

Review

Metabolism of Volatile Anesthetics

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CLINICAL anesthesia has been a reality for over a century and some of the agents used to produce anesthesia are among the oldest known drugs. Nevertheless, the information available to us concerning the biological disposition of anesthetic agents is extremely meager, while such information on other kinds of newer drugs is plentiful. One reason for this seems to be a universal acceptance of the notion that the volatile anesthetic agents are biochemically inert. For this and other reasons few workers have previously made any serious attempt to look for possible breakdown products of these agents.

Another possible explanation for this lack of interest in the biotransformation of the volatile anesthetics is the fact that most, if not all, theories of narcosis involve physical, rather than chemical, interaction. For this reason, investigators in this field may have felt little could be gained by investigating the chemical activity of the volatile anesthetic agents. However, Pittinger has recently commented¹ on the possibility that chemical reactivity plays a role in narcosis, and we shall attempt to add to this.

The information available in the literature is still extremely meager. However, we hope that the small fraction of work which has been completed will stimulate others to pursue this idea to its ultimate end.

Earlier studies on the disposition of the volatile anesthetics suffered in large part from lack of sensitivity of the methods employed. With the introduction of the use of radioactive isotopes, sufficient sensitivity has been achieved to perform studies on the biotransformation of these compounds. Indeed, it is only with radioactive isotopic techniques that such studies have been made successfully. An

inherent difficulty in these studies is the fact that following a single dose, which is about the only practical method, the volatile anesthetics are removed so rapidly from the body by exhalation that comparatively little metabolism is expected or found. With continuous administration of the anesthetic, the major portion of the anesthetic is present in areas of the body that do not participate in the metabolic process and therefore the extent of metabolism appears to be extremely small. In reality, certain organs metabolize a high percentage of the anesthetic presented to them. Therefore, in studies of this type the percentage of administered anesthetic which is metabolized is small but on an absolute basis the amount of metabolism is fairly large. In general those anesthetics which have been found to be metabolized are converted to CO₂ and urinary metabolites, to the extent of 1.5 to 12 per cent of a given dose.

It is important to note that the metabolism of volatile anesthetics occurs in microsomes for it is in the microsomes where most of the drug metabolizing enzymes now known are found. In addition, most of the drug metabolizing reactions require reduced nicotinamide adenine dinucleotide phosphate (NADPH), the cofactor responsible for hydrogen ion transfer. Thus, while it is not certain which of the many enzymes found in microsomes are involved in metabolism of volatile anesthetics, it is reasonable to assume they are subject to the same variation in amount and activity as has been found to be the case for other drug metabolizing enzymes.

A key, but often overlooked, consideration is the purity of the material used for study. It is extremely important that the purity of the labelled anesthetics be as high as possible and also that the impurities, if present, be known, for many such microcontaminants are unstable under any conditions and yield falsely positive results.

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Hydrocarbons

Ethylene is the simplest of the anesthetic hydrocarbons, and while previously it had been considered not to be metabolized² recent preliminary experiments³ suggest otherwise. In these experiments it has been found that ¹⁴C-ethylene in rats is converted to ¹⁴C-carbon dioxide and labelled urinary products but the exact amounts have not as yet been determined. The problem with the determination of the metabolism of ethylene is two-fold, for the gas is difficult to administer quantitatively and is rapidly eliminated from the lungs by exhalation.⁴ Because of these difficulties, any indication of metabolism may be significant but at the same time not easily quantitated.

Ethylene is not, strictly speaking, a material foreign to animal tissue: it has been found by several investigators to be produced by liver mitochondria under certain conditions, *in vitro*.^{5, 6} Thus, the metabolism that seems to occur is not surprising because the organism may have the ability to metabolize the ethylene endogenously produced. An analogue of ethylene, tetrafluoroethylene has recently been found to combine with cyanocobalamin, an active cofactor form of vitamin B₁₂.⁷ The combination occurs between the tetrafluoroethylene and the cobalt which indicates that this analog of ethylene is biochemically reactive.

Propylene has not undergone a study of transformation.⁸ It would be of interest to determine this if only to ascertain whether it is more readily metabolized than ethylene. Acetylene likewise has undergone no study of its metabolism,⁸ thus evidence for or against metabolism is lacking. A recent finding that acetylene combines with derivatives of vitamin B₁₂⁹ indicates a possible site of reactivity and a means of metabolism.

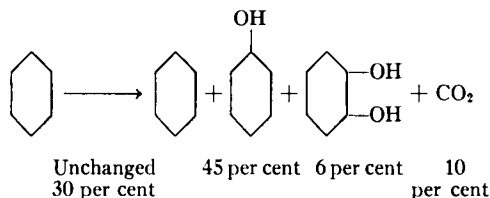
Alicyclic Hydrocarbons

The simplest of the cyclic compounds which produce anesthesia is cyclopropane. This compound has been found to be eliminated almost entirely by the lungs.⁸ Recent preliminary evidence has suggested that ¹⁴C-cyclopropane is converted to ¹⁴C-carbon dioxide in rats¹⁰; but again, as in the case of ethylene, quantitation is difficult as is maintenance of a par-

ticular concentration within the experimental animal. Therefore, until better methods are available, the data must remain preliminary. The cyclopropane ring is known to occur in nature (hypoglycin A, certain fats) and, if biosynthesized, it is reasonable to suppose it is degradable.

Cyclopentane and cyclohexane can also be considered at this point inasmuch as both can produce anesthesia.¹¹ Cyclopentane itself has not been studied *in vivo*. However, cyclopentylacetic acid and cyclopentenylacetic acid have been examined and appear to be metabolized completely.¹² Thus, it follows that the cyclopentane ring can be completely metabolized.

The metabolism of cyclohexane has been extensively studied.¹³ This compound has been found to be metabolized to the hydroxylated hexanol. According to Elliot, the break-down of cyclohexane is as follows:



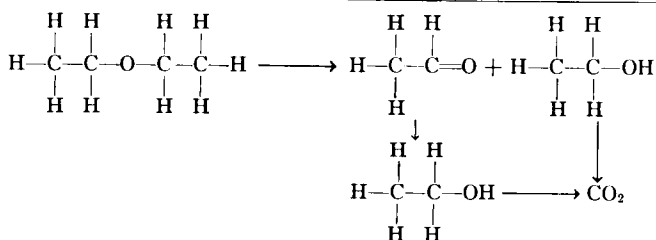
Ethers

It has been known for some time that certain ethers are metabolized by microsomes (Axelrod¹⁴). Until recently, the ether molecular structures ruptured did not include the anesthetic ethers. These short chain aliphatic ethers have been considered to be eliminated unchanged.^{15, 16} Recent work has indicated that, while a large amount of a given dose of ¹⁴C-diethyl ether is rapidly eliminated in the expired air, there is a measurable amount converted to ¹⁴C-carbon dioxide and labelled urinary metabolites.¹⁷ This provides the evidence that in some manner the short chain ether is cleaved. Additional evidence will be presented below, when the halogenated ethers are discussed.

There exists a certain, as yet unexplained, interaction between ethanol and anesthetics. Lee *et al.*¹⁸ have found that during the development of ethanol tolerance in rats, there is a stormy and prolonged induction period with diethyl ether and methoxyflurane. It has

also been stated that delirium is likely to occur in alcoholic patients¹⁹ when anesthesia is induced. This may be more than a fortuitous relationship since diethyl ether and ethanol are strong hydrogen bonding molecules. This may indicate a similar mode of action, or that they pass through the same intermediates during degradation.

It is highly likely that the mechanism of cleavage of the ether linkage in diethyl ether is by the enzymatic addition of a hydroxyl group at this point to form acetaldehyde and ethanol. The proposed mechanism is as follows:



The formation of an aldehyde intermediate is based on the fact that the product of O-demethylation, which is an ether cleavage, results in the formation of formaldehyde.²⁰

Other anesthetic ethers, such as methylpropyl ether, methylethyl ether, isopropylmethyl ether, ethylpropyl ether and divinyl ether have not been studied in terms of the metabolic transformation which they might undergo. However, since diethyl ether has been found to undergo metabolism it would not be surprising to find the others to be metabolized. Ethylvinyl ether is discussed later under the heading of halogenated ethers.

Paraldehyde, a cyclic polyether, has been shown to have mild hypnotic effects. Its metabolic fate has been studied to the extent that it is known to be excreted unchanged in rats, but in dogs the glucuronide is formed.¹¹ There are no data for man. Paraldehyde has been found to decompose readily to acetic acid when exposed to air and light.²¹ This may provide a clue as to the route of breakdown in biological systems.

Halogenated Hydrocarbons

The chloromethanes have been well studied as to distribution and metabolism. Several

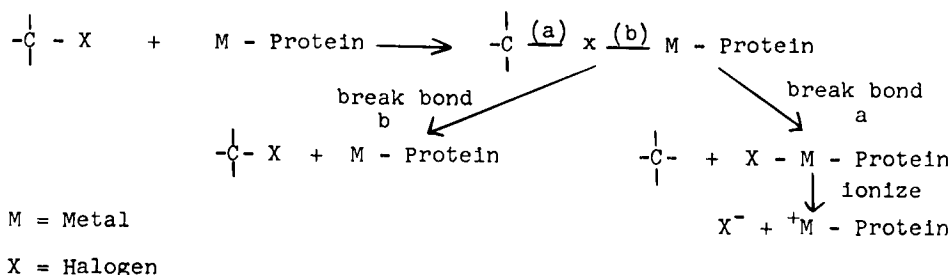
recent publications indicate that both chloroform and carbon tetrachloride are converted to carbon dioxide both *in vivo* and in the presence of liver slices.^{18, 22-25} The biochemical aspects pertaining to carbon tetrachloride poisoning have been particularly well covered in a recent review.²⁶

As early as 1947, it was shown by Heppel and Porterfield that a number of halogenated compounds are dehalogenated by enzymes found in a mixture of cell supernate and microsomes prepared from rat livers.²⁷ Furthermore, they found that the enzyme responsible could be concentrated by means of ammonium

sulfate fractionation. Unfortunately, it was not possible to state exactly where the enzymatic activity is located since no attempt was made to separate microsomes and cell supernate. Among the compounds found to be actively dehalogenated in this system were bromochloromethane, dibromomethane, dichloromethane, 1,2-dibromoethane, chloroform, bromoethane and 1-bromo-2-chloroethane. Bray *et al.*²⁸ have found that liver extracts are capable of a non-enzymatic liberation of chloride atoms from a number of aliphatic chlorine compounds. This appears to be the result of the formation of a carbon-sulfur bond with the loss of organic halogen. Bray also has shown a decrease in free sulfhydryl groups as a result of this alkylation. This type of reaction is interesting in that it evidently takes place for certain chloromethanes in the absence of enzymes: it also takes place for certain chlorinated aryl compounds, but only in the presence of an active cell supernatant enzyme which Boyland *et al.*²⁹ have labeled glutathionokinase. In the latter case, glutathione is required for the reaction but, in the former, any sulfhydryl containing compound will react as follows:

Because of the strong electrophilic nature of the halogens, halogen bridges of this type are known to occur³⁸ and the halogens may effect two bonds. If carbon 2 on intermediate no. 5 is attacked by a hydroxyl ion, bond (b) will be broken, resulting in trichloroethanol. This may even be enzymatically controlled. In addition, intermediate no. 5 may react directly with glucuronic acid to form the glucuronide of 1,1,1-trichloroethanol which is one of the urinary products; or it may react with the hydroxyl of water to yield the alcohol which is oxidized to the acid. In some cases, bond (a) may be broken with the addition of an hydroxyl at this point. Since this results in an unstable situation, chlorine would be detached from carbon and replaced with hydride ion, resulting in monochloroethanol, which is also an excretion product of trichloroethylene. As will be seen later, certain dechlorinations require NADPH which is able to transfer hydride ion; this mechanism is therefore, within the realm of possibility.

It is perhaps appropriate to indicate a general enzymatic mechanism for the removal of the halogens in these several halogenated materials. It is conceivable that the reaction occurs as follows:



In the above scheme the possibility of rupture exists of either of the bonds labelled (a) or (b). Which bond is broken is dependent upon two factors. One is the metal bound to protein. Of all the metals present in biological systems the metal most likely to foster this reaction is copper. Iron is also a possibility but is not as effective as copper. This reaction is similar to others which have been reported.³⁹

Secondly, the character of the replacement for X at C is equally important. If there are provisions for adding a hydroxyl to C, this will enhance rupture of bond (a). If the electron donating ability of the metal is great enough, this will cause break of bond (a) leaving $-\text{C}^{(-)}$, which in turn may react with a proton: the net result would be a reductive dehalogenation. If the two conditions as outlined above are not met, the bond (b) will not exist for very long and the complex will dissociate. However, if the concentration of the halogenated material is high, another molecule will instantly replace the first; in other words, a dynamic equilibrium is established.

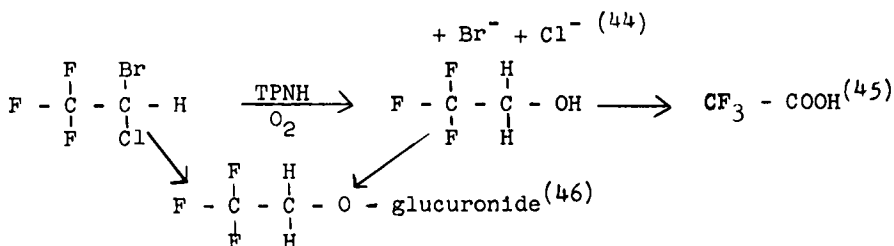
Halothane (1,1,1-trifluoro-2-bromochloroethane) has recently been shown to be metabolized both in rats *in vivo*⁴¹ and *in vitro*⁴² using 1-¹⁴C halothane and ³⁶Cl-halothane. Presumptive evidence for the removal of the bromide *in vivo* in man has also been reported,⁴³ while more definitive data have appeared recently.^{44, 45}

It is interesting to note that the carbon-fluorine bond in halothane is not easily broken⁴¹ as evidenced by the fact that 1-¹⁴C halothane

gives rise to very little ¹⁴CO₂. Using ³⁶Cl-halothane, it has been found that the carbon-chlorine bond is broken enzymatically by enzymes found in microsomes, this reaction requiring NADPH and oxygen.⁴² There is no chloride removal by the cell supernate alone. It is not known if the carbon-bromine bond is broken under the same conditions, although preliminary evidence indicates that this is so. Therefore, the major products of the metabo-

lism of halothane are chloride, bromide and trifluoroacetic acid.

Biotransformation of halothane is proposed as follows:



It has been assumed that dechlorination and/or debromination of halothane is the source of the urinary products found in the metabolism studies. Since the enzymes responsible for the dechlorination are microsomal enzymes requiring NADPH, it follows that these should be increased by pre-treatment with a known microsomal enzyme inducer such as phenobarbital, as found to be the case with other drugs.⁴⁷ This has been shown to occur using labelled halothane.⁴⁸ There is a two to four-fold increase in the urinary products following pre-treatment with phenobarbital. An increase of CO₂ production from halothane does not occur, probably because of the fact that the carbon-fluorine bond is still not easily broken, whether or not enzymes are induced.

Teflurane (1,1,1,2-tetrafluoro-2-bromoethane), a newer and still experimental volatile anesthetic, has not been studied in regard to metabolism. However, it is conceivable, in view of the findings with halothane, that the bromine would be removed biologically but the fluorines not.

Halogenated Ethers

The fluorinated ethylvinyl ether, fluoroxene, (1,1,1-trifluoroethylvinyl ether) has been studied with regard to metabolism using the 1-¹⁴C trifluoroethylvinyl ether.⁴⁹ This material has been found to be metabolized in a manner similar to halothane: that is, there is very little carbon-fluorine bond cleavage since little ¹⁴CO₂ is found, but the rest of the mole-

cule is extensively metabolized as evidenced by large amount of urinary metabolites. It appears that the vinyl portion of the ether is easily attacked by a biological system. It has

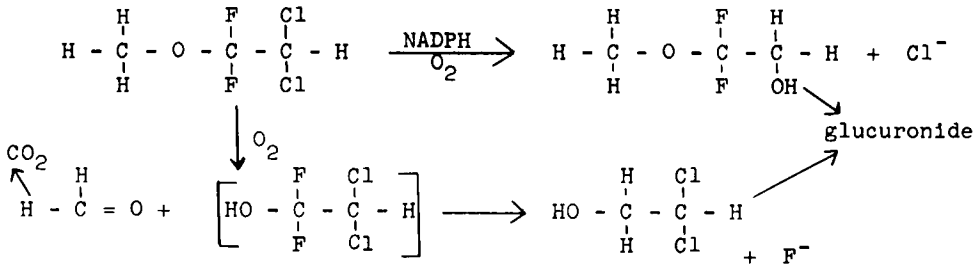
not been ascertained whether this is the result of an enzymatic reaction or if this represents a natural instability of the vinyl ether under biological circumstances.

The evidence for the metabolism of the trifluoroethylvinyl ether suggests that the non-fluorinated analog, ethylvinyl ether, is likewise metabolized. It may be assumed that the fluorines would have no influence on the biochemistry of the ether linkage; thus the products would be similar.

Methoxyflurane (2,2-dichloro-1,1-difluoroethylmethyl ether) has been extensively studied with regard to metabolism and distribution under several conditions.^{17, 42} ³⁶Cl and methyl-¹⁴C-methoxyflurane were used in these studies. These reports present evidence that both the ether linkage and the carbon-chloride bond are enzymatically cleaved. Furthermore, these reactions are mediated by microsomal enzymes and at least one, the carbon-chlorine bond cleavage, requires reduced NADP. This has been found by studying the reaction in a liver microsome preparation and by assaying the influence of known enzyme inducers on the extent of metabolism *in vivo*. Both the ether cleavage and break of the carbon-chlorine bond can be enhanced by pre-treatment with phenobarbital, 20-methyl-cholanthrene or chronic exposure to methoxyflurane vapors.⁴⁸ Evidence for some instability of the C-F bond in this compound was obtained by analysis of the long bones of rabbits chronically exposed to low concentrations. Inorganic fluoride levels were increased in these animals.

The following is a summary of the proposed route of biotransformation of methoxyflurane:

interaction of the nitrous oxide with a material normally found in bone marrow.



Inorganic Anesthetics

Under this category are found the "rare gases" xenon and krypton as well as nitrogen, nitrous oxide and carbon dioxide. All of these gases have in common the fact that they are comparatively weak anesthetics, that is, they require high partial pressures to produce anesthesia and in some cases elevated pressures are required.

It is difficult to imagine the rare gases, xenon and krypton, as being changed in any way *in vivo*. However, xenon can enter into chemical reactions.^{50, 51} While these reactions do not occur under conditions found in biological systems, nevertheless they point up the fact that xenon is not completely inert and may possibly have some slight biochemical reactivity. Furthermore, it is an accepted fact that xenon and krypton are capable of a weak association with certain metals.

Nitrogen narcosis has been extensively studied by Bennett.⁵² In his studies, he found that azacyclonol in a dosage of 150 mg./kg. prevents development of nitrogen narcosis in rats. Chenoweth⁵³ repeated this experiment but rather than using nitrogen as the anesthetic, used methoxyflurane. In these experiments he was not able to show an antagonism between the anesthetic and azacyclonol.

Little is known about the metabolism of nitrous oxide although it is actually quite a reactive chemical. Its distribution and excretion have been studied.⁵⁴ A pertinent series of papers has appeared^{55, 56, 57} describing a leukopenic effect of nitrous oxide, and it may be that this effect is the result of a chemical

Implication of Metabolism in Anesthesia

The fact that inhalation anesthetics undergo biotransformation perhaps, places them in the same position as many other drugs. The important questions to be asked are: what are the metabolites, how is the transformation carried out, *i.e.*, is it enzymatic or non-enzymatic, and does transformation influence the course of anesthesia? The answers to these questions for the most part must await further investigation, but an attempt can be made to answer them at this time. It must be emphasized that metabolic transformation is not necessary for the anesthetic properties of the volatile anesthetic, but their metabolism does indicate a biochemical reactivity.

Such metabolism of the volatile anesthetics as takes place in the liver probably does not influence the degree or extent of anesthesia as produced by these anesthetics. Since the volatile anesthetics are continuously administered insuring a constant blood concentration of unchanged anesthetic. If this were not the case, the effects of metabolism of volatile anesthetics would be as noticeable on the duration of anesthesia by these agents as it is on the duration of anesthesia by the barbiturates.

The metabolism of some of these volatile anesthetics has been found to occur in brain tissue although to a lesser extent than that found to occur in the liver. This metabolism in the brain tissue may be of extreme importance. This fact may require a re-evaluation in our thinking about theories of anesthesia. Could this mean that certain of the volatile anesthetics require a particular chemical reactivity to act as anesthetics?

Through the years several theories of action of anesthetics have been proposed and they have been summarized in a review.¹ These hypotheses have been based on the assumption that volatile anesthetics are biochemically inert and act because of certain physical properties they express. Recently Pauling⁵⁸ and Miller⁵⁹ have both proposed new and similar theories of the mode of action of anesthetics based on the ability of the anesthetic molecule to enter a clathrate structure. It is this clathrate structure which is assumed to block physically the passage of the impulse along a nerve fiber.

While the Pauling and Miller hypotheses, as well as certain of the other theories on mode of action of anesthetics may contain important considerations as to why a particular material may be anesthetic, they do not go far enough. For example, the clathrate concept may explain the process of transport from the lungs to the central nervous system, but at this point there must be inserted an additional factor to account for those materials with good hydrate forming ability which are non-anesthetic.⁶⁰

Since it is now known that many of the volatile anesthetics are not biologically inert, it may be necessary to consider that chemical reactivity on the part of the anesthetic molecule is essential for anesthesia to occur. There are certain considerations which make this an interesting theory. In the first place we have presented above, data indicating that the most potent anesthetics (as well as the most popular) are metabolized. If one looks carefully enough, probably most anesthetics can be shown to undergo some biotransformation. Secondly, there is the interesting, but as yet only partially explored observation, that ability to undergo metabolism parallels potency of the anesthetic. Thirdly, there is the fact that all anesthetics have in common the ability to associate or form weak bonds with metals, particularly copper and, to a lesser extent, iron. These facts when considered and studied further perhaps will bring new meaning to the state of anesthesia.

Implication of Metabolism to Toxicity

A great deal of discussion has taken place recently on the possible hepatotoxicity of certain volatile anesthetics.⁶¹ In this regard most

of the anesthetic show some hepatotoxicity although the degree of toxicity varies. One of the factors to be considered in this review is whether metabolism of anesthetic results in detoxification or the formation of toxic products.

In regard to the question of the formation of toxic products, it is evident from the preceding discussion that the various volatile anesthetics are metabolized by different routes which makes it difficult to consider a uniform mechanism of toxicity. As an example of metabolism by different routes one can always refer to the fact that the carbon-chlorine bonds are ruptured by different means depending on the type of molecule to which they belong. The fact that halothane and methoxyflurane are dechlorinated enzymatically while chloroform is partially dechlorinated by non-enzymatic means demonstrates clearly the possibility of obtaining different types of products. The problem is further complicated by the fact that a potentially toxic material may be either a stable intermediate or an intermediate of the free radical type. This type of reaction has been discussed by Butler.³⁰

In addition to these possibilities, there is also the question as to whether an observed toxic reaction is due to the unchanged anesthetic molecule. Such a mechanism could be similar to the one already discussed, in which an anesthetic molecule forms bonds or associates with certain metals. This could lead to interference with the results of reactions in which a metal such as copper or iron is important, as a change in oxidative phosphorylation.

The fact that toxic manifestations are relatively rare suggests that pharmacogenetics as proposed by Kalow⁶² is an important consideration. There may be an inborn difference among individuals, either in their ability to metabolize anesthetics, or a difference in the intermediary metabolism which renders some more liable than others to adverse anesthetic reactions.

Perhaps pertinent to this discussion is a series of articles concerning the toxicity of halothane in rats.⁶³⁻⁶⁶ These studies have shown that rats under certain conditions show an LD₅₀ to inhaled concentration of 2.8 per

cent for 10 minutes, while in other cases rats fail to show any adverse effect to an intraesophageal administration of 100 per cent. Furthermore, this variation shows daily periodicity according to Matthews, *et al.*⁶⁷ According to these workers, a simple manipulation of the daily exposure to light is sufficient to alter the periods of susceptibility. Presumably, this is a result of the daily fluctuation in the normal intermediary metabolism. If this same periodicity occurs in man, this would have a great influence on the possible toxicity of all volatile anesthetics. It was noted that the metabolism of diethyl ether, chloroform, halothane and methoxyflurane *in vivo* showed a large individual variation.¹⁷ It was not determined at the time if circadian rhythms played an important role in this variation in metabolism, but this may be worthy of consideration.

Summary

Evidence has accumulated to the effect that the volatile anesthetics are biodegradable. Direct evidence has been shown for the most popular and potent anesthetics, diethyl ether, chloroform, halothane and methoxyflurane. Indirect evidence for the biotransformation of other less potent anesthetics can be shown.

This information offers a fresh approach to certain questions concerning the action of anesthetics and the toxicity of the anesthetics. This will place the anesthetics in a position to be studied as chemical reactants rather than as physical inhibitors and offer a more positive approach to the study of their mechanism of action.

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