halothane.[7] In this preliminary investigation it was noted that the concentration of the impurity tended to increase under conditions of clinical use and that toxicity was a possible important consideration. The following study represents further investigations of the chemistry and toxicology of dichlorohexafluorobutene (DCHFB).

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Chemistry

Physical properties assigned to the cis and trans isomers of dichlorohexafluorobutene include:

Boiling point: trans 66.2° C. cis 67.9° C.[5]
Vapor pressure (25° C.): trans 157 mm. of mercury, cis 146 mm. of mercury [5]
n_D^{20}: trans 1.3477, cis 1.3471 [5]
Specific gravity (25° C.): 1.61 *
Water solubility (25° C.): 4×10^{-4} μg per cent *

Additional vapor pressure data have been experimentally determined with a mixture containing both isomers. These data for DCHFB may be compared with similarly observed vapor pressure data for halothane (fig. 1). The mass spectrum obtained for dichlorohexafluorobutene (DCHFB) is presented in figure 2.

Process of Manufacture. The presence of DCHFB as a contaminant of halothane is a consequence of manufacturing processes utilized in this country and in England. Under U. S. Patents 2-849-502 and 2-921-098, halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) is synthesized by high thermal reactions (400°–500° C.) either with exchange halogenation of 1,1,1-trifluoro-2-chloro-2,2-dibromethane and 1,1,1-trifluoro-2-chloroethane, or by direct bromination of 1,1,1-trifluoro-2-chloroethane. Both processes result in the presence of traces of cis- and trans-DCHFB as well as several other impurities. During the past year a number of gas chromatographic analyses of anesthetic halothane manufactured by Imperial Chemical Industries Limited and by Ayerst Laboratories have revealed the constant presence of at least five impurities (fig. 3). The major impurity represents trans-DCHFB, although the cis form is also consistently present in a ratio of 1:8.

* Unpublished experimental observations, this laboratory.

An alternative low thermal reaction for the synthesis of halothane has been described under U. S. Patent 2-959-624 whereby trifluorochlorethylene is reacted with hydrogen bromide and the product rearranged with aluminium chloride at 90° C., or by irradiation with ultraviolet light. In an examination of 9 halothane samples prepared by similar synthesis, no DCHFB has been found.†

Concentration of DCHFB in Halothane. It has previously been reported from this laboratory[8] that the average concentration of DCHFB in stock halothane is approximately 0.01 per cent and that one examined sample from a vaporizer in the operating room contained 0.1 per cent. Repeated gas chromatographic analyses, totalling over 50 freshly opened bottles of stock halothane manufactured in this country and in England, yielded a mean concentration for combined cis- and trans-DCHFB of 0.018 per cent (v./v.). Widest variations appeared (0.008 to 0.03 per cent) in the samples from England. These determinations were made with a flame ionization detector under conditions where the relative responsiveness of the detector for halothane was 0.741, with that of DCHFB taken as unity. It should be pointed out that for accurate quantitative analysis one must stay within the linear response range of the flame detector, otherwise concentrated solutions of halothane or DCHFB may give a false low integrated peak area response.[9] In an analysis of 83 specimens of halothane taken from operating room vaporizers under a variety of conditions, the mean concentration of DCHFB was 0.03 per cent. This exceeded by 0.012 per cent that average concentration of DCHFB measured in freshly opened stock halothane

† Halothane samples supplied by M. Hudlický, Research Institute for Pharmacy and Biochemistry, Prague, Czechoslovakia, by Farbwerke Hoechst Ag, Frankfurt, Germany, and by Dr. Eric Nilsson, Lund, Sweden.

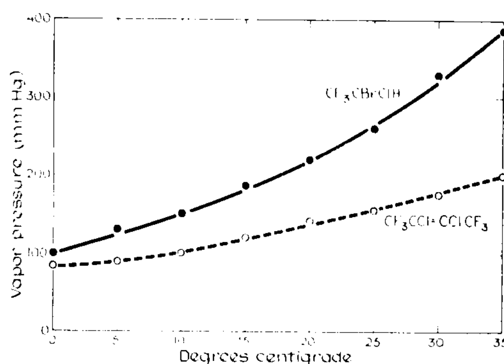

FIG. 1. Vapor pressure data determined with a mixture containing approximately equal parts of cis- and trans-DCHFB. Compare with vapor pressure data observed for halothane.

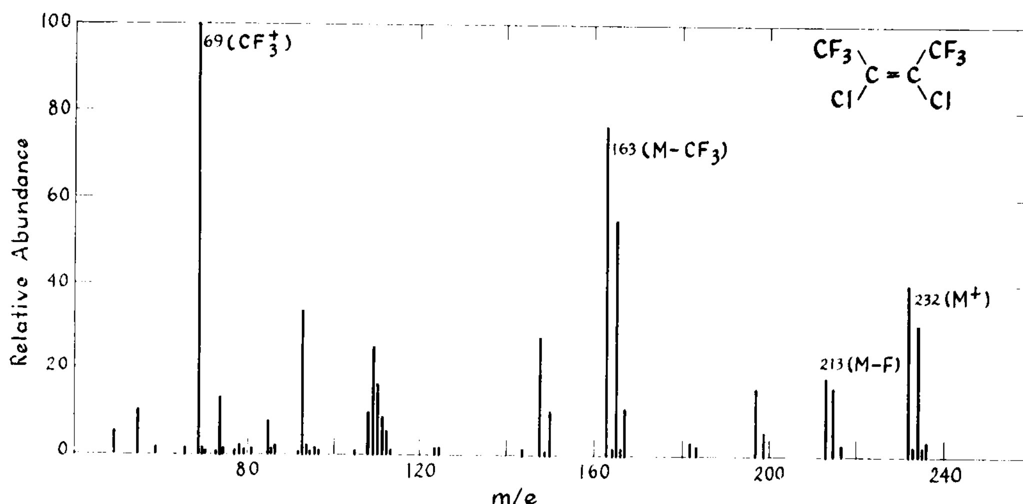

FIG. 2. Mass spectrum for DCHFB obtained with a Consolidated Electrodynamic Corporation Mass Spectrometer 21-103 C, using an all glass inlet system (200° C.). Ionizing energy 70 eV; ionizing current 50 μA. Mass unit (m./e.) is plotted versus relative abundance. (By permission of author.[8])

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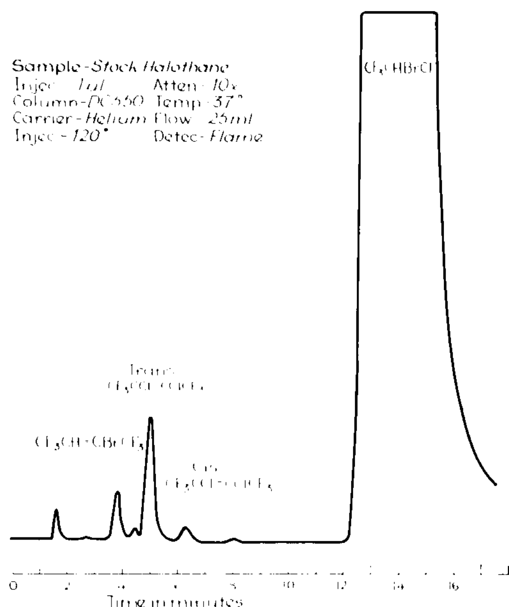

FIG. 3. Chromatogram obtained from the injection of 1 μl. of stock halothane (Ayerst Laboratories, lot D7892HF). Note separation of the three "butene" impurities.

(0.018 per cent). Occasionally, samples were found to contain as much as 0.05 to 0.07 per cent, as well as a single previously reported sample containing 0.1 per cent. These concentrations of DCHFB, observed under conditions of clinical use, are similar to those reported by Sexton and Hendrickson who analyzed halothane samples from 100 vaporizers and found a mean concentration of DCHFB of 0.029 per cent with one examined sample containing 0.058 per cent.[10]

A series of experiments have been performed to define conditions which could account for this increase in DCHFB content. Preliminary studies suggested the importance of two factors: *i.e.*, a concentration effect through selective evaporation of halothane (boiling point of halothane = 50° C.; boiling point of dichlorohexafluorobutene = 67° C.), and a second concentration effect resulting from the reaction of halothane with copper and oxygen to form DCHFB.[8]

Role of Evaporation. Slow evaporation of stock halothane at room temperature, rapid evaporation of halothane at high temperature (50° C.), distillation, and fractionation studies all produced a rapidly increasing concentration of DCHFB in the residual volume. Figure 4 details a representative experiment where 100 ml. of halothane were distilled through an all glass, water cooled distillation apparatus and each subsequent 10-ml. aliquot was analyzed for DCHFB content. It will be noted that with 90 per cent evaporation there is a radical enrichment in the residual 10 per cent volume, but little or no concentration effect is observed in the residual volume with evaporation of the initial 50 per cent. Evaporation experiments were also performed by adding additional amounts of DCHFB to halothane to form an enrichment of the mixture, and these studies provided confirmation of the previous experiments. Stock halothane (unenriched) evaporated to 10 per cent of its initial volume showed a 90 per cent increase of DCHFB content in the residue. Evaporation experiments with halothane containing a tenfold enrichment of DCHFB showed a 312 per cent increase over initial concentration. Table 1 indicates the concentration effect observed with these and with intermediate dilutions. These results are in agreement with observations by Ehrenfeld *et al.*[11] and can be shown to follow the Rayleigh equation for distillation of multicomponent mixtures.‡ Vapor pressure characteristics of the binary system for DCHFB and halothane become of significant importance with the widely variable tech-

‡ For more detailed treatment of the Rayleigh equation the reader is referred to: Perry, J. H.: Chemical Engineers' Handbook, ed. 3, New York, McGraw Hill Co., 1950, pp. 580–1.

TABLE 1. Increase in Content of DCHFB in Halothane Residue after Evaporation to 10 Per Cent Initial Volume

Initial Concentration DCHFB in Halothane	Final Concentration DCHFB in Halothane	Increase in DCHFB Content (%)
180	342	90
360	872	142
630	1,660	164
900	2,530	181
1,800	7,410	312

Relative increase is related to initial added concentrations of DCHFB. All values represent p.p.m. All studies in duplicate.

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niques employed in the administration and storage of halothane. Thus a continuous vaporization beginning from a larger initial volume of halothane would tend to create lesser concentrating effect (with respect to DCHFB) than an equal number of milliliters repeatedly and more completely vaporized from smaller initial volumes (fig. 4).

Role of Copper and Oxygen. Additional studies have suggested the possible role of copper in the presence of oxygen as a second contributing factor to the concentration effect observed. These studies were variously carried out in systems which both prevented and also combined with the effect of evaporation loss (table 2). In our initial studies 25 to 30-ml. aliquots of halothane were refluxed at high temperature with 2 to 3 g. of copper filings in a constant stream of oxygen (fig. 5). The flask was heated to just below the boiling point of halothane with an oil bath, and the copper filings maintained in the range of 60° to 250° C. with a heating mantle. Volume loss was minimized with a dry ice and acetone condensing system although it was impossible to prevent slight volume reduction. Initial data indicated an increase in DCHFB concentration of from 100 to 200 per cent within a two-hour period with reflux temperatures of 150° C. When significant loss by evaporation occurred, the concentration effect was even larger. An experiment at 250° C. showed no increase in DCHFB content, but the copper

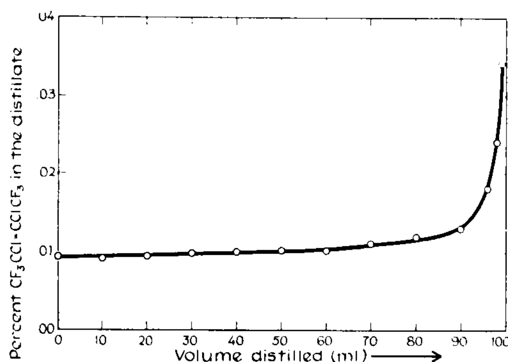

FIG. 4. 100 ml. stock halothane distilled through an all glass distillation apparatus (50° C.). Successive aliquots examined by gas chromatography for content of DCHFB. Note radical enrichment in residual volumes as volume distilled increased above 90 ml.

surface appeared blackened. Studies at 150° C. using a helium atmosphere instead of oxygen produced no increase in DCHFB content. Similarly, using an oxygen atmosphere at 150° C. but omitting the copper filings produced no increase in DCHFB. Separate experiments with hyperbaric oxygen, three atmospheres at room temperature, produced no change in DCHFB content.

Combined Roles of Copper, Oxygen, and Evaporation. Although the above data suggested a role for copper and oxygen in the concentration effect, it was difficult to separate completely the added influence of loss by evaporation. Furthermore, it was realized

TABLE 2. Changes in DCHFB Concentration in Halothane when Refluxed at Various Temperatures in the Presence and Absence of Copper or Oxygen

	Reflux Temperature (°C.)	Reflux Time (minutes)	Copper	Oxygen	Evaporation Loss	Increase in DCHFB Content (%)
Exp. 1	150	60	+	+	+	100
Exp. 2	150	60	+	+	+	82
Exp. 3	150	150	+	+	+	192
Exp. 4	150	60	+	He	0	5
Exp. 5	150	60	0	+	+	30
Exp. 6	200	150	+	+	+	100
Exp. 7	250	90	+	+	+	11
Exp. 8	150	60	+	He	0	0
Exp. 9	150	60	0	+	0	5
Exp. 10	60	60	+	+	0	20
Exp. 11	150	60	+	+	0	28

Twenty-five to 30 ml. samples placed in reflux apparatus (fig. 5) for 60 to 150 minutes. (Slight evaporation loss in earlier experiments).

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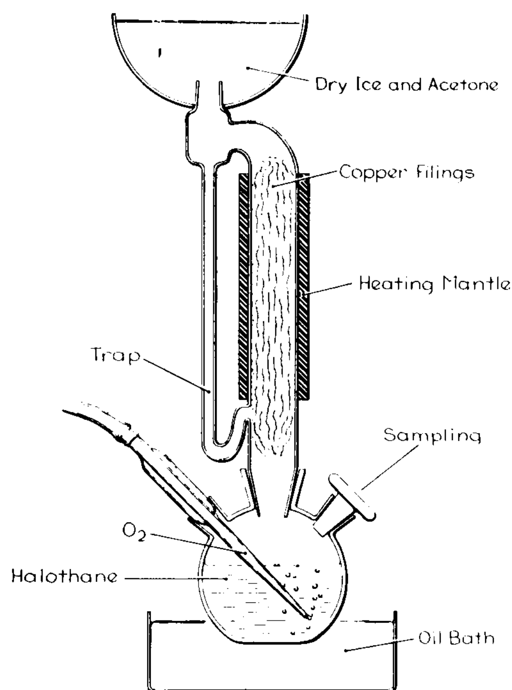

FIG. 5. Reflux system for high temperature reaction of halothane with copper filings in a stream of oxygen. Condensed halothane appearing in the "U" trap permits a constant recycling through the heated copper filings.

that while these laboratory experiments represented an observed effect at high temperatures, they did not necessarily reflect those circumstances present in the operating room. The following evaporation experiments performed at room temperature serve to isolate the relative concentrating role of evaporation, versus evaporation combined with an added copper effect. Forty-five milliliters of stock halothane were slowly evaporated in their original container to 5 per cent volume by exposure to air over a 72-hour period. A parallel experiment was carried out with the addition of 2.4 g. copper filings (previously washed and cleansed with halothane). Table 3 and figure 6 present the effect of evaporation alone on the concentration of cis- and trans-DCHFB, and also show an additional concentration effect due to added copper. In both experiments the cis isomer showed a greater relative increase in concentration than the trans isomer, and the total increase in DCHFB content observed with evaporation plus copper was 127 per cent. Evaporation thus accounts for 72 per cent of the combined increase, and 28 per cent is due to interaction with copper. This indicates a definite role for the copper, but of lesser importance than the concentration effect produced by evaporation.

Toxicology

In 1953 Lu and co-workers [7] studied the anesthetic properties of certain of the fluorinated hydrocarbons. Four rats were anesthetized with DCHFB producing convulsions at the point of anesthesia in two animals. All rats had "postanesthetic analgesia" and died within 18 hours. The acute toxicity of DCHFB in anesthetic concentration to the dog was reported from our laboratory and additional studies were presented by M. B. Chenoweth of the Biochemical Research Laboratory, Dow Chemical Company.[8] Toxic symptoms in Wistar strain rats followed a four-hour exposure to 100 p.p.m. with degenerative changes observed in the lung, liver and kidney. In a subsequent personal communication Chenoweth and his associates [12] reported that the

TABLE 3. Evaporation of Halothane to 5 Per Cent Volume in Original Container at Room Temperature over 72-Hour Period

	$CF_3CH{=}CBrCF_3$	Trans $CF_3CCl{=}CClCF_3$	Cis $CF_3CCl{=}CClCF_3$
Stock halothane	52 (±3.0)	154 (±4.5)	21 (±2.6)
Halothane evaporated to 5 per cent volume	38 (±3.1)	306 (±6.7)	52 (±4.8)
Halothane evaporated to 5 per cent volume with added copper filings	37 (±3.2)	359 (±5.0)	63 (±4.8)

"Butene" concentrations in p.p.m. are compared between stock halothane, evaporated halothane, and evaporated halothane plus copper (2.4 gm./45 ml.). Values represent mean (± S.E.) of at least three individual determinations of integrated peak areas.

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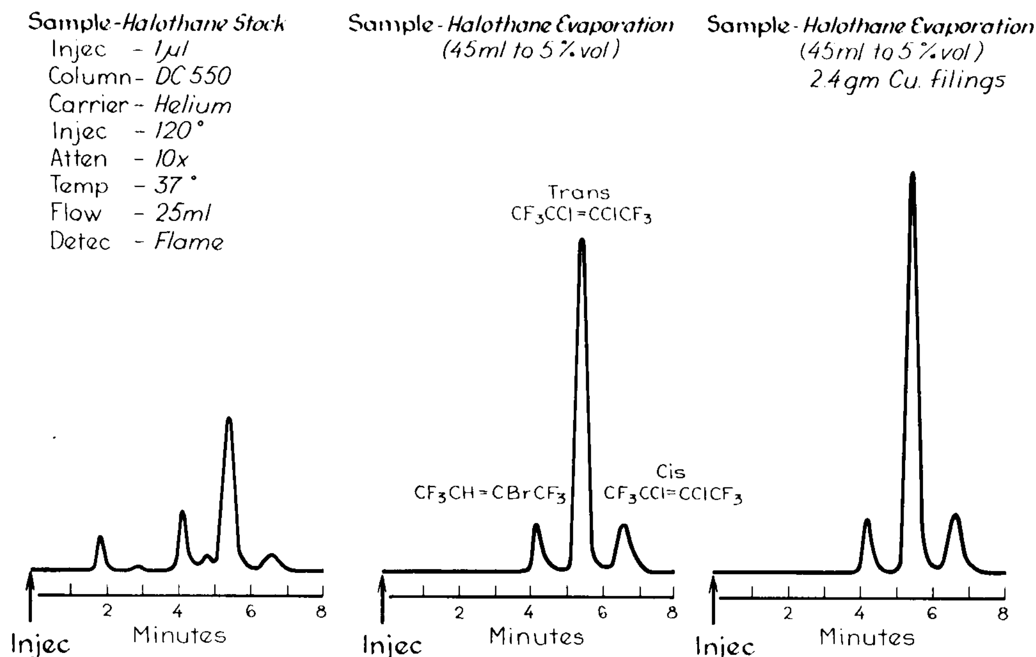

FIG. 6. Chromatographic tracings contrasting the effect of evaporation alone and evaporation combined with a copper effect. The comparison with stock halothane indicates an increased concentration of DCHFB in the residue. (Evaporation of halothane 72 hours at room temperature in original container.)

exposure of rats to 50 p.p.m. inhaled DCHFB for four hours resulted in severe injury and death. These investigators also fed DCHFB, 500 to 2,000 mg./kg. body weight, to 6 rats, and these animals developed "markedly fatty livers and severely necrotic kidneys." This liver damage was comparable to that produced by equivalent doses of carbon tetrachloride fed to a similar group of animals.

Toxicity Studies in the Rat. In a study carried out by the authors in cooperation with T. E. Shellenberger and G. W. Newell of the Department of Toxicology, Stanford Research Institute, 85 male Wistar strain rats were exposed to DCHFB in a dynamic system for three-hour periods. The inhaled concentrations in air ranged from 26 to 840 p.p.m. (v./v.). The LC_{50} for this exposure was 52 p.p.m., with 100 per cent mortality at 105 p.p.m. Of particular interest was the early respiratory death (6 to 24 hours) which occurred at 840 p.p.m., and a delayed death (4 to 14 days) resulting from exposures as low as 52 p.p.m. The latter group lost weight, slowly becoming lethargic, and expired. At postmortem examination, acute hemorrhagic changes in the lungs were found in those rats exposed to the higher concentrations of DCHFB, while a healing interstitial pneumonitis and central lobular necrosis of the liver was present in those animals exposed to 105–210 p.p.m. (fig. 7).

Toxicity Studies in the Dog. The toxicity of DCHFB was studied in 16 dogs. Anesthesia was induced with thiopental (150–200 mg.) followed by a three-hour inhalation of 1 per cent halothane with added DCHFB. Vaporization of the anesthetic was with 100 per cent oxygen. After insertion of the endotracheal tube, ventilation was supported with a Harvard respirator. Electrocardiogram, arterial blood pressure, and the electroencephalogram were monitored and intermittent determinations of *p*H, P_{O_2} and P_{CO_2} were made. Initially, control animals were paired with the experimental group, and the former maintained on 1 per cent halothane without added DCHFB. The LC_{50} in the dog was 200 p.p.m.

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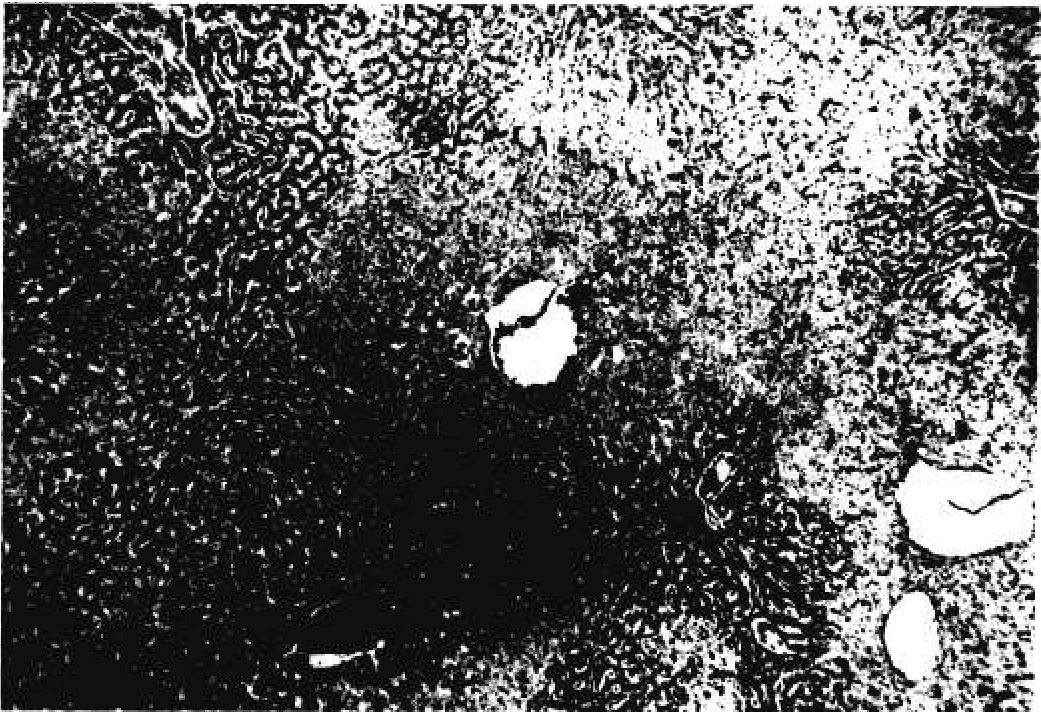

FIG. 7. Photomicrograph of rat liver (from 200 ×). Animal exposed 3 hours to 105 p.p.m. DCHFB and sacrificed on the eleventh post-experimental day. Note central lobular necrosis.

DCHFB in 1 per cent halothane for a three hour exposure, decreasing to 150 p.p.m. (v./v.) for a similar anesthetic repeated on four consecutive days. At 100 p.p.m. the recovery was similar to the control group with halothane alone. Frequent convulsive episodes were noted on the electroencephalogram (fig. 8). In one dog these appeared 45 seconds after exposure to 1,000 p.p.m. at a measured arterial DCHFB concentration of 0.031 mg./100 ml. Under halothane anesthesia, the external manifestations of central nervous system stimulation did not usually appear until 15 minutes after termination of the anesthesia when twitching movements increased and progressed to seizures of continuous duration. The animals left unprotected expired within six to eight hours. Intravenous sodium pentobarbital and diphenylhydantoin were given intermittently to one animal for a 24-hour period and maintained post-experimental control of seizures. As the effect of these medications wore off, the animal again began to convulse and subsequently expired. The preanesthetic administration of 500 mg. pyridoxine failed to protect a second animal. At 200 p.p.m. DCHFB the dogs were hyperirritable but did not show generalized seizures. Loss of appetite, loss of weight, and lethargy preceded demise which occurred by the seventh to tenth day. Postmortem examinations (brains excluded) were unremarkable except for renal tubular damage found in some animals.

Toxicity Studies in the Monkey. The above studies indicated that DCHFB was toxic to both the rat and dog, but that the target site and toxic dose varied in the two species. The rhesus monkey was selected for additional studies as a species more closely resembling man. Eight animals of both sexes were isolated for at least six weeks prior to the study. The experimental protocol followed was the same as that for the dog except that the LC_{50} for this expensive laboratory animal was determined by a reducing range of paired exposures according to the method of Venter.[13] The LC_{50} for the rhesus monkey was calculated by the method of maximum likelihood to be

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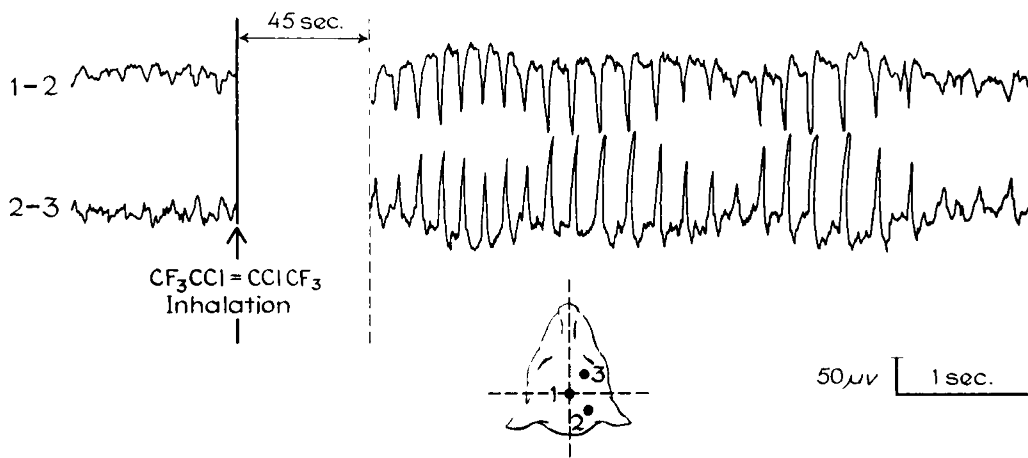

FIG. 8. Electroencephalographic tracing made in a dog inhaling 1000 p.p.m. DCHFB in halothane. Evidence of convulsive pattern appears within 45 seconds of first inhalation. Anesthetic was discontinued immediately after appearance of seizure.

54 p.p.m. (v./v.) for a three hour exposure. Death occurred within four to nine days (table 4). Convulsive disorders appeared in the 2 animals exposed to 350 and 400 p.p.m. Postmortem examinations revealed severe pulmonary changes in all animals. Grossly, areas of pneumonic consolidation were present, and microscopically the alveolar walls were edematous with occasional areas of necrosis and covered with a thick exudative layer. Relatively few polymorphonuclear cells were present and bacterial cultures from the lungs made in 2 animals were negative for a pathogenic organism. In animals living past the fifth day there was additional evidence of liver damage, the extent of which appeared related to the concentration of DCHFB. Following exposure to the lowest fatal concentration there were atrophic changes and fatty changes in the liver, while at the highest concentration central lobular necrosis of the liver was present. The above findings are in general agreement with a preliminary communication by Corrigan *et al.*[14] suggesting toxic manifestation not only in the rat, dog, and monkey, but also in the mouse and rabbit.

Uptake and Distribution

The uptake and distribution of DCHFB was studied to establish its intake into the body and to determine its selective distribution to specific organs. It was hoped that such information might assist in interpretation of the observed toxic effects and in the identification of possible sites of metabolic conversion. It

TABLE 4. Animals Exposed for Three Hours to Inhalations of 1 Per Cent Halothane with Added Amounts of DCHFB Vaporized with 100 Per Cent Oxygen*

	Concentration DCHFB Inhaled (p.p.m.)	Post Experimental Day of Death	Pathologic Change
Monkey 1	0	—	—
Monkey 2	25	—	—
Monkey 3	25	—	—
Monkey 4	50	9	Multiple areas of consolidation in both lungs. Mild fatty changes in the liver with areas of atrophy.
Monkey 5	50	—	—
Monkey 6	100	2	Massive consolidation right lung. (Klebsiella aerobacter isolated.)
Monkey 7	350	4	Multiple areas of consolidation in both lungs. Marked fatty changes in the liver.
Monkey 8	400	9	Multiple areas of consolidation in both lungs. Central lobular necrosis of liver.

* Ventilation supported with a Harvard respirator.

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TABLE 5. Mean DCHFB Content in Tissues of a Dog and 3 Monkeys Following Inhaled Concentrations of 0.1 Per Cent for 60 Minutes

	Concentration of DCHFB (μg. %)	
	Monkey (3)	Dog (1)
Arterial	136	123
Venous	10	3
Urine	0	0
Kidney	701*	57
Liver	0	0
Lung	181	—
Fat	897	—
Muscle	3	0
Pancreas	69	—
Thyroid	0	46
Adrenal	726	857
Heart	80	—
Bile	70	—
Brain	1,291	316

All samples corrected to equal 10 μl. or 10 μg. injection.

* A single specimen contained 1,840 μg. per cent. Average concentration in other 2 monkeys was 131 μg. per cent.

was realized that the most meaningful information would be that obtained in man. Since trace amounts of DCHFB were already present in stock halothane, it was possible to carry out studies of alveolar uptake in the surgical patient. Detailed studies of tissue and organ uptake were only available in the experimental animal. The following experiments in the dog and monkey preceded human studies of the alveolar uptake of DCHFB.

Uptake of DCHFB in the Experimental Animal. Studies were carried out with DCHFB administered alone and with DCHFB (0.1 per cent inhaled concentration) enriched halothane. The halothane itself was vaporized in oxygen and administered in a 1 per cent concentration. Partial sampling of tissue and body fluids was performed at one hour and complete sampling at two to three hours with termination of the experiment. Tissue was obtained by surgical biopsy, and the samples immediately stored at −4° C. At the time of analysis the tissues were biopsied with a specially constructed biopsy needle.[15] Content of DCHFB and of halothane in tissue was measured by gas chromatography with electron capture detection.§ Concentrations were calculated on the basis of the weighed tissue samples. Table 5 presents concentrations of DCHFB found in the dog and in the monkey. It was of particular interest that high concentrations of DCHFB were found in each of the highly perfused organ systems of the body but with the notable exception of the liver. The lack of discernable concentration of DCHFB in muscle is most likely related to the relatively reduced perfusion to this tissue (less than 5 per cent that of hepatoportal flow).[16] One cannot of course exclude the possibility of coincident metabolism within muscle. Further attempts to define the role of the liver were made by total hepatectomy and by exclusion of the liver (ligation of the hepatic artery with cannulation of the supra hepatic inferior vena cava, infra hepatic inferior vena cava, and portal vein.) There was a dramatic increase in the venous concentration of DCHFB (+ 40 per cent) observed in 3 monkeys during the 60 minutes post-shunt period. Precise evaluation of these results is, however, difficult due to the frequent hypotension associated with this operation.

The response to DCHFB in high concentration (2 per cent) and without halothane was determined in a single monkey. Following inhalation of DCHFB for one hour, all examined tissues, including the liver, contained a high concentration of DCHFB. The presence of DCHFB in the liver of this animal suggested that the threshold level for its metabolism has been exceeded.

Metabolism of DCHFB by the Liver. Studies of liver clearance were made to further evaluate the role of the liver in metabolism. DCHFB (0.15 per cent concentration) was inhaled for 90 minutes by 2 monkeys and blood samples simultaneously drawn from the inflow tract (infra hepatic inferior vena cava, hepatic artery, and portal vein) and from the outflow tract (supra hepatic inferior vena cava of the liver). The attained concentrations of DCHFB were then analyzed by gas chromatog-

§ Considerable increase in sensitivity is provided through the use of electron capture which in the case of DCHFB has 500 times the sensitivity of flame detection which in turn may afford 1,000 times the sensitivity of thermal conductivity detecting devices.

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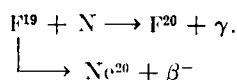
raphy. Using a portal vein to hepatic artery flow ratio of 2.8:1 [17] and assigning the relative contribution of infra hepatic inferior vena cava flow as 34 per cent of the combined portal vein and hepatic artery flow,[18] the inflow of DCHFB into the liver could be calculated at approximately 0.315 μg./ml. of blood flow. The outflow from the liver measured only 0.23 μg./ml. This provided a clearance rate of 0.085 μg./ml. of hepatic flow, and suggested that DCHFB was being destroyed within the liver. The mean hepatic clearance rate for DCHFB was calculated at 110.9 μg./kg. body weight using an estimated hepatic flow rate of 58 ml./100 g./minute [19] and an estimated liver size in the monkey equal to 2.5 per cent body weight. Despite these calculated rates of metabolism, no DCHFB could be demonstrated in samples of liver surgically removed and analyzed by gas chromatography. This implies that the compound no longer existed within the liver in its original (volatile) structural state, since earlier experiments had demonstrated that gas chromatography with electron capture provided a most sensitive method of tissue analysis for DCHFB (detection sensitivity with a 10-mg. sample equal to 1 part in 10^7).

Definitive evidence for the metabolism of DCHFB by the liver was established through the use of neutron activation analysis procedures. If it were possible to demonstrate the presence of fluorine in the liver which was not present prior to the administration of DCHFB, then the source of this material would necessarily represent metabolism of DCHFB. An attempt was made to chemically extract a fluorinated compound from the liver but this was unsuccessful due to the exceedingly small amounts of material present (approximately 4.4 μg. DCHFB/g. liver tissue). We then turned to the use of activation analysis which involves slow neutron irradiation of tissue samples for a period of 30 seconds (frozen and sealed within a polyethylene bag). Irradiation was accomplished next to the nuclear reactor core ($U^{235} - H_2O$) in a region of high thermal neutron flux. Neutron capture by F^{19} forms F^{20} via the reaction



$$F^{19} + N \longrightarrow F^{20} + \gamma, \quad F^{20} \longrightarrow Ne^{20} + \beta^-$$

F^{20} decays to Ne^{20} (stable) with an 11 second half-life and a γ ray energy of 1.64 million electron volts (Mev.). In practice, counting with a scintillation detector was carried out for a 24-second period beginning as soon as possible (10 seconds) after completion of the irradiation. The γ ray counts are stored in the memory of a multi-channel pulse height analyzer. The counting period was then followed by a subtract mode for 24 seconds to cancel out the long lived background peaks (Na and Cl γ rays), leaving the F^{20} γ ray spectrum (principally the 1.64 Mev. peak) intact. The analyzer memory permitted repeated irradiation and tabulation of accumulated counts. With this technique successive irradiations were repeated on an experimental liver sample (60 minute inhalation of 0.3 per cent DCHFB), a preliminary liver blank, and a 100 mg. sample of NaF crystals. Liver samples were irradiated three times to accentuate the 1.64 Mev. peak. Figure 9 shows presence of the fluorine peak at 1.64 Mev. both in the known NaF sample and in the experimental liver tissue. No fluorine peak was observed in the liver blank. Calculations of liver clearance for DCHFB similar to those detailed above indicated the presence of 30.1 μg. metabolic fluorine in the experimental liver specimen (13.9 g.).

Uptake of DCHFB in Man. Studies of the uptake of DCHFB in man have been limited to trace concentrations of DCHFB already present in commercial halothane. With 1 per cent halothane the arterial level of DCHFB may attain approximately 3×10^{-4} mg./100 ml.¶ With a 10 μl. injection sample these blood concentrations are just beyond the sensitivity of electron capture detection for DCHFB (1×10^{-9} g.). However, with a gas sampling port one can use large gas samples for injection into the gas chromatograph and thus measure the alveolar concentration of DCHFB.

Two series of investigations have been undertaken in man. In a group of 5 patients, 1 per cent halothane containing 0.018 per cent DCHFB was administered in a nonrebreathing system at a total flow rate of 12

¶ Blood/gas solubility coefficient = 0.445; oil/gas solubility coefficient = 61.9; rubber/gas solubility coefficient = 35.6. Data courtesy of Dr. E. I. Eger, II, Department of Anesthesia, University of California.

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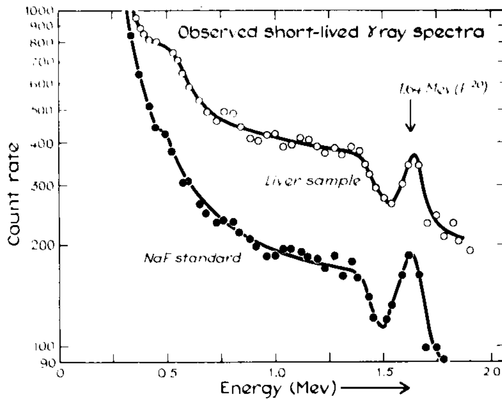

FIG. 9. Gamma-ray spectrum obtained by irradiation of NaF sample and liver tissue in area of slow neutron flux. Animal exposed to 0.3 per cent DCHFB alone by inhalation for 60 minutes. In both samples 1.64 Mev. peak represents F^{20}.

liters. The inhaled concentration of halothane was monitored with a U.V. meter || and maintained at a steady concentration. Ventilation was kept at fixed minute and tidal volumes through use of a Ventimeter.# A small polyethylene catheter passed through the endotracheal tube and terminated just above the carina permitted sampling of end-expiratory gases. At prescribed times inhaled and exhaled gas samples were collected and analyzed for content of halothane and for DCHFB by gas chromatography. The uptake of both substances may be compared in figure 10. It will be noted that the lower blood/gas solubility ratio of DCHFB permits rapid attainment of peak alveolar concentration for this compound. A 5–10 per cent gradient maintained between inhaled and exhaled concentrations of DCHFB provides evidence for its alveolar uptake.**

A second series of investigations were carried out in 5 subjects in which the inhaled concentration of halothane was again maintained at 1 per cent, but the rebreathing system was now completely closed with only sufficient oxygen added to meet basal metabolic requirements. A steady concentration of halothane was obtained by monitoring inhaled concentration with the U.V. meter and gradually reducing the oxygen flow diverted through the vaporizer. The previous study indicated than 90–95 per cent of the DCHFB was returned from the patient unchanged and that the halothane was being taken up by the patient in much larger amounts. It was thus anticipated that maintaining a constant 1 per cent inhaled concentration of halothane in a closed system would create an increasing concentration of DCHFB both in the vaporizer and in the patient. The data in figure 11 indicate, however, that despite a constant inhaled halothane concentration, there was a falling concentration of DCHFB in the vaporizer which is consistant with metabolism of DCHFB by the patient.

Discussion

Measurements of the alveolar concentration in man indicate that DCHFB is taken up together with halothane in both open and in closed systems of administration. In addition, data obtained with closed techniques of administration suggest that the impurity may be metabolized in man since the concentration of DCHFB continues to fall in the presence of a fixed inhaled halothane concentration. If such a process were not occurring, the less blood soluble DCHFB (blood-gas solubility coefficient = 0.446) would tend to build up in concentration within the vaporizer as it

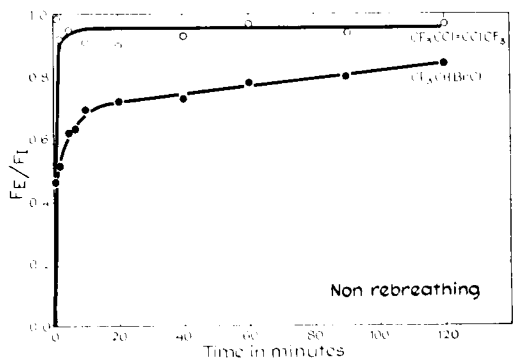

FIG. 10. Uptake of DCHFB compared to halothane in a nonrebreathing system. Inhalation of halothane maintained at constant concentration. Note rapidly rising concentration of DCHFB in exhaled alveolar gas. Uptake per breath remains steady at 5–10 per cent of inhaled concentration.

|| Analytic Systems, Inc., Pasadena, California.
Air Shields Inc., Hatboro, Pennsylvania.
** These data were in agreement with predicted end-expiratory values based on an analog model utilizing the determined solubility coefficients. This work done in collaboration with Dr. E. I. Eger, II.

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recycled. It is also possible that the DCHFB might be preferentially taken up by the rubber components of the rebreathing system, this may be ruled out by an even greater rubber/gas solubility coefficient for halothane = 121.2.[20]

Studies in the experimental animal indicate that DCHFB presents a high degree of toxicity, yet it is difficult to translate such data to man. It would, however, seem reasonable to assume that DCHFB could exert toxic effects in man. This follows from its proven toxicity in each of the 5 animal species that have been investigated (mouse, rat, rabbit, dog, and monkey). These toxic effects have been noted above and include primary pulmonary irritation producing severe chemical pneumonitis in the rat and in the monkey. In addition, central nervous system manifestations were observed in each of the three examined species. In the monkey these were present only at the higher exposed concentrations (350 p.p.m. DCHFB).

The demonstration in the monkey and rat of extensive fatty infiltration and central lobular necrosis from a halothane contaminant, *i.e.*, DCHFB, may have bearing on the suggested association of halothane and hepatic necrosis. Since the latter association itself remains unproven, the clinical importance of these relationships must await further study and interpretation. The absence of hepatic injury in the dog may represent a particular species variant associated with the presence of spiral and sphincter muscle fibers which surround all the hepatic veins of this animal,[21] and which are subject to pharmacologic influences.[22] The role of the liver in detoxification of DCHFB in the monkey has been demonstrated through studies of hepatic clearance and by activation analysis techniques proving the presence of a metabolic fluorine product. Although this fluorine metabolic product may represent a hepatoxic substance, evidence in this regard awaits further study. It is noted, however, that DCHFB does undergo metabolism within the liver.

Clayton has defined the toxicity of the fluorocarbons with special reference to their chemical constitution. He points out increased activity in the case of the fluoroalkenes and further toxicity with the addition of chlorine

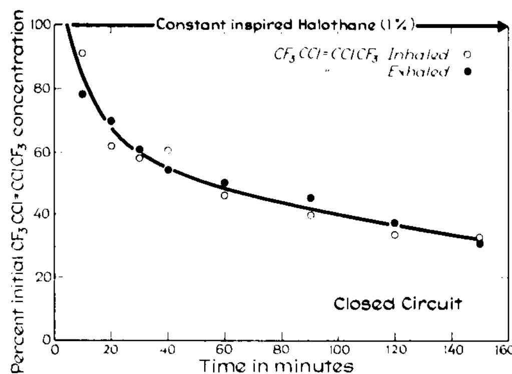

FIG. 11. Uptake of DCHFB in closed circuit system. Inspired halothane adjusted to maintain a steady 1 per cent concentration. Note falling concentration of inhaled and exhaled DCHFB despite its lesser blood solubility. (Points on curve represent percentage reduction from initial peak observed concentration.)

atoms.[23] On this basis he predicted an LC_{50} for DCHFB of 100 p.p.m. with some species variation.[24] Our finding of an LC_{50} of approximately 50 p.p.m. for a three-hour exposure in both the rat and monkey is in close agreement with this prediction. An even more hazardous relation to man might be predicted from experiments with the rat where the LC_{50} at six hours is 21 p.p.m.[14] This concentration represents only five to ten fold the 2–4 p.p.m. DCHFB which would be vaporized in a 1–2 per cent inhaled concentration of anesthetic halothane.†† Possibly a more crucial question than the determined LC_{50} would be the LC_{01} as it might apply in man since we must concern ourselves with the possibility of a fatal complication which occurs rarely, possibly as infrequently as 1 per 10,000 clinical administrations of halothane. Furthermore, while these calculations are based on lethal dosage, presumably even lower concentrations might produce serious though nonfatal effects. Repeated administration of halothane may yet contribute an added hazard.

Although it may eventually be possible to assign toxicity figures for an anesthetic such as halothane, similar information on the toxicity of DCHFB in man will in all probability never

†† These calculations are based on the mean concentrations of DCHFB reported in operating room vaporizers [10, 14] and from known and determined vapor pressure data for halothane and for DCHFB.

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be available. Nonetheless, the present investigations serve to define general concepts of toxicity relative to man and permit the interpretation of specific toxicity data determined in the experimental animal. Finally, the nature of these findings, plus the laboratory demonstration of increasing concentrations of the impurity during the clinical administration of halothane anesthesia, indicate that the presence of DCHFB in commercial halothane (even in trace amounts) must be considered to present a potential hazard, and it, therefore, should be removed from halothane utilized for anesthesia in human patients.

Summary

Dichlorohexafluorobutene (DCHFB) is a contaminant in halothane prepared by high thermal synthesis. Its concentration has been shown to increase under conditions of clinical use in the operating room, and experiments in the laboratory confirm this finding. It has been shown by measurement of alveolar concentration that DCHFB is taken up and presumably metabolized in man. Studies with animals indicate damaging effects in all species studied. Since species variation exist in the LC_{50} and in the organ systems affected, the ultimate clinical implications of these findings must await further study. It is evident, however, that the determined ranges in lethal concentration in animals are uncomfortably close to those concentrations of DCHFB actually present during clinical anesthesia. Thus a serious potential hazard is present which justifies the removal of DCHFB from anesthetic halothane.

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Addendum

Since completion of these studies, an examination of currently available halothane (Ayerst Laboratories Lot D36261D, B39221F, and C37781E) indicate that both cis- and trans-DCHFB, as well as several other minor impurities, have been removed from this product.

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INTESTINAL REFLEXES Local anesthetics such as cocaine, procaine, and dibucaine, applied to the serosal surface of the small intestine inhibit the peristaltic reflex but do not interfere with the graded reflex of the longitudinal muscle. The potency of local anesthetics in inhibiting the peristaltic reflex correlates with their anesthetic potency. In addition, morphine inhibits both the peristaltic reflex and graded reflex of the longitudinal muscle in the isolated ileum. This inhibitory action of morphine and morphine-like compounds on the gut can be antagonized both *in vivo* and *in vitro* by nalorphine and levallorphan. *(Kosterlitz, H. W., and Lees, G. M.: Pharmacological Analysis of Intrinsic Intestinal Reflexes, Pharmacol. Rev. 16: 301 (Sept.) 1964.)*

CARDIAC METABOLISM While the pump oxygenator is being used during intracardiac operations the heart performs neither pressure nor volume work. The coronary sinus was catheterized and the arterial-venous coronary differences of the following metabolites were determined: glucose, lactate, pyruvate, free fatty acids, beta-hydroxybutyrate, acetacetate, total aminoacids, glutamate, glutamine, alpha-ketoglutarate and ammonia. The results showed that 40 per cent of oxidative metabolism was supported by free fatty acids, more than 20 per cent by beta-hydroxybutyrate and other ketone bodies, and the remainder by carbohydrates. In spite of very high arterial lactate concentrations lactate contributes only a small part to the oxydative metabolism of the heart. In coronary venous blood the lactate/pyruvate ratio is higher than in arterial blood, although the oxygen supply of the heart is fully adequate. During fibrillation the extraction of free fatty acids decreases. The heart extracts glutamate continuously from the arterial blood and delivers glutamine into the coronary venous blood. Energy requirements of the heart were 98 per cent provided by oxidative metabolism and only 2 per cent by anaerobic, glycolytic glucose catabolism. *(Keul, J., and others: Metabolism of the Human Heart, Contracting against Zero Pressure and Delivering Zero Volume, Klin. Wschr. 42: 898 (Sept. 15) 1964.)*

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