TOXICITY OF INHALATION ANAESTHETIC AGENTS

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Although hundreds of compounds on the chemist's shelf are known to produce anaesthesia, relatively few are used clinically. The requirements for a clinically useful anaesthetic agent are necessarily stringent and include: a wide margin of safety, total reversibility of anaesthetic effect and an absence of long-term toxicity. In addition, a successful inhalation anaesthetic agent must combine the capacity for depression of the central nervous system with low levels of interference with respiration and the circulation. The central nervous system effects must be quickly reversible.

The acute depressant effects produced by the inhalation anaesthetics contrast with the slower development of long-term toxicity involving organs such as the liver, the kidneys or the reproductive system. Although such long-term toxicity associated with anaesthetics has been recognized for many years, in the past it has been considered to occur infrequently, to be of little importance and of undetermined aetiology. More recent findings suggest that not only are these long-term effects serious, but they occur more frequently than was previously recognized. In addition the aetiiological factors are now beginning to be understood.

RELATIONSHIP BETWEEN ANAESTHETIC METABOLISM AND TOXICITY

Several reports now suggest there is a significant association between the metabolism of anaesthetic agents and the development of toxicity. Whilst the majority of drugs are metabolized in the body to less toxic derivatives, this is not always so. Occasionally, chemically inert compounds can be transformed into reactive metabolites which can combine with tissue macromolecules. These combinations may be toxic. For example, bromobenzene is metabolized to an active epoxide (Reid et al., 1971; Mitchell, Jollow and Gillette, 1973). A second example is seen with the hepatic necrosis produced by acetaminophen U.S.P. (paracetamol) (Brodie et al., 1971; Gillette, 1974). The centrilobular necrosis produced by this drug is enhanced by pretreating animals with phenobarbitone which induces the mixed function oxidase system: in contrast, liver necrosis is prevented by prior use of an enzyme inhibitor such as piperonyl butoxide (Mitchell, Jollow and Gillette, 1973) or cobaltous chloride (Tephly and Hibbeln, 1971).

A similar relationship between drug metabolism and toxicity would appear to apply to many of our inhalation anaesthetics. Few anaesthetic agents, with the exception of chloroform, are capable of producing direct cellular damage by themselves, but the hepatic and renal toxicity is related to metabolism in a dose-dependent fashion. For example, Scholler (1970) found that the hepatotoxicity of chloroform was associated with its metabolism. Chloroform anaesthesia in rats pretreated with phenobarbitone which induces the liver enzymes led to extensive hepatic necrosis; pretreatment with the enzyme inhibitor disulfiram reduced or abolished the hepatic damage.

An association between anaesthetic metabolism and toxicity also occurs with other agents. Cascorbi and Singh-Amaranth (1972) observed that, whereas 20% of mice anaesthetized with fluroxene for 1 h died within 5–24 h, if animals were pretreated with phenobarbitone the mortality was 95%. However, pretreatment with carbon tetrachloride which depressed the non-specific hepatic enzymes markedly reduced the mortality following fluroxene anaesthesia. With halothane, the association between metabolism and toxicity has been more difficult to define. The lack of an adequate animal model was a serious problem. The important advance was the use of animals pretreated with an enzyme-inducing agent. Stenger and Johnson (1972) and Reynolds and Moslen (1974) observed sub-capsular hepatic necrosis following the administration of halothane to rats pretreated with phenobarbitone. More recently,
pretreatment of rats with the polychlorinated biphenyl Aroclor 1254, was found to lead to a widespread hepatic necrosis following a halothane anaesthetic (Reynolds, Moslen and Szalo, 1976; Sipes and Brown, 1976). Within a few hours of anaesthesia the serum transaminase concentrations were increased and there were severe morphological changes in the centrilobular parenchymal cells.

There are convincing reports that the nephrotoxicity seen following methoxyflurane is the result of the metabolic production of fluoride ions in both Fischer 344 rats and in man (Mazze, Cousins and Kosek, 1972; Cousins, Mazze and Kosek, 1974). In rats Mazze, Cousins and Kosek (1972) showed that the direct injection of sodium fluoride is capable of producing renal damage identical to that seen following methoxyflurane anaesthesia.

**MECHANISMS OF TOXICITY**

Three alternative pathways have been proposed to link metabolism of anaesthetic agents and their toxicity: the accumulation to toxic concentrations of metabolites normally readily excreted; the production of reactive intermediates that form irreversible bonds with tissue macromolecules and, finally, the formation of haptens that could produce hypersensitive or immune responses. In each case metabolism of the agent is implicated.

**Excreted metabolites**

A number of metabolites of anaesthetic agents which are excreted in the urine have been identified and their potential toxicity determined.

**Trifluoroacetic acid** is a major metabolite of halothane, fluroxene and isoflurane in several animals. However, chronic administration shows it to have a low level of toxicity (Schimmasek et al., 1966; Stier et al., 1972). Its low toxicity following oral, i.v. or i.p. administration is related to its poor absorption. In the physiological range of pH values it is ionized and is thus unlikely to penetrate cell membranes from the outside.

**Trifluoroethanol** is present in the urine following fluroxene anaesthesia and is a suggested (but unproven) metabolite of halothane. In mice its LD₅₀ varies from 158 to 350 mg kg⁻¹ (Airaksinen and Tammisto, 1968; Blake et al., 1969). Trifluoroethanol is produced in only small amounts in humans anaesthetized with fluroxene and has not been demonstrated after administration of halothane: it seems that it poses little clinical risk (Gion et al., 1974).

**Inorganic fluoride.** This toxic metabolite is released in large amounts during and following methoxyflurane anaesthesia and in lesser amounts with enfurane, halothane and isoflurane. The amount released with halothane and isoflurane is normally very small. Fluoride concentrations can increase to nephrotoxic values (in excess of 40–50 μmol litre⁻¹) following methoxyflurane (Mazze, Shue and Jackson, 1971). The amount of renal damage is related to the fluoride concentration.

**Oxalic acid.** There is an increased urinary excretion of oxalic acid following methoxyflurane anaesthesia and crystalline oxalate deposits have been demonstrated in the kidneys. Although renal toxicity as a result of tubular obstruction is known to follow high urinary oxalate concentrations, toxicity only occurs at concentrations 10 times greater than those found following the clinical use of methoxyflurane. The renal lesion produced by methoxyflurane is histologically and functionally different from that seen with oxalate (Cousins, Mazze and Kosek, 1974).

**Reactive intermediates**

Although the halogenated inhalation anaesthetic agents are normally considered to be unreactive, it appears that under appropriate conditions they can be metabolized to reactive free radicals capable of combining with cellular constituents. In most stable compounds the electron orbitals of all the atoms are filled with pairs of electrons of opposite spin. However, some physical or chemical stresses, such as those induced by metabolism, may disrupt this balanced state, producing compounds containing an atom with a single impaired electron in an outer orbit. This type of compound is a reactive free radical.

There are a number of ways in which such a free radical might be produced by metabolism of an anaesthetic drug. For example, hydroxylation and dehalogenation of halothane by its interaction with the cytochrome P-450 system could produce a reactive acyl halide:

\[
\text{F-Br} \stackrel{\text{P-450}}{\rightarrow} \text{F-C-OH} \stackrel{\text{HBr}}{\rightarrow} \text{F-C-Cl}
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**Trifluoroacetaldehyde** is a suggested urinary metabolite of halothane which has yet to be isolated in man or experimental animals. When given i.v. or i.p. in mice its LD₅₀ is 600 mg kg⁻¹, which places it between trifluoroacetic acid and trifluoroethanol (Airaksinen and Tammisto, 1968).
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It has also been suggested that, under some conditions, cytochrome P-450 is capable of removing a proton to form a reactive carbanion (Ullrich and Schnabel, 1973):

\[ F \text{Br} \quad F \text{Br} \]
\[ \downarrow \quad \downarrow \]
\[ F-C-C-H -H^+ \rightarrow F-C-C-\cdot \]
\[ \quad \downarrow \quad \downarrow \]
\[ F \text{Cl} \quad F \text{Cl} \]

By analogy with the work of Van Duuren and his colleagues (1972) with alpha-haloethers it is possible that the following reaction may occur:

\[ F \text{Br} \quad F \text{H} \]
\[ \downarrow \quad \downarrow \]
\[ F-C-C-H -\text{Br} \rightarrow F-C-C-\cdot \]
\[ \quad \downarrow \quad \downarrow \]
\[ F \text{Cl} \quad F \text{Cl} \]

The formation of free radicals and reactive intermediates is consistent with evidence showing that the metabolites bind covalently to liver microsomes and to tissue macromolecules (Uehleke, Hellmer and Tarbelli-Poplawski, 1973; Van Dyke and Wood, 1975).

The cellular damage produced as a result of the binding of a reactive metabolite varies depending on the molecular aggregate formed. The molecules with the highest susceptibility for damage include the unsaturated fatty acids and nucleic acids. Fatty acid complexes with phosphatidylcholine and phosphatidylethanolamine are the major components of the membranes of liver endoplasmic reticulum and mitochondria. Since inhalation anaesthetic agents are strongly lipophilic they can be expected to be closely associated with these lipoprotein membranes. The lipid constituents are particularly rich in unsaturated fatty acids and, thus, binding of the reactive intermediates would tend to occur within this milieu in the hepatic endoplasmic reticulum.

The action of the reactive intermediate may commence with an attack on the alpha-methylene carbon atom of the unsaturated fatty acid. The presence of the double bond weakens the carbon-hydrogen bond adjacent to the unsaturated bond. Free radical intermediates derived from the halogenated anaesthetics are thus able to initiate peroxidative decomposition of the lipids by abstracting allylic hydrogen from the alpha-methylene carbon atom, thereby rearranging the position of the double bonds.

A subsequent attack by oxygen produces cleavage of the radical (fig. 1) (Brown and Sagalyn, 1975). Unless terminated, this oxidative deterioration will be transferred sequentially to adjacent fatty acid complexes in a propagated autocatalytic chain (Recknagel and Ghoshal, 1966).

Brown (1972) produced evidence of hepatic microsomal lipoperoxidation in the rat following the administration of chloroform and halothane. Using rats pretreated with phenobarbitone, he measured the increase in diene conjugation resulting from lipoperoxidation in hepatic microsomes. Brown, Sipes and Sagalyn (1974) found, in animals pretreated with phenobarbitone, that chloroform anaesthesia produced a decrease in the liver content of glutathione and an increase in hepatic necrosis. Pretreatment with diethyl malate also decreases glutathione concentrations and is associated with liver damage. It appears that, if the tissue concentrations of such antioxidants reach critically low values, the reactive metabolites formed from chloroform are no longer

![Fig. 1. Possible mechanism for lipoperoxidation.](https://academic.oup.com/bja/article-abstract/50/7/665/580055)
quenched and become free to promote tissue damage by lipoperoxidation.

Wood, Gandolfi and Van Dyke (1976) investigated the binding of halothane metabolites to rat liver microsomes incubated in nitrogen. Under such anaerobic conditions greater diene conjugation occurred although the formation of malonaldehyde and other "non-peroxide-conjugated-dienes" was less in the anaerobic than in the aerobic state. Thus it seems that the initial step in lipoperoxidation occurs in the absence of oxygen, but peroxidation does not occur. In aerobic conditions the smaller amount of binding of the products of halothane to lipids may reflect a preferential oxidation of halothane and its radicals with the ultimate formation of trifluoroacetic acid.

Hypersensitivity and immune responses

It is possible that the rare hepatic injury seen following the use of halogenated anaesthetic agents might have an allergic or hypersensitivity basis. The reactions seen are usually considered to represent a delayed or cell-mediated sensitivity produced by specialized mononuclear cells, thymus-derived lymphocytes, or T cells which react with membrane-bound antigens. Whilst it is generally accepted that simple non-reactive molecules such as the halogenated anaesthetics cannot be antigens, their metabolites are reactive and capable of forming stable bonds with proteins or other macromolecules.

The presence in vivo of such metabolites which are covalently bound to macromolecules in the liver has been demonstrated by a number of workers. Rosenberg and Wahlstrom (1973) attempted to show whether trifluoroacetic acid and the other "presumed" halothane metabolites such as trifluoroethanol, trifluoroacetaldehyde and trifluoroacetic anhydride could act as hapten's in rabbits immunized with a chicken serum globulin complex. Although their results suggested that the immune serum formed similar precipitins against the complexes, the findings are not totally convincing. Later studies by Mathieu and his colleagues (1974) using a trifluoroacetyl guineapig albumin complex showed that guineapigs display a typical delayed-type hypersensitivity. There is therefore evidence to show that anaesthetic metabolites, when complexed to proteins, can act as hapten's. The fact that a delayed skin hypersensitivity can be induced does not mean that such hapten complexes are also capable of causing autoimmune liver destruction.

Specific organ toxicity

A direct relationship between the metabolism of anaesthetic drugs and their long-term toxicity has been firmly established. A reduction in the metabolism of such drugs by pretreatment with enzyme inhibitors or by antioxidants prevents the toxicity of chloroform and fluroxene in several species. In addition, increased toxicity with chloroform, halothane, methoxyflurane or fluroxene follows pretreatment with enzyme induction agents such as phenobarbitone or 3-methylcholanthrene. The aetiology of such toxicity may relate to the formation of either reactive metabolites which bind to macromolecules or toxic metabolic end-products such as trifluoroethanol or fluoride ions. Three organ systems appear to be the major targets: the liver, the kidneys and the reproductive organs.

Hepatotoxicity

Liver damage produced by chlorinated hydrocarbons such as carbon tetrachloride and chloroform is well known. Within 3 years of its introduction in 1847, Casper reported the first case of delayed chloroform toxicity (cited by Techendorf, 1921). Chloroform can be considered a true hepatotoxin, since its effects on the liver increase with dose and duration of exposure. The necrosis is preceded by fatty changes in the liver cells nearest the central veins. As the damage increases the necrosis spreads to involve the whole lobule with the last area of destruction being adjacent to the portal tracts.

An alternative explanation to a direct effect would be related to metabolism of the chloroform. Scholler (1970) found that pretreatment with the anti-metabolite disulfiram prevented the hepatotoxicity of chloroform. Similarly, in the newborn chloroform does not damage the liver, presumably as a result of the lack of development of the drug-metabolizing enzyme system (Kato and Gillette, 1965). In contrast, phenobarbitone pretreatment significantly increases the toxicity of chloroform (Brown and Sagalyn, 1974). The exact mechanism has not yet been defined, but it has been suggested that the damage is produced as a result of the binding of free radicals to cellular constituents of the liver.

Although one might suspect that most halogenated anaesthetic agents produce liver damage in an identical fashion, this does not appear to be the case. Admittedly, each is capable of causing hepatotoxicity, but the incidence and mechanisms are different. For example, halothane was introduced only after careful
animal experimentation had indicated it not to be metabolized and to lack hepatotoxicity. Millions of anaesthetics later, it would seem that halothane may be the cause of rare cases of hepatic necrosis. Although this relationship of halothane to liver damage is difficult to establish in the clinical situation, such a relationship has been found to occur in rats following pretreatment with the enzyme inducer, Aroclor 1254 (Reynolds, Moslen, and Szaló, 1976; Sipes and Brown, 1976). Similarly, there appears to be a relationship between trace-dose concentrations of halothane and hepatotoxicity. Stevens and his colleagues (1975) exposed rats in a chamber for 4 weeks to halothane concentrations equivalent to 1/30 MAC. The animals showed hepatotoxicity with vascular degeneration, lipidosis, focal and zonal necrosis. Similar animals exposed at 1/600 MAC showed no changes, and animals exposed to intermediate concentrations exhibited corresponding degrees of hepatic injury. These findings were interpreted as showing that halothane was capable of producing injury by a direct hepatotoxic action through the mechanism of anaesthetic metabolism.

There is some evidence that personnel working in operating rooms who are exposed to trace concentrations of inhalation anaesthetic agents may develop hepatotoxicity. Measurable concentrations of these gases can be found in all operating rooms where they are used (Linde and Bruce, 1969; Whitcher, Cohen and Trudell, 1971). Although the concentrations are small and measured in parts per million, continuing exposure can result in the absorption of a significant amount of anaesthetic. Once these highly lipid-soluble anaesthetics are taken into the body they are eliminated relatively slowly. While most are excreted intact within several hours, significant amounts remain in the body and are slowly biodegraded over days and weeks. The incidence of hepatotoxicity in exposed operating room personnel has been examined in several large surveys; it appears to correlate with the presence of trace anaesthetic gas concentrations and the duration of the operating room exposure (Cohen et al., 1974). The increase in hepatic disease among exposed physician anaesthetists was found to be approximately twice that of the unexposed control group. A similar study amongst dentists exposed to higher waste anaesthetic gases in their practice showed an even greater incidence of hepatotoxicity (Cohen, Brown et al., 1975).

Halothane. Although the precise cause of halothane hepatotoxicity remains to be established, its metabolism appears to play an essential role whether the presumed cause is hypersensitivity, the accumulation of toxic metabolites, or the conjugation of reactive intermediates.

The clinical picture of fever, malaise, arthralgia, eosinophilia or lymphocytopenia supports the diagnosis of a hypersensitivity-type response. Unfortunately, these signs do not always appear and, in addition, similar symptoms accompany viral hepatitis. A number of in vitro tests which depend on the development of cell-mediated sensitivity have been designed to diagnose "halothane hepatitis". Such tests include lymphocyte transformation and the appearance of antimitochondrial antibodies (Rodrigues et al., 1969; Paronetto and Popper, 1970). Although both tests were reported to be highly specific, subsequent investigators have failed to confirm these findings.

The presence of a previous exposure to halothane in more than half the reported cases of hepatitis is in accord with the sensitization hypothesis. However, with the prevalent use of this anaesthetic, multiple exposures would be expected to occur with increasing frequency. A small number of individuals with previous episodes of hepatitis have actually undergone a challenge test with halothane (Belfrage, Ahlgren and Axelsson, 1966; Klatskin and Kimberg, 1969). Two well-known cases of anaesthetists challenged with small doses of halothane attest to the existence of a hypersensitivity reaction, although the significance of these reports has recently been questioned (Simpson, Strunin and Walton, 1973). Finally, there are data which suggest a significant relationship between repeated exposures to halothane and the rapidity with which jaundice develops following subsequent exposures (Inman and Mushin, 1974). Although there appears to be a decreasing time interval for the development of jaundice following repeated exposures to halothane, these data have also come under question. Thus, while it is tempting to postulate a causal relationship between the hypersensitivity response and hepatotoxicity to halothane, the data are equivocal.

The metabolism of halothane to toxic end-products has also been suggested as an explanation for the liver damage. Studies in the mouse and guinea pig with parenterally administered trifluoroacetic acid, as well as with some presumed halothane metabolites, indicate varying degrees of damage (Airaksinen and Tammisto, 1968). However, the route of administration employed in these studies may not have allowed the metabolites to reach certain critical intracellular
areas, which would be directly accessible to metabolites produced in vivo. There also appears to be only limited toxicity to the final metabolites of halothane. These metabolites, although weakly toxic, may conceivably accumulate to hazardous concentrations as a result of repeated administrations of the anaesthetic, depression of excretory pathways, or increased production of the metabolites following induction of the drug-metabolizing enzymes.

Of more serious concern are the studies which indicate covalent binding of metabolites of halothane to macromolecules within the liver. The reactive intermediates are able to bind to both lipid and protein. Gandolfi and Van Dyke (1978) showed that the binding to phospholipids is through the fatty acid moiety. This binding is enhanced at lower oxygen tensions, following diethyl maleate-induced glutathione depletion or after pretreatment of the animal with a diet rich in polyunsaturated fatty acids. Enzyme induction increased the production of reactive intermediates and tended to promote increased binding. Hypoxia and a reduced antioxidant concentration also act to prolong the half-life of the intermediates and thus produce increasing binding.

Recent studies have described the final urinary metabolites of halothane in man and also indicate the presumed toxicity of the reactive intermediates (Cohen, Trudell et al., 1975). Although trifluoroacetic acid has been recognized as the final metabolite with limited toxicity, it may be that the oxidative formation of the trifluoroacetyl radical represents the key step in the metabolism of halothane. There is evidence that this radical may bind covalently with phosphatidylethanolamine, for N-trifluoroacetyl-ethanolamide is present as a urinary metabolite. The most likely source of the ethanolamide would be the phosphatidylethanolamine, which is a normal lipid constituent of cell membranes. Presence of the urinary metabolite suggests conjugation of the radical with the phosphatidylethanolamine in the cell membrane. The urinary metabolite would be produced as a result of enzymatic cleavage of this molecule. The accumulation of this metabolite within the cell could result from either the increased production of the radical following increased metabolism of halothane or the reduction in the amount of the phosphatase enzyme available for cleavage of the conjugated molecule.

The appearance of a cysteine conjugate of halothane in the urine is of considerable importance. The conjugate found in human urine was 2-bromo-2-chloro-1,1-difluoroethylene. It was suggested that this occurred following the reaction of glutathione with a reductive dehydrofluorination product of halothane. The highly reactive bromochlorodifluoroethylene intermediate could be formed as a result of proton abstraction from halothane by cytochrome P-450 (Ullrich and Schnabel, 1973).

It has been suggested that glutathione plays an important part in the body by its preferential conjugation of the reactive intermediates formed during metabolism of foreign compounds. In vivo studies in rats pretreated with phenobarbitone indicate that anaesthesia with chloroform, but not with halothane, decreases the concentration of glutathione in the liver (Brown, Sipes and Sagalyn, 1974). This latter finding may represent a species difference. Although the protective role of glutathione conjugation in man remains to be established, the administration of nucleophiles such as cysteine, cysteamine and dimercaprol prevents the liver damage produced in mice by paracetamol (acetaminophen) (Boylan and Chasseaud, 1967; Judah, McLean and McLean, 1970). Theoretically, the use of these agents offers a possibility for the prevention and treatment of halothane-induced hepatitis.

The pathways described in the preceding discussion indicate that the metabolism of halothane may proceed along either an oxidative or a reductive route. Under aerobic conditions, metabolism preferentially leads to fluoride, bromide and trifluoroacetic acid. Widger, Gandolfi and Van Dyke (1976) investigated the effects of a reduced oxygen tension on the metabolism of halothane. Rats exposed to a 7% inhaled oxygen concentration responded with significantly increased concentrations of fluoride ions in the plasma. This increase in dehalogenation under hypoxic conditions was also accompanied by a more than three-fold increase in the covalent binding of metabolites to the microsomal lipids. This greater binding during hypoxia supports the hypothesis that reductive metabolism will produce a more reactive chemical species.

Methoxyflurane. Hepatitis associated with methoxyfluorane has also been reported. Joshi and Conn (1974) reviewed 24 cases. In most respects the clinical aspects of this syndrome were indistinguishable from those found following halothane. Histologically, the lesion is identical with that of viral hepatitis. The majority of cases were in women. More than half had a previous exposure to either halothane or methoxyfluorane, and a cross sensitization between these two anaesthetic agents seems likely. Although most
authors support an immunological basis for this hepatitis, no patients have been purposely challenged with methoxyflurane to induce a recurrence. Several case reports, however, discuss patients with previous hepatic injury from methoxyflurane who have received a subsequent anaesthetic with a recurrence of the hepatic injury (Rosander, 1970; Brenner and Kaplan, 1971). There is also one report of hepatic injury following methoxyflurane administered in subanaesthetic concentrations for obstetric analgesia on each of two occasions (Rubinger, Davidson and Melmed, 1975). At present there are no data which define the specific reactive metabolites of the methoxyflurane that might act as possible haptons, although their existence seems possible.

**Fluroxene.** This anaesthetic drug has enjoyed a remarkable history of clinical safety in over 500,000 administrations. However, recent reports suggest the occasional occurrence of liver damage in man. Two fatal cases have been described in which the patients were on enzyme-inducing drugs, and in one individual this included both phenobaritone and diphenylhydantoin (Reynolds, Brown and Vandam, 1972; Tucker et al., 1973). The overall toxicity of fluroxene noted in several animal species appears to correlate with its metabolism to trifluoroethanol. However, humans metabolize fluroxene to trifluoroacetic acid, and only a very small amount is present in the urine as trifluoroethanol (Gion et al., 1974). There is no evidence which defines the cause of the hepatic injury in man following fluroxene anaesthesia; this syndrome does not correspond with the toxicity seen in the experimental animals. However, it does seem likely that fluroxene toxicity is associated with its metabolism, although perhaps along different pathways.

**Nephrotoxicity**

In the broadest sense all anaesthetic agents are nephrotoxic, since they characteristically produce a generalized depression of renal functions. These changes are largely secondary to effects of anaesthesia on the cardiovascular, sympathetic and endocrine systems. Renal blood flow and glomerular filtration rates usually return to normal within a few hours after the termination of the anaesthetic, although the ability to excrete a water load may be impaired somewhat longer. Persistent abnormalities of renal function, however, have been associated with the prolonged use of fluorinated anaesthetics.

**Methoxyflurane.** There is abundant evidence in man to show that renal toxicity is associated with high-dose methoxyflurane anaesthesia and that the syndrome of high output renal failure follows metabolism of the drug to inorganic fluoride ions. Evidence that it is the inorganic fluoride ion which produces the nephrotoxicity in man may be seen with the production of similar abnormalities in the rat following the i.v. injection of sodium fluoride in amounts calculated to produce concentrations similar to those seen with methoxyflurane anaesthesia (Cousins, Mazze and Kosek, 1974).

Although the mechanism by which the fluoride ion causes renal toxicity is unknown it is likely to be related to an interference in the transport of sodium in the proximal convoluted tubules (Kosek, Mazze and Cousins, 1972). The toxicity may also be produced from the potent inhibitory effects of fluoride ions on enzyme systems involved with cellular energy transfer (Wiseman, 1970). An additional possibility is that inhibition of adenylyl cyclase leads to interference with the action of antidiuretic hormone (Orloff and Handler, 1964). A fourth possibility is that inorganic fluoride, which is a potent vasodilator, interferes with the counter-current system of the kidney, leading to an increase in medullary blood flow, washout of sodium from the interstitium and loss of hypertonicity (Caruso, Maynard and DiStefano, 1970).

Although ethical considerations preclude studies in humans, the induction of the drug metabolizing systems in the rat with phenobarbitone has been shown to result in an increased production of inorganic fluoride and an increase in toxicity (Cousins, Mazze and Kosek, 1974). Pretreatment of the rats with the antimetabolite, SKF 525A-A, was shown to decrease the metabolism of methoxyflurane and to diminish its nephrotoxicity. There can be little question that the renal damage produced by methoxyflurane is associated with its metabolism and the production of inorganic fluoride ions.

**Enflurane, halothane, isoflurane and fluroxene.** Since each of these anaesthetic agents liberates fluoride ions during its metabolism, concern has arisen as to whether there is an associated nephrotoxicity. The metabolism of enflurane to inorganic fluoride is considerably less than that for methoxyflurane, with a peak serum inorganic fluoride concentration averaging 22 μmol litre⁻¹ following administration of a clinically anaesthetic concentration (Cousins et al., 1974). This peak concentration is significantly below that known to be associated with nephrotoxicity. Nonetheless, at least two case reports have appeared indicating nephrotoxicity following enflurane. In one,
histological evidence of renal damage appeared with only a modest increase in the serum inorganic fluoride concentration (Loehning and Mazze, 1974). Since this patient had severe pre-existing renal disease with a failing kidney transplant, the authors suggested that the toxicity threshold may have been lowered in the diseased kidney. In the second case it was reported that, following 6 h of uneventful enflurane anaesthesia, the serum inorganic fluoride ion concentration reached 93 μmol litre⁻¹ coinciding with evidence of renal failure (Eichhorn et al., 1976). Since this patient had received an enflurane anaesthetic 6 weeks earlier, the possibility of enzyme induction was suggested; no alternative causes for the renal failure were found.

Isolated cases of renal damage have also been reported in association with fluroxene anaesthesia (Reynolds, Brown and Vandam, 1972; Tucker et al., 1973). These patients suffered from hepatic involvement and in addition were receiving enzyme-inducing drugs. Under usual clinical conditions, one would not anticipate renal symptoms in conjunction with the limited release of fluoride seen with this anaesthetic agent.

Toxicity to the reproductive organs

The clinical observations of an increase in the spontaneous miscarriage rate, of a decreased fertility, and an increase in foetal abnormalities amongst children of operating room personnel exposed to waste anaesthetic gases suggest an effect of inhalation anaesthetics on the reproductive system (Knill-Jones et al., 1972; Cohen et al., 1974). Epidemiological surveys show that exposed female anaesthetists and their progeny may show a doubling of spontaneous miscarriage and foetal abnormality rates when compared with control groups of unexposed female paediatricians. Whilst there are no data which confirm a cause–effect relationship, there remains the possibility that these effects result from chronic exposure to waste anaesthetic gases. Furthermore, it is likely that the metabolism of such anaesthetics will be causally implicated. Cascorbi, Blake and Helrich (1970) suggested, in a small-scale study, that anaesthetists metabolized halothane more efficiently than did a control group of pharmacists, although the differences were not statistically significant. It is possible that the anaesthetists underwent an induction of their microsomal enzymes as a result of the chronic exposure to anaesthetic gases in the operating room.

Ghoneim and his colleagues (1975) found evidence to show that operating room exposure may also exert an inhibitory effect on other enzyme systems. Their study of the kinetics of Warfarin in anaesthesia residents showed a prolongation of the primary half-life by 53% following 4 months of operating room exposure. Recent studies with radioactive halothane indicate an unusually high concentration of the non-volatile metabolites in the ovaries and testes of heart transplant donors (Cohen and Van Dyke, 1977). The role of these metabolites in the previously mentioned toxicity in anaesthetists has not been defined. Despite the possibly serious implication of these studies, much further information is needed to identify the precise role of anaesthetic agents and their metabolites in the toxicity associated with the reproductive system.

ANAESTHETICS AND CARCINOGENICITY

Although there is legitimate concern regarding the relationship between cancer and anaesthesia, the evidence is fragmentary and only limited studies are at present available. Eschenbrenner (1945) reported the production of hepatoma in mice following the repeated oral administration of chloroform; however, the hepatic lesions appeared only if the anaesthetic dosage was large enough to produce liver necrosis. Kunz, Schaudie and Thomas (1969) demonstrated that a different histological picture of rat liver carcinoma resulted from the administration of nitrosamines, depending on whether the animals were anaesthetized with phenobarbitone, halothane or methoxyflurane. On the other hand, Peraino, Fry and Staffeldt (1971) showed a protective influence of phenobarbitone on the hepatocarcinogenesis induced in the rat by 2-acetylaminofluorene. Using an in vitro microbial system, Baden and his group (1976) were unable to produce mutagenicity in two histidine-dependent strains incubated with halothane in concentrations ranging from 0.1 to 30%. In an extension of these studies, mutagenicity was also absent following incubation with methoxyflurane, enflurane, trichloroethylene and isoflurane; in contrast, exposure to fluoroxyne in concentrations of 0.1 and 30% produced a six-fold increase in the numbers of mutant colonies (Baden et al., 1977).

In another approach, Saffiotti (1978) found that trichloroethylene, when administered in large intragastric doses, was capable of producing hepatic cancer in mice in a dose-dependent fashion. Concern over the effects of chronic exposure to inhalation anaesthetic agents led Corbett (1976) to investigate the offspring of mice exposed transplacentally and
after gestation to sub-anaesthetic concentrations of isoflurane. The results obtained after 15 months indicated a significant increase in hepatic neoplasms, with no increase in the control groups.

Evidence for the direct induction of human cancer by anaesthetic drugs is largely epidemiologic. At least two current studies suggest a relationship between female cancer and operating exposure, presumably causally related to the waste anaesthetic gases. A limited study by Corbett and his colleagues (1973) showed the incidence of cancer in nurse anaesthetists in Michigan to be three times that of the control group. Evidence from a large-scale national survey (Cohen et al., 1974) showed the incidence of cancer in exposed female physician anaesthetists to be twice that of unexposed female paediatricians. Somewhat smaller increases in the cancer rates were seen in operating room nurses. Of considerable interest is the as yet unexplained finding that this increase in cancer does not apply to the exposed male. In the exposed women, there was little differentiation in the type or location of cancer that developed, with the exception that the frequencies of leukaemia and lymphoma were increased three-fold.

The strong alkylating property of certain haloethers has been shown to be closely related to their carcinogenicity (Van Duuren et al., 1969, 1972). Of these compounds, bis(chloromethyl)ether was shown to be a strongly active carcinogen. An even higher degree of carcinogenicity has been assigned to vinyl chloride and its reactive by-products (Block, 1974). The ability of both these groups of hydrocarbons to form reactive intermediates is a property shared with many halogenated anaesthetic agents.

Although only limited data are available with regard to nitrous oxide there is some evidence that it is metabolized (Matsubara and Mori, 1968). It is known to be biologically active and an enzyme inducer (Remer, 1961; Eastwood et al., 1963). The following equilibrium serves to illustrate the oxidation states of nitrogen in aqueous solution:

\[ \text{NO}_3^- \rightleftharpoons \text{HNO}_2 \rightleftharpoons \text{NO} \rightleftharpoons \text{N}_2\text{O} \rightleftharpoons \text{N}_2 \rightleftharpoons \text{NH}_4^+ \]

Nitrous oxide is an intermediate (+) 1 oxidation state and could conceivably be metabolized by either an oxidative or a reductive mechanism. There is ample evidence to indicate the hepatic oxidation of amines and the reduction of nitro compounds (Mitchard, 1971; Weisburger and Weisburger, 1971). If the human liver can be shown to be capable of metabolizing nitrous oxide, particularly to nitric oxide or to the nitrite ion, the additional steps converting the latter compounds to alkyl nitrosamines and diazo alkanes have already been demonstrated (Wolfe and Wasserman, 1972).

**CONCLUSION**

It would appear that a number of mechanisms exist for the potential toxicity of volatile anaesthetic agents. Although the pathways are as yet incompletely defined there can be little doubt that metabolism plays a central role. It is thus essential that we continue our search for new and non-metabolizable anaesthetic agents. Investigations into the possible therapeutic use of anti-metabolite and antioxidant drugs would seem to be of interest. With persistent efforts we shall move closer towards the realization of our goal: to provide the safest possible anaesthetic to each of our patients.

**REFERENCES**


TOXICITY OF INHALATION ANAESTHETIC AGENTS


