Review Article

Biophysical Mechanisms of Anesthetic Action:
Historical Perspective and Review of Current Concepts

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The study of the mechanisms of anesthetic action occupies the attention of a remarkably diverse group of disciplines. This diversity is reflected in the dispersion of articles relevant to the study of anesthetic action throughout the chemical and biomedical literature. The goal of this paper is to review and place in historical perspective the current concepts of anesthetic mechanisms at the molecular and subcellular level.

Introduction

Neural activity can be disrupted in many ways. General anesthesia is a pharmacologically induced reversible disruption resulting in a coordinated sequence of changes in neural activity. What is most remarkable, and perhaps least appreciated, is the diversity of the large number of compounds that produce general anesthesia. The attempt to develop a unifying principle that would reconcile this diversity characterizes much of the work on anesthetic mechanisms of action.

A unitary theory of anesthesia proposes that all general anesthetics act by the same mechanism at the same molecular or subcellular site. Although there is much convincing evidence for a common biophysical mechanism underlying all anesthetics, it would be well to remember that the unitary theory is not without detractors. Metcalfe et al. argue that a large number of molecular interactions could produce the same nonfunctional conformation of an essential subcellular component, probably membrane protein (degenerate perturbation hypothesis).1 Wall suggests that intracellular neuronal structure or synaptic organization is such that anesthetics acting on a variety of sites would produce the same pattern of dysfunction.2 Paton questions the unitary theory because of variability among anesthetics in excitant phenomena, respiratory and blood pressure effects, anesthesia/analgesia ratios, and electrophysiologic effects.3 Some of these differences among anesthetics probably result from differences in rates of anesthetic uptake by the brain, which in turn result from differences in blood–gas partition coefficients, redistribution, metabolism, and excretion. Some anesthetics may have pharmacologic effects, e.g., sympathoadrenal stimulation,4 direct stimulant action on vascular smooth muscle, and vasopressin release,5 that are separate from and not inherent to their anesthetic properties. Variability in excitant phenomena may result from differential effects of anesthetics on a specific convulsant site of action. The unitary theory may require modification to include anesthetic sites of slightly different properties in neurons of different pathways.

The membrane actions of inhalational general anesthetics and local anesthetics are probably not due to the same basic mechanism.6 Therefore, the mechanisms of anesthetic action discussed below may not pertain to local anesthetics. The relationship of the barbiturates and steroid anesthetics7 to the inhalational agents is less clear. Unlike the inhalational anesthetics, certain regularities of structure–activity relationship have been observed for the barbiturates.6,8 Comparison of barbiturates and inhalational anesthetics is difficult because the potencies of barbiturates cannot be related to the MAC's of inhalational anesthetics and no comprehensive data relating the potencies of barbiturates to their partition coefficients are available. Comparisons among barbiturates are complicated since the rate of induction can be confused with potency.

The relationship that a small molecule such as N₂O or an atom of xenon has to a steroid anesthetic is certainly obscure. The steroid anesthetics also have definite structure–activity relationships and fail to manifest a relationship between potency and partition coefficient.9 Possibly only a part of the barbiturate or steroid participates in the molecular interaction that results in anesthesia. Although many authors have related investigations of barbiturates and volatile agents, perhaps the following theories of anesthetic action should be extended to encompass the noninhalational anesthetics only with some caution.

Theories of anesthesia at the molecular and subcellular level are discussed on two levels:

A. Physical chemical
1. Lipid theories
2. Aqueous-phase theories

B. Biophysical
1. Critical-volume theory
2. Protein conformational change theory
3. Microtubule theory

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Physical Chemical

General anesthesia is produced by a wide range of compounds with no common chemical structure or activity: noble gases, aliphatic, alicyclic, aromatic, halogenated hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers. Even the air we breathe is anesthetic.\textsuperscript{11,12} The stereoisomers of halothane are equipotent,\textsuperscript{13} indicating that anesthetic interaction at receptor sites necessitating specific molecular conformation is unlikely. General anesthesia must be a physical phenomenon working solely by forces acting between molecules. These electrostatic intermolecular forces, known as van der Waals forces, are much weaker than the intramolecular chemical bonds between atoms and between ions. The physicochemical approach looks for correlations between anesthetic potency and physical properties of a series of anesthetics. From these correlations, insight into the molecular environment at the site of action may be obtained.

Correlations between anesthetic properties (vapor pressure, boiling point, van der Waals constant, polarizability) and anesthetic potency can be misleading. These properties depend upon forces exerted between anesthetic molecules; however, anesthesia must be mediated by interactions between anesthetic molecules and some cell constituent (probably membrane), rather than by interactions between anesthetic molecules.

Correlations between several anesthetics and a model (olive oil, for example) are more useful. A good correlation implies that the same intermolecular forces are experienced by the anesthetic at the site of action and in the model phase. However, this approach may also be misleading. An homologous series of compounds often obeys a regular relationship with a second molecule or phase. Even if these same regular relationships exist with the molecules at the anesthetic site of action, there is no proof that the particular property of the anesthetic being measured is causing the state of anesthesia. Therefore, compounds with a wide range of structures, and especially those with anomalous properties, enhance our ability to discriminate between various models. In particular, the fully-fluorinated compounds (for example SF\textsubscript{6}, CF\textsubscript{3}), which have very weak intermolecular forces and consequently anomalous solubility behavior, have been found useful. Note that this approach does not permit any statement about where the anesthetic site of action is, or how the anesthetic interacts with the site. For this reason, many investigators prefer to refer to physicochemical theories as rules.

LIPID THEORY

At the turn of the century, H. H. Meyer and Overton independently published essentially identical theories of anesthesia.\textsuperscript{14} In Meyer’s words:

“The narcotizing substance enters into a loose physicochemical combination with the vitally important lipoids of the cell, perhaps with the lecithin, and in so doing changes their normal relationship to the other cell constituents, through which an inhibition of the entire cell chemism results.” They also found that anesthetic potency was directly related to the agent’s oil–water partition coefficient.

The Meyer-Overton lipid solubility theory was formulated in modern form by K. H. Meyer\textsuperscript{15} in 1937: “Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipoids of the cell. This concentration depends on the nature of the animal or cell, but is independent of the narcotic.” Formally the Meyer-Overton hypothesis is:

$$x_A P_{90} = \text{constant} = X_{90}$$  \hspace{1cm} (1)

where $x_A$ is the mole fraction solubility per unit pressure of the anesthetic at its site of action (or some analogous such as olive oil), $P_{90}$ is the partial pressure of the anesthetic that anesthetizes 50 per cent of a group of subjects, and $X_{90}$ is the mole fraction solubility of anesthetic that anesthetizes 50 per cent of a group of subjects. Equation 1 derives directly from Henry’s law, $P_A = k_A X_A$. Henry’s law states that the partial pressure of solute A is proportional to its mole fraction solubility, or equivalently, the mole fraction solubility of solute A is proportional to its partial pressure. The constant of proportionality $k_A$ is called the Henry’s law constant. A few texts define Henry’s law as $X_A = k_A P_A$, in which case $x_A = 1 / k_A$.

In 1939 Ferguson pointed out that the partition coefficient, the vapor pressure of anesthetics in solution, and various solubility relationships of anesthetics are all derivable in principle from the thermodynamic activity.\textsuperscript{16} The activity of A, represented by $a_A$, is defined as $a_A = P_A/P_A^*$ where $P_A$ is the measured vapor pressure of dissolved A (A is one component of a solution) and $P_A^*$ is the vapor pressure of pure A in some standard state, most conveniently, liquid A. Recall Raoult’s law: $P_A = X_A P_A^*$ where $X_A$ is the mole fraction of A, which states that the partial vapor pressure of each component of a solution is equal to the mole fraction of that component times the vapor pressure of the pure component.

The activity coefficient, $\gamma$, is defined as: $\gamma_A = a_A X_A = P_A / P_A^*$. If the solution is ideal $\gamma = 1$ and $a_A = X_A$. In dilute solutions $\gamma$ is usually close to 1. Referring to figure 1, line I represents $\gamma = 1$, curve N represents a negative deviation from Raoult’s law, $\gamma < 1$, and curve F a positive deviation from Raoult’s law, $\gamma > 1$. Ferguson’s principle states: equal degrees of anesthesia are attained when equal thermodynamic activities of the anesthetic are applied to the cell or organism. Most substances can be shown to produce physiologic effects at an activity of about 3 per cent.
Recently, a thermodynamic description of the effect of anesthetics on protein conformation has been advanced as an explanation of general anesthesia. Hill has proposed a thermodynamic description of general anesthesia in terms of the Gibbs free energy of the anesthetic site. He argues that other theories are deductible from the Gibbs free-energy hypothesis. Gibbs free energy is a thermodynamic quantity that represents the net, i.e., external, work available from a system undergoing an isothermal process at constant pressure. Thermodynamic descriptions are very powerful in that they can predict the outcome of a process without knowledge of the mechanistic details of that process.

**Aqueous-phase Theories**

Pauling and S. L. Miller independently proposed (1961) that the site of action of anesthetics lies in the aqueous phase of the CNS. Pauling hypothesized the formation of gas hydrates. Hydrates, once formed, could increase the impedance of the neural network or occlude pores in membranes. Hydrates could also impair the reactivity of enzymes they surround by a cage effect. Miller did not invoke the formation of hydrates but based his theory on the ordering that simple solutes are supposed to induce in nearby water molecules ("iceberg effect"). These "icebergs" would function in the same manner as Pauling's hydrates. However, both theories seem to have died from a lack of evidence. Both theories predict that anesthetic potency of a substance is related to the stability of its hydrates, as represented by the reciprocal of its dissociation pressure at 0 C. An examination of figure 2 (with key numbers from table 1) reveals hydrate dissociation pressure to be considerably inferior to the oil-gas partition coefficient as a predictor of ED50. ED50 in man (MAC) also correlates better with lipid solubility. A recently reetermined value for the dissociation pressure of CF4 hydrate improves the picture somewhat. SF6 still shows marked deviation, however. Attempts to improve the correlation by correcting the hydrate dissociation pressure to 37 C using the heats of formation of the hydrates have been unsuccessful. The formation of hydrates of a number of common anesthetics has been unsuccessful to date. That helium and neon do not manifest anesthetic behavior and also do not form hydrates lends little support to the hydrate theory. Other theories suggest that these gases would be anesthetic at pressures above 100 ATA; however, hydrostatic pressure reversal intervenes at these pressures.

**Biophysical**

These theories make a statement about the site of anesthetic action and the site-anesthetic interaction.

**Critical-volume Theory**

The critical-volume theory is a modification of the Meyer-Overton theory. Mullins, in 1954, proposed that anesthesia commences when a certain critical-volume fraction of an inert substance is attained in membranes. Mullins believed that anesthetics filled up holes (free volume) in membranes. Subsequent work has not supported free-volume theories of liquids, but the critical volume of anesthetic entering the membrane can be thought of as expanding the membrane (rather than filling up holes in the membrane). The resulting disorder caused by a volume increase in the membrane bilayer would result in compression of the entities embedded in it or the proteins binding it. Thus, sodium or potassium channels, acetylcholine receptors, or enzymes could be modified. If we treat the site of anesthetic action as a bulk phase, we may write

$$E_a = \frac{\bar{V}_a x_a P_a}{V_m}$$  \hspace{1cm} (2)

where $E_a$ is the fractional expansion, $\bar{V}_a$ is the partial molar volume of the anesthetic in the anesthetic site, $x_a$ is the anesthetic's mole fraction solubility per unit pressure in the site, $V_m$ is the molar volume of the site, and $P_a$ is the partial pressure of the anesthetic. Mullins proposed that at each anesthetic's $P_{50}$ (the pressure necessary to anesthetize 50 per cent of a group of subjects), $E_a$ would be a constant (the same for all anesthetics). Thus, we may formally write the critical volume theory:

$$\text{Constant} = E_{50} = \frac{\bar{V}_a x_a P_{50}}{V_m}$$  \hspace{1cm} (3)

where $E_{50}$ is the fractional expansion corresponding to the ED50. Examining Eq 1 and Eq 3 reveals that the Meyer-Overton theory predicts a linear relation between $\log P_{50}$ and $\log x_a$, and the critical volume theory predicts that a plot of $\log P_{50}$ with respect to $\log (\bar{V}_a x_a)$ should yield a straight line of unit negative slope. Figure 3 demonstrates that the data are
consistent with either model. The difficulty in differentiating between the two theories arises because the size range of anesthetic molecules is insufficient. The molar volumes of the anesthetics vary only by a factor of about 2. This small variation is difficult to separate from experimental error. Also, because the linear relationship in figure 3 is between the logarithms of $P_{50}$ and $V_2$, $V_2$ varies $V_2$ by a factor of 2 would move the points very little.

The critical-volume theory is very appealing in that it offers a physical mechanism for the lipid theory. It also offers an explanation for the phenomenon of pressure reversal. In turn, pressure antagonism of anesthesia offers a test for delineating between the Meyer-Overton and critical-volume theories.

**Pressure Reversal.** For many years it has been known that hydrostatic pressure results in stimulation of the CNS of aquatic animals at pressures above 50 atm. The effects of pressure on mice are fourfold:

1. Tremors whose onset lies in the pressure range of 70 ATA and may be dependent on the rate of compression.
2. Convulsions at higher pressures.
3. Respiratory distress has been reported to occur at pressures above 90 ATA.
4. At still higher pressures, spontaneous muscle contraction and paralysis (most clearly observed in newts), and finally death. A high-pressure neurological syndrome in man characterized by onset of coarse tremors of the limbs at 25 ATA has recently been discovered.

Johnson and Flagler (1950) first made the observation that hydrostatic pressure could modify an organism’s response to drugs. They found that tadpoles narcotized with 2–5% per cent ethanol would
## Table 1. ED<sub>50</sub> Pressures (Atmospheres Absolute, ATA) for Righting Reflex of Mice

<table>
<thead>
<tr>
<th>Key Number</th>
<th>Results of Miller et al.&lt;sup&gt;a&lt;/sup&gt; ± SE</th>
<th>Results of 1987 Study&lt;sup&gt;*&lt;/sup&gt; ± SE</th>
<th>Epstein et al.1 ± 95 Per Cent Confidence Limits</th>
<th>Others&lt;sup&gt;†&lt;/sup&gt; ± SE</th>
<th>Value Adopted (ATA)</th>
<th>Corrected Value&lt;sup&gt;‡&lt;/sup&gt; (See ref&lt;sup&gt;7&lt;/sup&gt;) (ATA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 He</td>
<td>&gt;120</td>
<td>&gt;100</td>
<td>&gt;140</td>
<td>&gt;140</td>
<td>&gt;146</td>
<td>&gt;146</td>
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<tr>
<td>2 Ne</td>
<td>&gt;110</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Ar</td>
<td>15.2 (± 0.31)</td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Kr</td>
<td>4.50 (± 0.399)</td>
<td></td>
<td></td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Xe</td>
<td>0.95 (± 0.033)</td>
<td></td>
<td></td>
<td>0.95</td>
<td>0.95</td>
<td></td>
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<tr>
<td>6 H&lt;sub&gt;2&lt;/sub&gt;</td>
<td>129 (± 7.3)</td>
<td>34 (± 1.3)</td>
<td></td>
<td>33</td>
<td>130</td>
<td>138</td>
</tr>
<tr>
<td>7 N&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>32 (± 2.4)</td>
<td></td>
<td>33</td>
<td></td>
<td></td>
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<tr>
<td>8 N&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>1.50 (± 0.036)</td>
<td>1.45 (± 0.019)</td>
<td>1.51 (± 0.043)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>9 C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.36 (± 0.051)</td>
<td></td>
<td></td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.164 (± 0.0053)</td>
<td></td>
<td></td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 CF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18.8 (± 0.88)</td>
<td></td>
<td></td>
<td>19</td>
<td>17.5</td>
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<tr>
<td>12 C&lt;sub&gt;2&lt;/sub&gt;F&lt;sub&gt;6&lt;/sub&gt;</td>
<td>17.7 (± 0.62)</td>
<td></td>
<td></td>
<td>18</td>
<td>15.5</td>
<td></td>
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<tr>
<td>13 C&lt;sub&gt;2&lt;/sub&gt;F&lt;sub&gt;4&lt;/sub&gt;</td>
<td>18 (± 4)</td>
<td></td>
<td></td>
<td>18</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>14 SF&lt;sub&gt;6&lt;/sub&gt;</td>
<td>5.4 (± 0.21)</td>
<td>6.8 (± 0.12)</td>
<td></td>
<td>6.1</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>15 CCl&lt;sub&gt;4&lt;/sub&gt;F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.40 (± 0.012)</td>
<td></td>
<td></td>
<td>0.40</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>16 CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.0084 (± 0.00053)</td>
<td></td>
<td></td>
<td>0.0084</td>
<td>0.0084</td>
<td>0.0084</td>
</tr>
<tr>
<td>17 Halothane</td>
<td>0.0174 (± 0.00040)</td>
<td></td>
<td></td>
<td>0.0077</td>
<td>0.0077</td>
<td>0.0077</td>
</tr>
<tr>
<td>18 CH&lt;sub&gt;3&lt;/sub&gt;CClF&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.029 (± 0.00030)</td>
<td></td>
<td></td>
<td>0.029</td>
<td>0.029</td>
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<tr>
<td>20 Ether</td>
<td>0.032</td>
<td></td>
<td></td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Methoxyflurane</td>
<td>0.0022 (± 0.00019)</td>
<td></td>
<td></td>
<td>0.0022</td>
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<sup>‡</sup> For other references see reference 53.

Resume swimming in an apparently normal manner following application of 200–300 ATA pressure. Similar effects have been observed in newts and mice anesthetized with such diverse anesthetic agents as ether, nitrous oxide, halothane, pentobarbital, and phenobarbital. The workers who investigated phenobarbital constructed dose–response curves and thus were able to determine the ED<sub>50</sub>. Application of 102 atm of He increased the ED<sub>50</sub> of phenobarbital by an amount similar to that found for gaseous anesthetics. Although the phenobarbital dose–response curves were not parallel, this evidence lends support to similar mechanisms of action of barbiturates and gaseous...
anesthetics. Furthermore, mice anesthetized with nitrogen or pentobarbital manifested a significant increase in the lethal pressure\(^2\) (see figure 4).

The critical-volume theory obviously offers an explanation for pressure reversal. We may hypothesize that the fractional expansion of the membrane caused by absorption of an inert substance is reversed by increased pressure. We may rewrite Eq 2 so as to take into account the effects of pressure:

\[
E = \frac{V_{2}x_{2}P}{V_{m}} - \beta P = \left(\frac{V_{2}x_{2}}{V_{m}} - \beta\right)P
\]  

where \(\beta\) is the coefficient of isothermal compressibility.\(^3\) The first term represents expansion due to solution of the gas, and the second term represents the compression due to pressure. Depending upon the relative values of the constants in Eq 4, a gas may cause net expansion, compression, or no change in the volume of the membrane.

Assume that a subject is anesthetized by a gas at pressure \(P_{50}\), and then the partial pressure of anesthetic is raised to \(P_{a}\). Simultaneously, the increased membrane expansion is exactly counterbalanced by raising the total pressure \(P_{r}\) by the addition of helium. Then:

\[
\left(\frac{V_{2}x_{2}}{V_{m}} - \beta\right)(P_{a} - P_{50}) = - \left(\frac{V_{tc}x_{tc}}{V_{m}} - \beta\right)(P_{r} - P_{a})
\]

Dividing by Eq 4 with \(P = P_{50}\)

\[
\frac{\left[\left(\frac{V_{2}x_{2}}{V_{m}} - \beta\right)(P_{a} - P_{50})\right]}{\left(\frac{(\frac{V_{2}x_{2}}{V_{m}} - \beta)}{P_{50}}\right)} = - \left[\left(\frac{V_{tc}x_{tc}}{V_{m}} - \beta\right)(P_{r} - P_{a})\right] E_{50}
\]

we obtain

\[
\frac{P_{a}}{P_{50}} = \left(\frac{\beta}{E_{50}} - \frac{V_{tc}x_{tc}}{E_{50}V_{m}}\right)(P_{r} - P_{a}) + 1
\]  

\(\frac{P_{a}}{P_{50}}\) is linear in \((P_{r} - P_{a})\) with a Y intercept of +1. Figure 5 provides considerable justification for the critical-volume theory.

The Meyer-Overton theory also predicts pressure reversal because \(x_{a}\) the mole fraction solubility per unit pressure of the anesthetic, is pressure-dependent (decreases with increased pressure). The relation between the true mole fraction solubility per unit pressure \(x_{a}\) and the predicted \(x_{a}\) as a function of pressure is:

\[
\ln x = \ln x_{a} - \frac{V_{tc}P}{RT}
\]  

where \(R\) is the gas constant and \(T\) the absolute temperature.\(^3\) Substituting \(x_{a}\), \(P_{r}\) and \(x_{50}\), \(P_{50}\) respectively into Eq 6 obtains:

\[
\ln x_{a} = \ln x_{a} - \frac{V_{tc}P_{r}}{RT}
\]

\[
\ln x_{50} = \ln x_{a} - \frac{V_{tc}P_{50}}{RT}
\]

Recalling Eq 1, if the \(ED_{50}\) partial pressure is \(P_{50}\) at a given total pressure, and the \(ED_{50}\) partial pressure is \(P_{a}\) at an increased total pressure, then

\[
P_{50}x_{50} = P_{a}x_{a}
\]  

where \(x_{a}\) is the mole fraction solubility per unit pressure at the increased pressure. Subtracting Eq 8 from Eq 7 and eliminating \(x_{a}/x_{50}\) using Eq 9 we obtain:

\[
\frac{1}{V_{tc}}\ln \frac{P_{r}/P_{50}}{P_{a}/P_{50}} = \frac{1}{RT} (P_{r} - P_{50})
\]  

Comparing figure 6 with figure 5 clearly demonstrates that the Meyer-Overton prediction is inferior to that of the critical-volume theory. Furthermore, the Meyer-Overton rule offers no explanation for the lack of anesthetic effect of helium and neon.

The above derivations of Eq 5 and Eq 10 are not rigorous. At high pressures, gases deviate from the gas laws. A corrected pressure, the fugacity, should be substituted to correct for the nonidealities of gases at high pressure. In addition, Eq 5 should be corrected by Eq 6.

The critical-volume theory suffers from an interesting deviation: The members of an homologous series of anesthetic molecules have progressively less physiologic effect with increasing size ("cut off").\(^3\) For example, methane through hexane behaves predictably. As one progresses past hexane, larger and larger thermodynamic activities are necessary to produce the same degree of anesthesia. Saturated decane vapor is nonanesthetic. Apparently, the anesthetic site is composed in such a way to exclude large molecules, or alternatively, the inclusion of large molecules does not result in the structural transformation necessary for anesthesia.

More recently, the critical-volume theory has been extended to treat the high pressure neurological
syndrome (HPNS). Calculations have shown that anesthesia occurs at an expansion of 1.1 percent and convulsions at a compression of 0.85 per cent using a benzene model (solvent). There may be separate sites that produce anesthesia and convulsions because the compressibilities $\beta$ associated with convulsions are two to five times greater than those associated with anesthesia.\textsuperscript{39} The inference of separate sites depends on the appropriateness of the model. Benzene is an isotropic solvent that may not truly represent the nerve membrane.

Certain fluorinated ethers are potent stimulants of the central nervous system. Cohen \textit{et al.} have studied a series of structurally similar fluorinated ethers that have a spectrum of effects.\textsuperscript{40} The series progresses from compounds that are convulsants, through those that have mixed convulant-anesthetic effects, to compounds that are anesthetic (included are fluoroxyne, enfurane, and methoxyfluorane). One ether is a convulsant, but its structural isomer is an anesthetic. These authors found that the only reliable indicator that could predict the type of activity of fluorinated ethers was the partial molar volume. Knowledge of the partial molar volume allows derivation of the solubility parameter $\delta$ (see below) and the partition coefficient. The convulsants were associated with low $\delta$ values while the anesthetics were associated with higher values in a relatively narrow range. Thus, if there are separate (or multiple) anesthetic and convulsant sites (as suggested by the compressibility data), assuming the sites have different $\delta$ values, then the low-$\delta$ convulsants would partition into one site and the high-$\delta$ anesthetics would partition into the other site. Alternatively, the convulsants may act through a non-phase-specific mechanism.

The compression term of Eq 4 dominates for helium and neon, and thus these gases should produce net compression of membranes. Therefore, the lack of narcotic effect of helium and neon is predicted by the critical-volume theory.\textsuperscript{28,41} That high-pressure helium–oxygen atmospheres produce excitant phenomena in animals and the HPNS in man is further support. One would predict that addition of a narcotic gas to helium–oxygen would re-expand the membrane and antagonize the HPNS. This has been found to be true in animals\textsuperscript{42} and man.\textsuperscript{43}

**Protein Conformational Change (PCC) Theory**

It is generally believed that anesthetics act by depressing synaptic transmission. There is evidence that transmitter release and postsynaptic membrane conductance are mediated by membrane lipoproteins.\textsuperscript{44,45} The membrane expansion associated with anesthesia is about ten times the occupying volume of anesthetic in the membrane; therefore, extensive conformational changes in membrane proteins may be involved.\textsuperscript{46} The disordering of structure or membrane fluidization invoked by the critical-volume theory could disorder the membrane proteins indirectly. The PCC theory proposes that
anesthetics directly inactivate lipoproteins, i.e., the site of anesthetic action is protein. A protein may have hydrophobic regions. If the site of anesthetic–protein interaction is in these regions, then the direct relation between anesthetic potency and lipid (hydrophobic) solubility would be predicted.

Indirect evidence in support of the PCC theory includes studies of anesthetic effects on chemiluminescence and optical rotation of protein solutions. Chemiluminescence is dependent on an enzyme, luciferase, and a substrate, luciferin. In 1942, Johnson et al. demonstrated that anesthetics inhibited bacterial luminescence and that this inhibition was reversible by the application of pressure. Recently, in-vivo inhibition of light output of luminous bacteria with modern general anesthetics has been confirmed. Ueda and Kamaya have demonstrated inhibition by anesthetics of luminescence in cell-free firefly-tail extract in vitro. These effects may be the indirect result of anesthetic action on lipids. However, luciferase is a water-soluble enzyme and unlikely to be associated with intracellular lipid. Furthermore, anesthetics have been shown to reduce the optical rotation of solutions of bovine plasma albumin and crystallized β-lactoglobulin approximately in direct relation to their anesthetic potencies. However, chloroform produced a considerably greater reduction in optical rotation than halothane. This would not be expected since halothane is slightly more potent than chloroform. The anesthetics were equilibrated with the protein solutions at 25 C. In the case of anesthetics with boiling points above 25 C, the effects were normalized to 1 atm assuming a simple linear relationship between partial pressure and change of optical rotation.

MICROTUBULE THEORY

Microtubules are widely distributed and abundant intracellular structures. Although theories of microtubule function are still speculative, they are probably concerned with mechanical functions of the cell. Microtubules may be responsible for maintaining the shapes of cells and the rigidity of cellular processes, acting motile systems such as cilia, and transporting selected constituents along axons.

Allison and Nunn proposed that general anesthesia results from reversible depolymerization of microtubules in nerve cells. Subsequently, Allison et al. demonstrated that five inhalational anesthetics produced reversible dispersion of microtubules in heliozoa at concentrations of 2 to 4 MAC. Nitrous oxide also caused dispersion of microtubules, but at concentrations below 1 MAC. Furthermore, recovery was seldom satisfactory in that cell lysis often occurred some minutes after withdrawal of the nitrous oxide. Diethyl ether had no effect in concentrations less than that which produced lysis (20 percent). Barbiturates caused a considerable loss of microtubules in ganglionic cell bodies and sciatic axons of the frog. However, the relevance of these findings to anesthesia is in question since the exposure to barbiturate lasted five to 17 hours and fairly high concentrations of anesthetic were utilized. Other investigators have reported the failure of pentobarbital and halothane to depolymerize axonal microtubules in mouse optic nerve.

Squid axons continue to conduct action potentials following extrusion of 95 per cent of the axoplasm and subsequent perfusion with potassium solutions. Although microtubules may have occupied the remaining 5 per cent of axoplasm, these data suggest that microtubules are not necessary for axonal conduction. Colchicine, podophyllotoxin, vinblastine, and griseofulvin have the ability to disrupt microtubule systems. Katz found that colchicine and podophyllotoxin decreased transmitter release from the frog neuromuscular junction, while griseofulvin increased and vinblastine had no effect on transmitter release. These drugs were shown to act on the nerve terminal membrane, and their effects on transmitter release did not result from action on microtubules. Colchicine or vinblastine inhibits acetylcholine-induced catecholamine release from perfused bovine adrenal glands and blocks transmission through the superior cervical ganglion of the cat. However, both of these actions result from an anticholinergic effect of these drugs and cannot be interpreted as evidence that microtubules participate in the release of amines. Hinkle and Green demonstrated that low concentrations of halothane (3 mM and 10 mM) increase the number of microtubules in rabbit vagus nerve while blocking axonal conduction, and colchicine decreases the number of microtubules but does not affect the electrical activity.

High pressure reversibly depolymerizes microtubules of a range of organisms, as demonstrated by light and electron microscopy and observation of birefringence. These studies, utilizing protozoa and annelid oocytes, suggest that the microtubule theory is inconsistent with pressure reversal of anesthesia. However, neuronal microtubules in a variety of nerve cell types have recently been observed to be stable in the presence of high pressure. Evidence for the variability of microtubules within individual cells and between cell types or species is recognized; thus, generalization of studies involving non-neural and non-mammalian cells may not be warranted.

Weisenberg has shown that elevated cytoplasmic Ca++ concentration inhibits assembly of tubulin subunits. If narcosis depends on intracellular Ca++ release, microtubule depolymerization may be incidental to anesthesia.
The Anesthetic Site

The anesthetic site may be better defined by modeling with a series of solvents with graded solvent powers. Solvents may be characterized by the solubility parameter \( \delta = \frac{-E_c}{\sqrt{V}} \) where \( E_c \) is the energy of vaporization of the solvent and \( V \) is its molar volume. The solubility parameter is a measure of the strength of intermolecular forces within the solvent. The model that best predicts the correlation of anesthetic potency with solubility is a relatively nonpolar solvent with \( \delta = 9 \pm 1 \) [cal. cm\(^{-3}\)]\(^{1/2} \), (benzene \( \delta = 9.2 \) [cal. cm\(^{-3}\)]\(^{1/2} \)). The best model using volume fractions (critical volume theory) is a solvent with \( \delta \sim 10 - 11 \) [cal. cm\(^{-3}\)]\(^{1/2} \). Present evidence supports the general assumption that the site of anesthetic action is on the cell membrane.

Nuclear Magnetic and Electron Spin Resonance Techniques

Nuclear magnetic resonance (NMR) and electron spin resonance (ESR) are two relatively new and increasingly important methods for defining the nature of the anesthetic site and the anesthetic-site interaction. Electron spin resonance is also called electron paramagnetic resonance (EPR).

An electron possesses angular momentum as a consequence of both its orbital motion and its spin. Analogously, the earth has components of angular momentum arising from its orbital motion about the sun and its spin about its axis. The electron also possesses charge, which in conjunction with its angular momentum causes the electron to have a magnetic moment (magnetic field). A macroscopic analogy is the magnetic field produced by moving charge (an electric current) in a coil of wire.

However, unlike the macroscopic world (a spinning top, for example) atomic particles can possess only a certain number of discrete observable values of angular momentum. The spin angular momentum of the electron can take on only two values. Since the magnetic moment is directly proportional to the angular momentum, the magnetic moment can also take on only two observable values. In isotropic space the two spin states of the electron are associated with the same energy, and the electron is said to be spin degenerate. Applying a magnetic field makes space anisotropic (manifest properties that depend on the orientation of the measurement) and the electron spin can be thought of as aligning parallel or antiparallel to the magnetic field. The two spin states now correspond to two different energy levels. Transitions between these levels can be caused by applying a quantum of energy equal to the energy separation of the levels. These quanta can be supplied by radio or microwaves, and the absorption of the energy measured. The frequency of radio waves necessary to cause a transition is directly proportional to the magnetic field seen by the electron. However, the magnetic field seen by the electron is the sum of the applied field and fields in the immediate environment (nearby nuclei and electrons) of the electron. The environment also determines the time necessary for the electrons to return to the ground state (spin-lattice and spin-spin relaxation). Thus, the magnetic resonance absorption spectrometer is a tool that can explore the physical environment on the molecular scale.

Since electrons in each molecular orbital exist in pairs with anti-parallel spins (and therefore they possess zero net spin magnetic moments), ESR spectra can be observed only in species that contain unpaired electrons (paramagnetic). Biological systems can be made paramagnetic with minimal disturbance of structure by inserting into the system a stable free radical whose chemical structure is very similar to that of some component of the biological molecules. These paramagnetic molecules are called spin labels.

The orbital geometry of the unpaired electron on nitro oxide spin labels is highly anisotropic. This condition gives directional properties to spin labels. Thus, it is possible to determine whether the spin label is constrained in its motion, thereby inferring the degree of order and structure within the spin label's environment.

Nuclear magnetic resonance is similar to ESR, except that the spin transitions take place within the nucleus. Some nuclei are non-magnetic and do not manifest NMR spectra (\( ^{12}C, ^{16}O \)), other nuclei have many observable components of their magnetic moment. \( ^1H, ^11B, ^13C, ^19F, \) and \( ^3P \) possess just two energy levels (like the electron) and are commonly studied. For further discussion of the underlying physics and biological application of NMR and ESR see McLaughlin,11 Kieth et al.,12 and Smith.73

Membrane Models

Many models of membrane structure postulate a lipid bilayer in which the long hydrophobic hydrocarbon chains of the fatty acids are aligned towards the center of the membrane, and the polar head groups are on the surface. For example, the classic Danielli-Davson model postulates a lipid bilayer coated on each side by protein. Other models propose complex networks of lipid and protein. However, because of the enormous complexity of biological membranes, simpler and better characterized models have been extensively studied.

Artificial bilayers can be formed by evaporating a phospholipid onto a container; the films are then hydrated by filling the container with aqueous salt solutions. Alternately, a phospholipid in an aqueous solution is sonicated to form a suspension of microscopic spheres (vesicles or liposomes) that have a lipid bilayer as a wall.
Hubbell and McConnell have found fluid-like hydrophobic regions in both biological membranes and bilayers. Furthermore, in both systems the hydrophobic region becomes increasingly fluid (disordered) as one moves away from the polar head groups toward the center of the bilayer. The order of phospholipid bilayers has been shown to be markedly increased by the addition of cholesterol up to approximately 50 mole per cent. The order begins to decrease with the addition of further cholesterol. The order of bilayers is also markedly affected by the charge and concentration of cations in the aqueous phase. The remainder of this discussion deals with the effects of anesthetics on biological and model membranes as determined by ESR and NMR techniques. Study of these systems has important implications for theories of anesthetic action.

**Effects of Inhalational Anesthetics**

Halothane, chloroform, enflurane, and methoxyflurane cause fluidization and expansion of dipalmitoyllecithin (a phospholipid bilayer), as measured by NMR. However, halothane and chloroform produce a molecular environment in the phospholipid somewhat different from that produced by enflurane and methoxyflurane.

The effects of halothane and methoxyflurane on phosphatidylcholine-cholesterol bilayers have been studied by ESR using spin-labeled phosphatidylcholine. These anesthetics produce disorder (fluidization) in phospholipid bilayers with a linear dose-response relationship. Because the ratio of molar volumes for methoxyflurane and halothane is only 1.096, it was not possible to determine whether the two anesthetics produce equal disorders at equal molar concentrations (Meyer-Overton) or at equal volume fractions (critical-volume theory). Measurement of the ESR spectra of spin labels that localize the nitroxide in different regions of the bilayer revealed that the anesthetics produce a general fluidization of the hydrophobic region rather than local disorder at one level. In addition, pressure increases the order of these phospholipid bilayers with or without halothane present. Halothane at 128 atm reverses the effect of 60 mmol halothane per mole of lipid. Thus, phospholipid bilayers would appear to be good models with which to characterize the site of action of anesthetics. Also, the agreement between the behavior of the pure lipid and whole animal systems lends support to the theory that the primary site of anesthetic action is in the lipid phase.

The spin label TEMPO, although not an inhalational anesthetic, does behave like an anesthetic in that it produces reversible depression of synaptic transmission at concentrations lower than those necessary to block axonal conduction. The distribution of TEMPO between the lipid and aqueous phases changes by only 5 per cent with the application of 4,000 psig of helium. Other work involving measurement of cation permeability of liposomes also strongly suggests that the amount of anesthetic dissolved in the bilayer is the same for all pressures. Thus, the phenomenon of pressure reversal appears to result from reordering of the hydrophobic region rather than displacement of the anesthetic molecules from the region. This mechanism of pressure reversal supports the critical-volume theory over the Meyer-Overton lipid-solubility theory.

The 3F NMR spectrum of the fluorine on halothane was studied in an aqueous phosphatidylcholine bilayer suspension. Halothane rapidly achieves equilibrium and exchanges throughout the bilayer and aqueous phases. No more than a very small amount of halothane is permanently held in the bilayer. These findings are inconsistent with the aqueous-phase theories of Pauling and Miller since they postulate stable anesthetic-water structures.

Recently, Boggs et al. have questioned much of the previous work concerning the fluidization by anesthetics and reordering by pressure of phospholipid bilayer membrane models. These investigators studied phosphatidylcholine-cholesterol bilayers, but used 8-doxylpalmitate and 12-doxylstearate spin labels. They also found a dose-related disordering of the bilayers and increased ordering with pressure. However, they maintain that anesthetics produce no detectable change in bilayer order at clinical concentrations. The controversy would appear to revolve about the definition of clinical concentrations. Boggs et al. state that the aqueous concentration of halothane necessary to anesthetize a newt is 0.4 mM (this information is originally from Miller et al.). Using the halothane phosphatidylcholine-cholesterol-water partition coefficient of Trudell et al., they calculated the corresponding concentration of halothane in the lipid (actually they incorrectly used the partition coefficient of methoxyflurane, 19 ± 4, rather than the coefficient for halothane, 13 ± 3). Boggs eg al. found no detectable effect on bilayer order below concentrations of halothane of 2.0–3.6 mM aqueous (5–9 times the ED90 in newts). Per liter of lipid, 3.6 mM aqueous corresponds to 47 mmol halothane. Trudell et al. could also measure bilayer disordering at a similar concentration (61 mmol halothane per liter of lipid). However, Trudell et al., quoting Meyer and Overton, state that anesthesia occurs at concentrations of 30–60 mmol per liter of lipid. The MAC of halothane is 0.0077 and the gas partition coefficient is 224. The concentration necessary for anesthesia, ignoring temperature, is (0.0077)(224)/224 1/mole = 77 mmol halothane per liter of oil. The problem is that both phosphatidylcholine and olive oil are model systems. Whether the anesthetic site has a partition coefficient like olive oil or phosphatidylcholine is uncertain.
Rosenberg et al. studied the effects of halothane on dipalmitoyllecithin and palmitoylauraylecithin bilayers and rat brain synaptic plasma membranes using ESR. They found that low concentrations of halothane, ~1 mmol per liter of membrane suspension (the partition coefficients were unknown), increased order, while higher concentrations, ~9.3 mM, decreased order. The authors suggest that this biphasic effect of halothane may be due to filling of free space in the lattice of membrane structure.

**Steroid and Barbiturate Effects**

The effects of steroid and barbiturate anesthetics on membrane model systems have also been examined by magnetic resonance techniques. 3α-hydroxy-5α-pregnan-11,20-dione (Alphaxalone) is a very potent anesthetic, whereas the 3β-hydroxy compound (Betaxalone) is inactive. The only difference between the compounds is that the 3α-hydroxy is approximately perpendicular to the plane of the rings, whereas the 3β-hydroxy is coplanar with the rings. The ring system of either compound is in the all-trans configuration and is approximately planar. A similar, though smaller, difference in anesthetic activities is found between 3α- and 3β-hydroxy-5α-pregnan-20-one. The members of each isomeric pair do not differ significantly in their lipid solubilities or molar volumes. Lawrence and Gill studied the effects of these and other compounds on lecithin–cholesterol bilayers by ESR. The potent anesthetics all produced marked dose-related increased fluidity of the bilayers. The inactive compounds, at the same concentrations or volume fractions as their active isomers, produced much less disordering. The good correlation of anesthetic potency and ability to disorder spin-labeled bilayers lends support to a lipid site of anesthetic action. For the compounds that were potent anesthetics, the ability to disorder bilayers correlated better with volume fraction than with concentration. These drugs produced approximately the same degree of fluidization as halothane at the same volume fraction. These data support the critical-volume theory. However, for the steroid anesthetics not only the volume fraction of anesthetic in the membrane but also apparently the orientation of the steroid molecule is critical. The steroid anesthetics are large molecules compared with the lipid bilayer, and one steroid molecule perpendicular to the plane of the membrane would penetrate halfway through the bilayer. Lawrence and Gill suggest that the difference in activity between the 3α- and 3β-hydroxy isomers could be that the formation of a hydrogen bond between the hydroxyl and polar groups at the bilayer surface causes a difference in the orientations of the two molecules in the bilayer.

Novak and Swift employed 1H, 13C, and 31P NMR spectroscopy to investigate the nature of the barbiturate–phospholipid interaction. These studies specifically identified the molecular site of interaction as an N–H moiety of the barbiturate and the phosphate group of the phospholipid. The NMR spectra are consistent with hydrogen bonding as the mode of association of the barbiturates (phenobarbital and pentobarbital) with the phospholipids (phosphatidyicholine, sphingomyelin, phosphatidyl ethanolamine, and cardiolipin). Inhalational anesthetics are thought not to form hydrogen bonds, so that the mechanism of anesthetic action of the barbiturates may be fundamentally different from that of the inhalational agents. Furthermore, marked differences in potencies of stereoisomers of barbiturates have been reported, whereas there is no difference in potencies of stereoisomers of halothane.

The increased membrane fluidity produced by anesthetics may reflect a transition from organized gel to randomized liquid-crystal phase. Anesthetics have been shown to lower the phase transition temperature of phospholipids, as measured by both ESR and differential scanning calorimetry. Furthermore, pressure is able to antagonize the effect of anesthetics on these transition temperatures. Trudell and Cohen have advanced the theory that anesthetics perturb lateral phase separations that are necessary for solvated membrane protein function.

**Other Models**

Erythrocyte membranes have been used extensively as models for drug interaction. Hubbell et al. have examined the effects of some local anesthetics on erythrocyte membranes with ESR. The predominant effect is a fluidizing of the membrane. However, all the spin labels examined were bound both by separated membrane protein and lipids, so the authors were unable to distinguish protein and lipid binding sites in the intact membrane. Comparison of neural and erythrocyte membranes suggest that the latter are more ordered and tightly packed. For example, the neural membrane binds TEMPO, while the erythrocyte membrane does not. Cholesterol, which has a pronounced ordering effect on neural membrane, has little effect on the erythrocyte membrane. Probably the erythrocyte membrane is so tightly packed that little additional ordering is possible. The ESR experiments on erythrocyte membranes are supported by comparable studies using NMR.

Hemoglobin has been used as a model of anesthetic–protein interaction. Barker et al., using NMR, examined the effects on hemoglobin of clinical concentrations of anesthetics and came to the following conclusions: Anesthetic drugs at clinical concentrations interact with hemoglobin. At clinical concentrations, the interactions with hemoglobin of halothane and methoxyflurane are localized and specific; at higher concentrations the interactions are more general and nonspecific.
although reversible. The conformational changes produced by high concentrations of halothane, methoxyflurane, and diethyl ether are dependent on the agent as well as on the concentration. These data suggest that anesthetics may be capable of interacting directly with membrane proteins, thus lending support to the protein conformational change theory.

Summary

The large number and diversity of anesthetic agents were evident to investigators 80 years ago, and suggested a physicochemical theory of anesthesia. Meyer and Overton were the first to offer a quantitative relationship between a physicochemical property and potency of anesthetic agents. They also focused attention on the lipid phase as the site of anesthetic action. Ferguson realized that the concentration of an agent at its site of action bears a generally unknown relation to the concentration in the external phase. However, at equilibrium the activity of an agent is the same in every phase, motivating Ferguson to suggest that activities rather than concentrations be used as indices of dosage. The critical-volume theory resulted from modification of the Meyer-Overton theory to include the molal volume of the anesthetic. The allowance for molal volume resulted initially from an attempt further to regularize the experimental data. The concept of a critical-volume fraction of anesthetic being necessary for narcosis was discussed in most detail by Mullins. Subsequently, the concept of the effect of the anesthetic has changed from filling of free space to expansion and fluidization of the membrane. The ability of pressure to cause excitant phenomena and antagonize anesthetics is predictable from the critical-volume theory and is therefore highly significant evidence. K. W. Miller and associates are perhaps most prominent in the recent quantification and formalization of the critical-volume theory and HPNS. The existence of a separate convulsant site(s) is suggested by the demonstration of significantly different compressibilities associated with anesthesia and convulsions. Work corroborating a separate convulsant site involved measurement of the partial molal volumes of a series of related convulsant and anesthetic ethers and calculation of each compound's solubility parameter. Multiple convulsant sites may exist, and these two methods may not have accessed the same site. Understanding the anesthetic-convulsant duality will have important practical application to deepwater diving, and may well offer important insight into the neurophysiologic and electrophysiologic effects of anesthetics. The application of ESR and NMR allows investigation at the molecular level of effects of anesthetics on biological and model membranes. Magnetic resonance techniques have generally supported the concept of membrane fluidization by anesthetics.

Some investigators have recently attempted to displace the focus of attention from the lipid phase. However, the evidence is clearly against the aqueous-phase theory of Pauling and S. L. Miller. The microtubule theory of Allison and Nunn has not accumulated supporting evidence comparable to the lipid theories. Contradictory evidence makes any evaluation of this theory speculative. Additionally, the interspecies and intracellular variability of microtubules raises questions of the relevance of many studies. Although the majority of investigators appear to favor a lipid anesthetic site, the PCC theory is certainly viable. A hydrophobic protein site of action could certainly cause many of the physicochemical relationships that implicate the lipid phase. Further work will be necessary to distinguish the anesthetic site of action unequivocally.

Biophysical or molecular processes are just one level at which anesthetic mechanisms can be investigated. Although biophysical processes are in many ways most accessible and quantifiable, anesthetic mechanisms can also be discussed relative to cellular and intermediate neural actions, and modification of consciousness. Millar has pointed out that the question "How does anesthesia occur?" is unanswerable in the absence of a definition of consciousness.

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