TOXICOLOGY OF INHALATION ANESTHETICS AND METABOLITES

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Several reviews that bear on the subject of the toxicology of inhalation anesthetics have appeared in recent years. A partial list includes biotransformation (1–4), the biochemical basis of chemical injury (5–9), and anesthetic toxicity (10–13). The proceedings of two symposia (14, 15) and another monograph in the series of the *Handbook of Experimental Pharmacology* (16) have been published.

Patterns of exposure to inhalation anesthetics may be divided into two broad groups: (*a*) acute, single or multiple exposures to relatively high levels, typical of the clinical application of general anesthetics, and (*b*) chronic, low-level exposure to which operating room personnel are subjected.

This review is divided into three sections: (*a*) biotransformation of anesthetics, (*b*) mechanism of toxic injury, and (*c*) toxicology of chronic exposure to anesthetic gases.

BIOTRANSFORMATION

Halothane

Species differences in the biotransformation of halothane ($CF_3CHBrCl$) have been demonstrated to be largely quantitative. No firm evidence of significant qualitative differences has been published at this writing.

Halothane at anesthetic concentrations has been demonstrated to inhibit its own dehalogenation (17). The rate of halothane metabolism in miniature swine has been equated with the rate of hepatic extraction and suggested to be related inversely to the rate of delivery of halothane to the liver (18). The implication was that the anesthetic impaired its own metabolism. Topham & Longshaw (19) conducted related studies but measured the biliary excretion of halothane in rats and dogs as well as the accumulation of nonvolatile metabolites in the whole bodies and organs of rats and mice. They concluded that a significant fraction of hepatic halothane extraction may be accounted for by the biliary excretion of unchanged halothane, thus calling for cautious interpretation of data equating hepatic extraction with

metabolism. They further offered support for a postulated enterohepatic circulation of halothane and/or metabolites that would contribute to a prolongation of the half-time of hepatic excretion. Halothane in trace amounts was identified in venous blood 44 hr after the induction of anesthesia in humans (17). Such persistence could be attributable to a combination of enterohepatic circulation and redistribution of the anesthetic. An enterohepatic cycle would not seem likely to be of great significance for the highly lipid-soluble halothane and the putative polar conjugates of its metabolites. Even if the conjugates were hydrolyzed in the gut, the principal nonvolatile metabolite of halothane, trifluoroacetate (20, 21), would not be likely to be reabsorbed by the gut, since it remains mostly ionized at body pH (pK = 0.25) unless a carrier-mediated mechanism were involved in its reabsorption.

No evidence has been presented to date that any species tested is able to defluorinate halothane. Fiserova-Bergerova (22) detected no increase in bone fluoride levels following the intraperitoneal (i.p.) injection of halothane in olive oil to mice and rats. One would hope that similar studies would be pursued using the inhalational route of exposure. The lung represents the usual route of clinical exposure, and recognition of the potential significance of this organ as a site of xenobiotic biotransformation suggests the possibility of a role for it in the formation and disposition of metabolites of anesthetics as well as the fate of the parent compounds themselves (23, 24).

Creasser & Stoelting (25) detected no increase in serum fluoride levels in five patients anesthetized with halothane-N_2O.

Current concepts in the biotransformation of halothane are summarized in Figure 1.

Dechlorination (26) and debromination (27) require NADPH, are mediated by enzymes of the hepatic endoplasmic reticulum, and may be stimulated by the prior administration of phenobarbital. In the presence of O_2, dehalogenation is assumed to be oxidative (1).

Trifluoroacetate has been recovered from the urine of men (20, 21, 28) and squirrel monkeys (29) following exposure to halothane. Trifluoroacetaldehyde has been proposed as an intermediate in the formation of trifluoroacetate during the oxidative dehalogenation of halothane (12). Implicit in this scheme is the participation of liver dehydrogenase systems following the model of the biotransformation of trichloroethylene (30). Once the concept of the participation of these enzymes has been invoked, the formation of trifluoroethanol from trifluoroacetaldehyde or trifluoroacetate reduction by liver dehydrogenases (31) becomes reasonable (1).

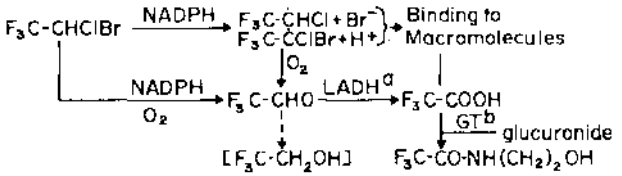

Figure 1 Current concepts in the biotransformation of halothane: (*a*) liver alcohol dehydrogenase, and (*b*) glucuronyl transferase.

The fact that neither trifluoroethanol nor its glucuronide has ever been identified in any species as a metabolite of halothane weakens the force of this argument. Were the alcohol formed in significant quantity it probably would have been detected by now as it has been following the administration of fluroxene (32). It is unlikely that trifluoroethanol is an endproduct of halothane metabolism in man (33), but that trifluoroethanol is not formed cannot be excluded absolutely. Nonvolatile metabolites of halothane are formed that remain to be identified.

The possibility that halothane undergoes reductive dehalogenation is being investigated by Van Dyke (34). He is following the lead of Stier (35) who suggested a pathway involving formation of a radical that is converted directly to trifluoroacetate. The possibility that enzymes of the hepatic endoplasmic reticulum mediate reductive dehalogenation in the absence of O_2 is being investigated (34).

Cohen & Trudell (29) have presumptive evidence in the squirrel monkey exposed to halothane of the urinary excretion of trifluoroacetate and glucuronic acid, although not necessarily as a single molecule. Blake et al (28) have provided evidence that in man trifluoroacetate is excreted in the free form rather than conjugated with glucuronic acid. Other glucuronides, possibly that of trifluoroethanol, could be involved. The significance of the inhibition of UDP glucuronyl transferase that has been shown to occur in the presence of not only halothane, but also of methoxyflurane, chloroform, and diethyl ether (36) is uncertain.

Trifluoroacetyl ethanolamide has been reported in the urine of men exposed to halothane (3). The implications of this observation as well as the binding of halothane metabolites to macromolecules are discussed later.

Methoxyflurane

A scheme for the biotransformation of methoxyflurane ($H_3C\text{-}O\text{-}CF_2CHCl_2$) that includes the known metabolites identified by both in vitro and in vivo studies of mammalian systems is presented in Figure 2.

Holaday et al (37) identified products of the biotransformation of methoxyflurane in man as CO_2, inorganic fluoride, dichloroacetic acid, and methoxyfluoroacetic acid. Formaldehyde that is rapidly oxidized to CO_2 has been shown to be formed by human and rat hepatic microsomes in the presence of methoxyflurane (38).

The bones of rats and mice accumulated fluoride following the intraperitoneal injection of methoxyflurane in olive oil (22). The apparent defluorination of methoxyflurane in this series of experiments was enhanced by the prior administration of phenobarbital. Blood serum levels of inorganic fluoride have been demonstrated to

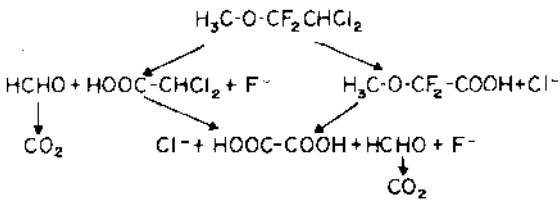

Figure 2 Proposed pathways for the metabolism of methoxyflurane.

become elevated during and after surgical anesthesia in man (25, 39, 40). Mice, rats, guinea pigs, rabbits, dogs, and monkeys also defluorinate methoxyflurane (41).

Blood oxalate levels increased (42), calcium oxalate crystals were detected in renal biopsy specimens (43), and urinary oxalate excretion was increased significantly (43, 44) after methoxyflurane anesthesia in man.

Fluroxene

The principal metabolites of fluroxene (F_3CCH_2–O–CH=CH_2) in mice, dogs, and man are CO_2 (derived exclusively from the vinyl moiety), trifluoroethanol (largely as the glucuronide), and trifluoroacetic acid (32, 45). No inorganic fluoride is liberated from the trifluoromethyl group (25, 32, 45). The principal urinary metabolite in the mouse and dog is trifluoroacetate (32).

The probable scheme for the biotransformation of fluroxene is illustrated in Figure 3.

Enflurane and Isoflurane

Enflurane (F_2HC–O–CF_2CHClF) and isoflurane (F_2HC–O–$CHClCF_3$) are structural isomers and will be considered together.

Of the nonflammable halogenated agents isoflurane represents the clinically useful general anesthetic that has most nearly approached the "ideal" with respect to biotransformation in man. Isoflurane has been reported to be only minimally defluorinated (46, 47) or not defluorinated (48; D. A. Holaday et al, personal communication) in man, minimally defluorinated in the Fischer 344 rat (47, 49, 50), and not defluorinated in the miniature pig (51), C-57 and white Swiss mice, and Wistar rat (22).

Enflurane, on the other hand, is apparently defluorinated to a somewhat greater extent than isoflurane. Elevated serum fluoride levels have been reported in man (48, 52), the Fischer 344 rat (53), and in an unidentified species (4). Halsey et al (51) inferred from hepatic extraction studies in the miniature pig that enflurane was not metabolized.

Nonionic fluoride has been detected in the urine of man (46, 47) and the Fischer 344 rat (47) following exposure to isoflurane, and in the Fischer 344 rat (53) following exposure to enflurane.

Animal pretreatment with phenobarbital has been shown to stimulate the defluorination in vitro of both isoflurane and methoxyflurane by Fischer 344 rat liver microsomes, but only of methoxyflurane when tested in vivo (50). This apparent

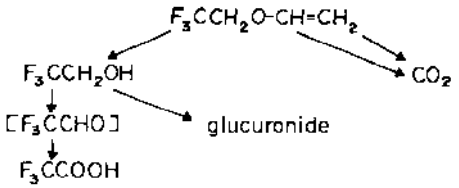

Figure 3 Proposed pathway for the biotransformation of fluroxene.

inconsistency was suggested to be a consequence of the difference in lipid solubilities of the respective compounds. Ostwald solubility coefficients for methoxyflurane in blood at 37°C have been reported to be 8–14, depending on the species, and 1.0–1.4 for isoflurane in human blood (54). The implication was that the relatively lower lipid solubility of isoflurane limited its delivery to the site(s) of biotransformation.

In this connection it is appropriate to point out an often overlooked physiological adaptation to the metabolic demands placed on the liver by the stimulation of drug-metabolizing enzyme systems. Liver blood flow was shown to increase within 24 hr in rats treated with phenobarbital, antipyrine, but not benzpyrene, and to remain elevated for 2–8 days after treatment (55). Increases amounted to 33–175% of control rates. Increases in liver perfusion would have the effect of increasing the rates of delivery of all blood-borne substances to the liver and should therefore be expected to influence the processes of xenobiotic biotransformation as well as their measurement in vivo. The potential effects of hepatic hypoperfusion, on the other hand, have been considered by Van Dyke & Wood (34).

Unidentified fluoro-organic metabolites of enflurane and isoflurane are known to be produced in man and the rat (46, 47). In an interesting theoretical study Loew et al (56) have proposed pathways of biodegradation for the respective compounds (Figures 4 and 5) within the context of their structural relationship to methoxyflurane (Figure 2).

Loew et al (56) concluded that the susceptibility of the three anesthetics to O-dealkylation or dechlorination was methoxyflurane > enflurane > isoflurane. This agreed with observed behavior. The rank order correlation was based on the insertion of active oxygen into C–H bonds, which is more likely to occur the more electron-rich each atom is.

THE MECHANISM OF TOXIC INJURY

The results of the national halothane study (57) did not rule out the possibility that massive, postoperative hepatic necrosis might, in rare instances, be attributable to halothane anesthesia. The study did reveal, however, that the highest incidence of

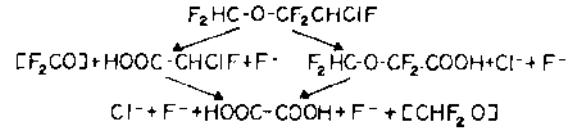

Figure 4 Proposed pathway for the biotransformation of enflurane (56).

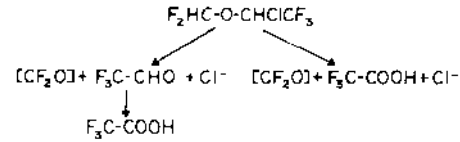

Figure 5 Proposed pathway for the biotransformation of isoflurane (56).

postoperative liver complications not otherwise explicable, occurred following the use of cyclopropane.

"Halothane hepatitis" remains an enigma. Various etiologic factors contributing to, and theories explaining the occurrence of hepatic failure attributable to halothane have been proposed (10, 58). Among these are liver hypoxia, liver disease, impaired liver nutrition, sepsis, viral hepatitis, multiple exposures, obesity, factors affecting biotransformation, direct hepatotoxicity, immunosuppression, toxic metabolites, and hypersensitivity. In spite of massive efforts to solve this problem, the diagnosis of halothane hepatitis still cannot be made with certainty and remains "a diagnosis of exclusion" (58).

Dykes et al (11) found little support for the hypothesis that an immunologic mechanism is important in the development of halothane hepatitis. Some animal studies fail to support the notion that a toxic mechanism is involved (59, 60); indeed, the failure to detect any dose-response relationship in man further undermines the argument for a toxic mechanism.

Whether or not halothane hepatitis is a genuine clinical entity remains to be determined. That halothane is metabolized, though, is beyond doubt. Nonvolatile metabolites, presumably bound to macromolecules, are known to remain in animal bodies for weeks following exposure (29).

The tissue binding of drugs and their products of biotransformation is presumed to be a necessary prerequisite for not only drug-receptor interactions, but also for the metabolism and potential manifestations of toxicity (8, 9). The multiplicity of physicochemical properties of the wide variety of macromolecules that occur in the body confers a highly variable specificity on the potential drug-metabolite-receptor interactions. This leads to the requirement of considering interactive schemes such as the one represented in Figure 6.

Radioautographic and fractionation studies in mice (29) have shown that several halothane metabolites are accumulated in the liver and that these seem to be preferentially distributed to the mitochondria. Possible halothane metabolites have been demonstrated to decrease hepatic ATP/ADP ratios in mice (61). Reduced ATP/ADP, AMP ratios could interfere with protein synthesis (62), which would be accompanied by the disaggregation of polyribosomes. This has been observed in rat liver cells following exposure to halothane (63).

The inhibition of protein synthesis could lead to a failure of the synthesis of lipoproteins (64) and result in the inability of the liver to transport triglycerides. Such a mechanism has been proposed for the development of fatty livers following ethionine and CCl_4 (65), and has not been ruled out for halogenated anesthetics. The connection, if any, between fatty livers and necrosis is uncertain. Fatty infiltration is often a prelude to necrosis but the degree of insult sufficient to cause cellular death remains unknown (5).

If a nonvolatile metabolite of an anesthetic is able to accumulate in the mitochondria to concentrations sufficient to interfere with respiration and/or phosphorylation (66–68), this process might, under certain circumstances, result in the disruption of the mitochondrial membranes with the release of serologically active phospholipids (69, 70) or lipoproteins (71). Furthermore, anesthetic metabolites could be bound to liberated fragments of subcellular membranes, whether of mito-

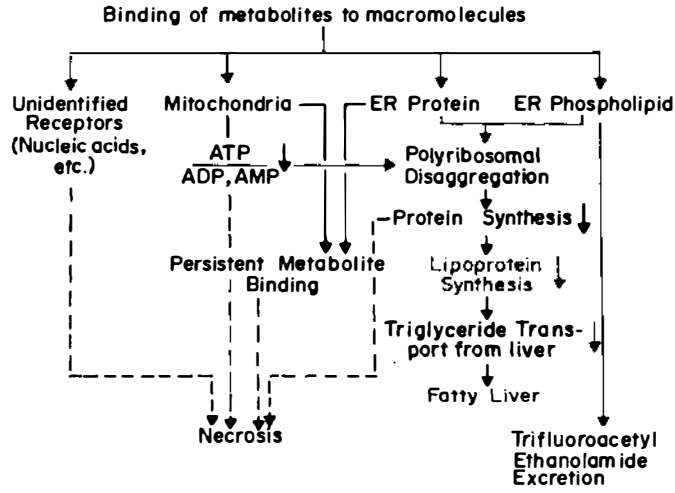

Figure 6 Possible mechanisms of the toxic action of the metabolites of inhalation anesthetics.

chondrial or endoplasmic reticular origin (34) and acting as haptens give rise to other immunologically active species (72–74). Thus, the trifluoroacetyl ethanolamide excreted by man following halothane anesthesia (3) would be expected to be of a subcellular membrane origin.

Recent work suggests that the condition of the liver determines its response to the presence of anesthetics (59). Centrolobular necrosis has been observed in rats pretreated with phenobarbital followed by exposure to halothane (75). Another model of liver injury has been investigated in which rats were pretreated with polychlorinated biphenyls prior to exposure to halothane (E. S. Reynolds, personal communication).

Possible interactions among halothane metabolite free radical formation, glutathione, and lipoperoxidation are illustrated in Figure 7.

Hepatic NADPH and reduced glutathione (GSH) levels decreased following i.p. injection of very large doses of halothane (2000 mg/kg) and proposed halothane metabolites (76, 77). The decrease in tissue levels of NADPH and GSH could have been attributable to the formation of glutathione-metabolite conjugates with GSH in the role of a free-radical scavenger (77). A proposed inhibition of glutathione reductase (77) would not account for the NADPH depletion, but the profound respiratory insufficiency that undoubtedly accompanied the injection of 2000 mg/kg of liquid halothane could.

Excess free radical formation from halothane, accelerated by pretreatment with phenobarbital (75), could result in the covalent binding of metabolites to macromolecules (Figure 6) and the triggering of peroxidation of phospholipids of subcellular membranes (78). This would explain the detection of diene conjugates in the urine of animals exposed to halothane (59, 75, 78).

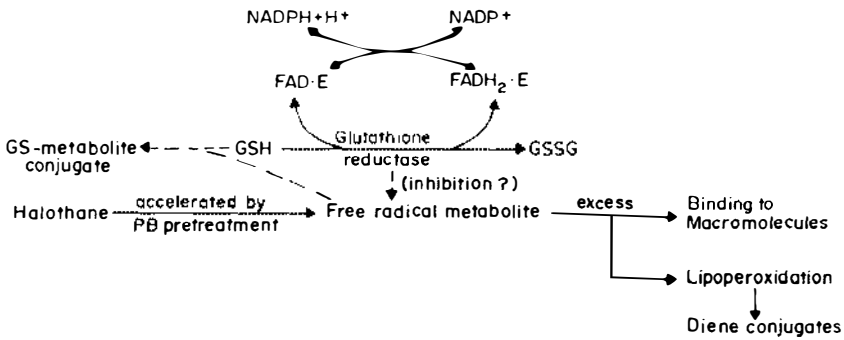

Figure 7 Possible interactions of halothane metabolites with glutathione and their relationships to lipoperoxidation.

Not only has a methoxyflurane-induced nephropathy been identified as a clinical entity, but the etiology also has been defined with a reasonable degree of certainty (79). Of the principal metabolites, inorganic fluoride has been incriminated as the probable cause of the usually reversible, vasopressin-resistant polyuria associated with methoxyflurane anesthesia (79–81). Oxalate probably is not formed in sufficient quantity to be of clinical significance in the pathogenesis of clinical methoxyflurane nephropathy (79, 82, 83). A persistent renal insufficiency has been reported following methoxyflurane, but the pathogenesis is not well understood (84).

The impairment of the ability of the kidney to concentrate urine is thought to be the consequence of a reduction of the corticomedullary concentration gradient (80).

TOXICOLOGY OF CHRONIC EXPOSURE TO ANESTHETIC GASES

Overview

The report by Vaisman (85) in 1967 of an increased incidence of reproductive failure in women working in the operating room (OR) has sparked a growing interest in the potential consequences of occupational exposure to anesthetic gases. Linde & Bruce (86) measured an average of 1.8 ppm of halothane in the end-expired air of 24 anesthetists within 1 hr of leaving the OR following exposures of 1–2 hr. In another series involving eight subjects they detected end-expired mean levels of halothane of 3.7 ppm after an average exposure to 5.3 ppm for 2 hr. A significant correlation ($r = 0.79$) existed between the end-expired levels of the anesthetic and the room air concentration times the duration of exposure. Corbett & Ball (87) made similar observations on personnel exposed to methoxyflurane and further detected significant elevations in urinary fluoride excretion within 5 hr after exposure. They also were able to reduce OR air levels of 1.3–9.8 ppm of methoxyflurane to 0.015–0.095 ppm through the use of a waste anesthetic gas scavenging system. Halothane was detected in the expired air of anesthesiologists for 7–64 hr after exposure to 1–10 ppm for 20–390 min (88).

Table 1 Methoxyflurane-induced nephrotoxicity; dose-response relationships

Serum inorganic F^- μmol/liter	MAC-Hr[a]	Toxic response	Reference
< 40	—	0/11, none	83
< 40	< 2.0	0/7, none	81
50–80	2.5–3.0	3/4, subclinical 1/4, mild clinical	81
80–175	> 5.0	3/7, mild clinical 3/7, clinical	81

[a]Minimum (alveolar) anesthetic concentration (end-expiratory) times duration of anesthesia.

Bruce et al (89) in a retrospective study of mortality among anesthesiologists over a period of 20 yr found significantly higher death rates from malignancies of lymphoid-reticuloendothelial origin, but no difference in death rates from leukemia when compared to rates among US males in general. Corbett et al (90) reported a higher than expected incidence of malignancies among Michigan nurse-anesthetists during 1971.

Reproductive histories of operating-room personnel have been surveyed (91, 92), and the results of two such studies are summarized in Table 2. In addition to higher incidences of spontaneous miscarriage among the OR personnel, both studies revealed that the miscarriages occurred earlier in pregnancy in the exposed versus the control groups. The conclusion by one group (92) that the increased rate of abortion among the exposed women was probably attributable to stress rather than to some other variable such as exposure to traces of anesthetic gases was not well supported by their evidence, particularly in view of the fact that they took reasonable care to match their controls on the basis of occupational stress.

Corbett et al (93) have suggested the possibility of a teratogenic hazard associated with occupational exposure to anesthetic gases. A significantly higher than expected incidence of cavernous skin hemangiomas and musculoskeletal anomalies appeared in the offspring of 641 female nurse-anesthetists surveyed.

Pregnant hamsters (94) and pregnant rats (95) have been exposed repeatedly late in the first third of gestation to anesthetic concentrations of N_2O-halothane. Results from both studies suggested that exposure to halothane early in pregnancy may have

Table 2 Incidence of spontaneous abortion among operating-room personnel

Occupationally exposed	Controls	Reference
29.7%[a] (67)[b]	8.8% (92)	91
37.8%[a] (50)[b]	10.3% (81)	92
19.5%[a] (182)[b]	11.4% (118)	92

[a]Incidence among total number of pregnancies occurring during periods of employment in OR.

[b]Number of individuals surveyed.

increased the incidence of reproductive failure. These results cannot be extrapolated directly to man, but along with the foregoing discussion they should serve to alert the medical community to the possible harmful effects of inhalation anesthetics on human reproductive biology.

Report of the American Society of Anesthesiologists Ad Hoc Committee on Effects of Trace Anesthetic Agents on Health of Operating Room Personnel

This retrospective study (96) was based on the responses to 73,496 mailed questionnaires. Of these, 49,585 represented exposed operating room personnel and 23,911 represented the control group. The survey provided statistically significant evidence that the risk of spontaneous abortion was increased by exposure to the operating-room environment during the first trimester of pregnancy. The liveborn offspring of both the women directly exposed and the wives of men exposed to the OR environment had an increased incidence of congenital abnormalities. Higher rates of cancer and hepatic and renal diseases were found in exposed females but not in exposed males. The committee concluded that the hypothesis that exposure to the OR atmosphere posed a significant health hazard to operating room personnel be weighed carefully.

Central Nervous System Correlates of Chronic Exposure to Inhalation Anesthetics

An interesting and thus far totally inexplicable finding in the study by Bruce et al (89) was that anesthesiologists had a higher than expected death rate from suicide during the period of 1947–1966. Whether or not any connection exists between this observation and their occupational exposure to waste anesthetic gases is a matter of conjecture. Exposure of volunteers to trace levels of N_2O-halothane for 4 hr caused six of twenty to fall asleep during psychological testing and significantly increased psychomotor reaction time (97).

Chronic exposure of young rats to 90 ppm of halothane resulted in deficits in responses to negatively and positively reinforced operant training (98). Exposure as adults was without a similar effect. Acute exposure of volunteers to higher, but subanesthetic, levels of methoxyflurane, enflurane, or isoflurane suggested that the anesthetics as a group caused impairment of memory functions (99). The evidence supports the idea that the possibility of behavioral modification in the presence of very low levels of anesthetic gases is a matter deserving critical examination.

Furthermore, it is possible that a connection between structure and function ultimately will be found. Ultrastructural changes in the rat central nervous system were detected after exposure for 8 wk to 10 ppm halothane (100), and differentiation in cultured mouse neuroblastoma cells exposed for 72 hr to 0.3–2.1% halothane was impaired (101).

On Cleaning Up the Environment

Evidence has been presented suggesting that chronic exposure to traces of anesthetic gases may constitute a significant occupational hazard to operating-room personnel. It would, therefore, seem prudent to take steps to reduce such pollution in the

operating room pending further investigation. Escape of significant quantities of anesthetic gases from both closed and open systems is inevitable. This may be controlled by active scavenging systems (102, 103) that capture waste gases to be disposed of through venting systems or by the incorporation of activated charcoal adsorbent cannisters to the expiratory circuit (104). Such measures are easily instituted, and the hardware is relatively cheap to install. The potential benefit would certainly seem to be worth the investment while we are waiting for the results of further studies of the problem.

CONCLUSION

A large fraction of the halothane absorbed by the liver is strongly bound to subcellular fractions. The halothane is presumably in the form of metabolites and probably covalently bound. Furthermore, liver injury sustained in the presence of halothane may be dependent on the prior condition of the liver, that is, macromolecular constitution as an expression of genotype and the effects of chemical agents other than halothane. The identification of metabolites and their interaction with subcellular constituents may provide the basis for the future resolution of the problem of "halothane hepatitis."

The search continues for a better general anesthetic. All tested so far are biotransformed to a measurable extent. Isoflurane is apparently metabolized to a lesser extent than the others and in this respect may represent a step forward in the search for the "ideal" inhalation anesthetic.

Presumptive evidence has been presented that exposure to trace levels of anesthetic gases may adversely affect human reproductive and behavioral processes. The further investigation of these potential problems is of paramount importance. Meantime the prudent course of action would seem to be to institute measures to reduce the contamination of the operating-room atmosphere by waste anesthetic gases.

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