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Kinetic and Thermodynamic Aspects of the Mechanism of General Anesthesia in a Model System of Firefly Luminescence in Vitro

Issaku Ueda, M.D., and Hiroshi Kamaya, M.D.†*

Cell-free firefly-tail extract emits light when ATP is added. The flash intensity follows first-order kinetics. Methoxyflurane, chloroform, halothane, enflurane, and fluroxene inhibited this reaction. Tensions which inhibited flash intensity 50 per cent were 2.1×10^{-3} atm, 9.0×10^{-3} atm, 1.04×10^{-2} atm, 2.0×10^{-2} atm, and 5.1×10^{-2} atm, respectively. These values correlate better with oil/gas partition coefficients than with hydrate-dissociation pressures. Thermodynamic analysis showed that the inhibition mechanism of anesthetics is identical to reversible thermal inactivation of the enzyme. In this reaction the original "folded" enzyme became inactive by transformation into the "unfolded" or expanded type. Inhibition was accompanied by a high heat of reaction (ΔH: −80,000 to −89,900 cal/mol) and a large entropy change (ΔS: −277 to −320 entropy units), and was noncompetitive with ATP binding. The magnitudes of the heat of reaction and the entropy change support the theory that anesthetics act at a hydrophobic site of luciferase, inducing a structural change of the enzyme to form the "unfolded" type. The correlation between the ED_{50}'s of the anesthetics and their oil/gas partition coefficients together with the enzyme kinetic data indicates that the site of anesthetic action may be hydrophobic. (Key words: General anesthetics; Firefly luminescence; Unfolding theory of anesthesia.)

LUMINESCENCE measures the metabolic energy output of light-emitting organisms. The idea that metabolic inhibitors may suppress this luminescence was tested by Harvey[1] in 1915, in a study of luminous bacteria and alcohols, with positive results. Since then, inhibitory actions of anesthetics upon bacterial luminescence *in vivo* have been extensively studied by the investigators from Princeton.[1–9] They have used urethanes, alcohols, and barbiturates except for Johnson *et al.*,[4] who also studied diethyl ether and chloroform. Recently White and Dundas[10] and Halsey and Smith[11] reported inhibitory effects of modern gaseous anesthetics upon bacterial luminescence *in vivo*.

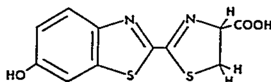
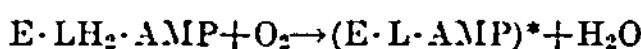


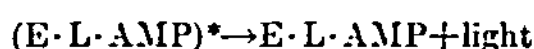
FIG. 1. Structure of firefly luciferin.

Firefly tails contain a light-emitting substance, luciferin, and an enzyme, luciferase. Luciferin and luciferase can be solubilized cell-free from the homogenate of firefly tail, and emit light *in vitro* when mixed with adenosine triphosphate (ATP). The flash intensity is proportional to the amount of ATP added, which limits the reaction rate. The luminescent reaction in firefly extracts has been described by McElroy, Seliger, and White.[12,13] The structure of firefly luciferin (fig. 1) has been clarified, and total synthesis was accomplished by White *et al.*[14] Firefly luminescence is induced in stages. Luciferin (LH_2) and ATP form a complex with luciferase (E) to release pyrophosphate (PP), resulting in an enzyme–AMP–luciferin complex. This complex attains its high-energy state (represented by an asterisk) in the presence of oxygen. Upon returning to the low-energy state, the complex emits a burst of light while releasing adenosine monophosphate (AMP) and dehydroluciferin (L).

$$LH_2 + E + ATP \rightleftharpoons E \cdot LH_2 \cdot AMP + PP \quad (1)$$



$$E \cdot LH_2 \cdot AMP + O_2 \rightarrow (E \cdot L \cdot AMP)^* + H_2O \quad (2)$$



$$(E \cdot L \cdot AMP)^* \rightarrow E \cdot L \cdot AMP + \text{light} \quad (3)$$

$$E \cdot L \cdot AMP \rightleftharpoons E + L + AMP \quad (4)$$

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Although bacterial luminescence *in vivo* is sensitive to ambient oxygen tensions, the light intensity of the firefly system *in vitro* is not influenced by oxygen tensions except in extreme anoxic conditions. The firefly cell-free system has an advantage over live luminous bacteria in that the cell-free system does not consume oxygen to support nonluminescent cellular metabolism. Furthermore, the bacterial system has two pathways for luminescent reaction, one of them having a higher yield of light output than the other. The second, low-yield, pathway has not been detected in the firefly system.

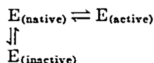
Previously, Ueda[15] reported the inhibitory actions of diethyl ether and halothane upon the initial flash intensity of firefly-tail extract. The present study was intended to analyze the mode of anesthetic action upon cell-free firefly luminescence according to the theory of absolute reaction rates described by Eyring and Magee.[16] These authors expressed the light intensity of luminous bacteria as follows†:

$$I = \frac{cTe^{-\Delta H^{\ddagger}/RT}}{1 + e^{-\Delta H/RT}e^{\Delta S/R}} \quad (5)$$

I = light intensity
$\Delta H^{\ddagger}$ = activation enthalpy
ΔH = enthalpy change
ΔS = entropy change
R = gas constant
T = absolute temperature
c = constant
e = exponent

This equation may be applied to data obtained with either purified single enzyme systems or complex physiologic processes. All numerical values can be calculated by observing intensities at various temperatures.

Light intensity of biological luminescence increases with temperature elevation, reaches its maximum at a certain temperature (optimal temperature), and then decays as the temperature is further elevated. The decay of light intensity at supraoptimal temperatures is readily reversible by decreasing the elevated temperature. This indicates that more luciferase attains its high-energy state ($E_{(active)}$) with temperature elevation, and is thermally inactivated ($E_{(inactive)}$) above the temperature optimum. This relationship is illustrated as follows:



$$E_{(native)} \rightleftharpoons E_{(active)}$$
$$\downarrow\uparrow$$
$$E_{(inactive)}$$

Metabolic inhibitors may suppress light intensity either by interacting with active sites of the luciferase or by accelerating the transition of $E_{(native)}$ to $E_{(inactive)}$. In other words, the inhibition may be either independent of thermal inactivation or dependent upon it.

Johnson, Eyring and Williams[3] designated the former inhibition "type I" and the latter "type II." They formulated the following relationships for each type of inhibition. (The theoretical basis for the equation is included in the Appendix.)

Type I

Inhibition is independent of thermal inactivation of the enzyme.

$$\ln(I_1/I_2 - 1) = r\ln X - \Delta H/RT + \Delta S/R \quad (6)$$

Type II

Inhibitor promotes thermal inactivation of the enzyme.

$$\ln(I_1/I_2 - 1)(1 + 1/K) = s\ln U - \Delta H/RT + \Delta S/R \quad (7)$$

The notations for the equations are:

I_1 = intensity of light without inhibitor
I_2 = intensity of light with inhibitor
K = equilibrium constant for $E_{(inactive)}$ and $E_{(native)}$
X = concentration of type I inhibitor
r = number of molecules of type I inhibitor combining with each enzyme molecule
U = concentration of type II inhibitor
s = number of molecules of type II inhibitor combining with each enzyme molecule

The expression $(I_1/I_2 - 1)$ may be arranged as $(I_1 - I_2)/I_2$, which indicates the ratio of per cent light output inhibited to per cent light output uninhibited.

Equation 6 indicates that when the temperature is varied while keeping the concentration of type I inhibitor (heat-independent) con-

† Descriptions of enthalpy and entropy appear on pages 416 and 419.

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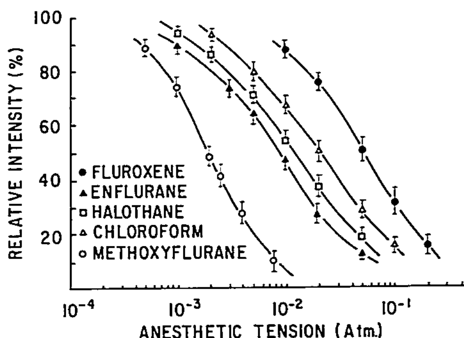

FIG. 2. Dose-response curves. Flash was induced at 21.5 ± 0.1 C by mixing ATP to a final concentration of 0.1 mM. Each point is a mean of three observations; vertical bars represent standard deviations. *Abscissa*: Logarithm of tensions of anesthetics in the gas phase equilibrated with the reaction mixture, expressed as atmospheric pressure. *Ordinate*: Per cent flash intensity compared with controls without anesthetics.

stant, the plot of $\ln(I_1/I_2 - 1)$ *vs.* $1/T$ is straight and the slope of the line gives the value of ΔH. Equation 7 indicates that under the identical conditions, the plot of $\ln(I_1/I_2 - 1) \times (1 + 1/K)$ *vs.* $1/T$ becomes straight when the inhibitor belongs to type II (heat-dependent). Therefore, type I and type II inhibition are distinguishable by plotting $1/T$ against $\ln(I_1/I_2 - 1)$ and $\ln(I_1/I_2 - 1)(1 + 1/K)$; the straightness of the line determines the type of inhibition to which the inhibitor belongs, and the slope of the straight line gives ΔH. When the inhibitor concentration, rather than the temperature, is varied, a linear relationship between the logarithm of inhibitor concentration ($\ln X$ or $\ln U$) and $\ln(I_1/I_2 - 1)$ or $\ln(I_1/I_2 - 1) \times (1 + 1/K)$ is observed. In this case, however, K becomes a constant value because K is dependent upon the temperature only. Therefore, the plot of $\ln(I_1/I_2 - 1)$ *vs.* $\ln U$ (as well as $\ln X$) becomes straight; this plot does not distinguish between the two types of inhibition. The slope of the line gives the number of inhibitor molecules (r and s) combining with each enzyme molecule.

Johnson, Eyring and Williams[3] have shown with luminous bacteria that type II inhibition was accompanied by large ΔS (−165 to −304 entropy units) and ΔH (−56,000 to −70,000 cal/mol) values, while type I inhibition showed considerably lower values for ΔS (−36 to −40 entropy units) and ΔH (−12,000 to −16,980 cal/mol).

The results of the present study have shown that the inhibition of firefly luciferase by general anesthetics conforms to type II. The thermodynamic numerical values are presented below.

Method

Cell-free extracts of luciferin and luciferase were prepared from dried firefly tail (Sigma) by homogenizing it in 0.1 M arsenate buffer, pH 7.4, in all-glass tissue grinder, and centrifuging the homogenate at 30,000 × g for one hour, as previously described.[15] The final concentration was 5 mg of firefly tail (dry weight) per 1.0 ml of 0.1 M Na arsenate and 0.04 M $MgCl_2$ at pH 7.4. The extract was made fresh daily and kept in an ice-water bath during the experiment.

Luminescence was measured with a Hitachi Perkin-Elmer 139 spectrophotometer equipped with a photomultiplier with the light source turned off, and recorded on a Brown Electronic recorder. The temperature of the system was controlled by using a water-jacketed cuvette compartment and circulating water from a large-volume water bath which had a heating element and mercury switch thermoregulator (Precision, Chicago, Illinois). The temperatures of the water bath and the cuvette compartment were monitored by thermocouples connected to a digital thermometer readable to 0.01 C (Digitec, Dayton, Ohio). The cuvette compartment was modified so that two hypodermic needles could be inserted into it. One was used to inject ATP solution and the other to deliver anesthetic gas or compressed air. The needles were coated with

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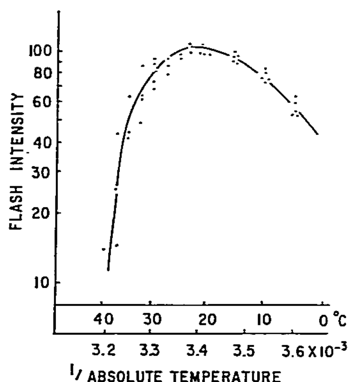

FIG. 3. Arrhenius plot of flash intensity without anesthetics. Flash was induced by mixing ATP to a final concentration of 0.1 mM at various temperatures. *Abscissa*: Reciprocal of absolute temperature. Corresponding temperatures in degrees Celsius are shown. *Ordinate*: Logarithm of flash intensity in arbitrary values.

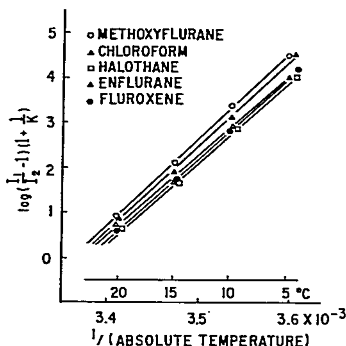

FIG. 4. Type II inhibition of firefly luminescence by anesthetics. Flash was induced by mixing ATP to a final concentration of 0.1 mM at various temperatures. Anesthetic tensions were ED_{50} values interpolated in figure 2. *Abscissa*: Reciprocal of absolute temperature. Corresponding values in degrees Celsius are shown. *Ordinate*: $\log (I_1/I_2 - 1) \times (1 + 1/K)$ where I_1 equals flash intensity without anesthetics at a certain temperature, I_2 equals the same with anesthetics, and K equals equilibrium constant between inactivated and native luciferase.

silicone antifoam (General Electric) to prevent foaming of the firefly tail extract during bubbling. Disodium ATP (Sigma) was dissolved in 0.1 M Na arsenate buffer, *p*H 7.4, in appropriate concentrations.

A 2.0-ml sample of firefly-tail extract was transferred to a standard 1.0-cm light-path cuvette and warmed for 5 minutes to the desired temperature in the water bath.

The anesthetic gas mixture was bubbled through the extract during the last 3 minutes of the temperature equilibration period. The cuvette was transferred to the water-jacketed compartment, and the anesthetics were again administered to the cuvette through the hypodermic needle attached to the compartment. After one minute, 400 μl of ATP solution were administered through the second needle. Flash intensity was recorded and compared with control values. Control values were obtained by treating the extract with air.

Methoxyflurane, chloroform, halothane, enflurane, and fluroxene were vaporized in a Copper Kettle and diluted with oxygen. The concentrations of anesthetic gas were estimated from Kettle temperature and flow of diluent oxygen. The accuracy of the concentrations obtained was confirmed by gas chromatography with a thermal conductivity sensor. Concentrations of anesthetics were expressed as partial pressures in the gas phase equilibrated with the firefly-tail extract.

Results

The study was performed with three variables: temperature, ATP, and anesthetic tension. The effect of each variable was tested by fixing the remaining two.

The effects of anesthetics upon flash intensity are illustrated in figure 2. All anesthetics inhibited flash intensity in a dose-dependent fashion. The experiment was undertaken at 21.5 ± 0.1 C and 0.1 mM ATP. The tensions of anesthetics which produced 50 per cent inhibition (ED_{50}) were: methoxyflurane, 2.1 $\times 10^{-3}$ atm; chloroform, 9.0 $\times 10^{-3}$ atm; halothane, 1.04 $\times 10^{-2}$ atm; enflurane, 2.0 $\times 10^{-2}$ atm; fluroxene, 5.1 $\times 10^{-2}$ atm. At lower anesthetic tensions, excitation rather than inhibition was observed with all agents (not shown in the figure).

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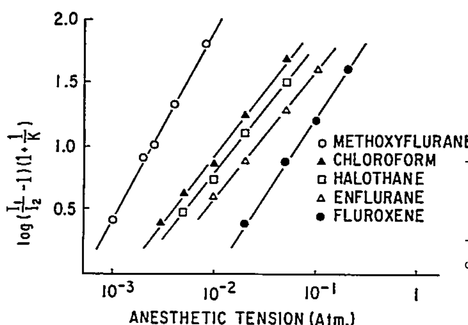

FIG. 5. Inhibition as a function of anesthetic tensions. Flash was induced at 21.5 C by mixing ATP to a final concentration of 0.1 mM. *Abscissa*: logarithm of tensions of anesthetics. *Ordinate*: see figures 3. The slopes of the curves give the numbers of anesthetic molecules combining with luciferase: methoxyflurane 1.5, chloroform 1.0, halothane 1.1, enflurane 1.0, fluroxene 1.2.

Flash intensity of the firefly-tail extract increased with temperature elevation between 5 and 22 C. Maximal flash intensity was observed at 22 C (optimal temperature); flash intensity declined above this temperature. This thermal inactivation was reversible. The firefly-tail extract was heated to supraoptimal temperature for 3 minutes and cooled to the temperature optimum and flash intensity was compared with that obtained from the extract maintained at the supraoptimal temperature. More than 80 per cent reversal of thermal inactivation was observed.

Figure 3 shows the Arrhenius plot in which the logarithm of flash intensity was plotted against the reciprocal of absolute temperature. From the slope of the low temperature range, the activation energy ($E_{(native)} \rightarrow E_{(active)}$) was estimated as 9,800 cal/mol. The activation enthalpy ($\Delta H^{\ddagger}$), obtained by subtracting RT (gas constant times absolute temperature) from activation energy, was 9,200 cal/mol. The heat of reaction (ΔH) of thermal inactivation ($E_{(native)} \rightarrow E_{(inactive)}$), estimated from the slope of the high temperature range, was 76,400 cal/mol. The entropy change (ΔS) for thermal inactivation, calculated from equation 5, was 257 entropy units.

The fitness of the data to type I (equation 6) or type II (equation 7) was determined by observing I_1 and I_2 between 5 C (278 K) and 20 C (293 K) by mixing ATP to a final concentration of 0.1 mM. Anesthetic tensions which gave 50 per cent inhibition of flash intensity at 21.5 C were selected from figure 1.

The values of $(1 + 1/K)$ at each temperature were estimated from the equation:

$$\ln K = (-\Delta H + T\Delta S)/RT \qquad (8)$$

where ΔH and ΔS were obtained from the preceding experiment (fig. 3). Straight lines were obtained when $\log (I_1/I_2 - 1)(1 + 1/K)$ was plotted against the reciprocal of absolute temperature (fig. 4) in conformity with type II inhibition. The type I plotting did not produce straight lines. The anesthetics promoted the thermal inactivation of luciferase.

Figure 5 shows the relationship between $\log (I_1/I_2 - 1)(1 + 1/K)$ and the logarithm of the anesthetic tensions. Flash intensities

TABLE 1. Summary of Thermodynamic Characteristics of Type II Inhibition of Luciferase by Anesthetics*

	Methoxyflurane	Chloroform	Halothane	Enflurane	Fluroxene
ΔH (cal/deg)	−89,700	−89,900	−83,270	−80,000	−88,410
ΔS (entropy units)	−320	−312	−291	−277	−306
s†	1.5	1.0	1.1	1.0	1.2

* These data were estimated from figures 4 and 5.
† The number of type II inhibitor molecules combining with each luciferase molecule.

were measured at 21.5 ± 0.1 C. The heat of reaction, the number of molecules of each anesthetic combining with luciferase, and the entropy change were estimated and are summarized in table 1. Large values of both ΔH (−80,000 to −89,900 cal/mol) and ΔS (−277 to −320 entropy units) were obtained. The numbers of molecules combining with luciferase ranged from 1.5 for methoxyflurane to 1.0 for chloroform and enflurane.

Figures 6 and 7 are Lineweaver-Burk plots in which the reciprocal of flash intensity is plotted against the reciprocal of ATP concentration of several tensions for methoxyflurane (fig. 6) and halothane (fig. 7). The temperature was 21.5 ± 0.1 C. From these figures the Michaelis constant (K_m) for ATP was estimated as 0.35 mM. The presence of methoxyflurane or halothane did not change K_m, showing a noncompetitive type of inhibition.

Discussion

Before we discuss the interaction of anesthetics with firefly luciferase, it may be argued that the anesthetics act on luciferin rather than luciferase. However, the molecular structure of firefly luciferin (fig. 1) leaves little possibility of chemical bonding of this small molecule to the fairly nonreactive general anesthetics of the present study. Furthermore, prompt reversal of the inhibition by simple washout with air disputes the possibility of chemical bonding, except for a very loose association between the two molecules.

Because the present study refers to the reversible thermal inactivation of luciferase, it would be appropriate to start the discussion by describing its characteristics.

Working with bacterial luminescence *in vivo*, Brown, Johnson, and Marsland[17] found that when the temperature was below optimum, a pressure of several hundred atm decreased the light intensity. However, when the temperature was above optimum, the same pressure increased the light intensity. This apparent paradox was solved as follows. By plotting the logarithm of the rate of reaction against pressure, these authors and Eyring and Magee[16] estimated that $E_{(active)}$ is 50 ml more voluminous per mole than $E_{(native)}$ at 0 C. At 35 C, $E_{(inactive)}$ is 64 ml per mole larger than $E_{(native)}$. These volume changes are accompanied by large ΔH (55,260 cal/mol) and ΔS (180 entropy units) values compatible with the conformational change of the enzyme structure.

According to the Braun-Le Chatelier principle, pressurizing the system shifts the equilibrium in the direction that would reduce the pressure and favors the existence of a smaller volume. Therefore, below the optimal temperature, pressure antagonizes the transition of $E_{(native)}$ to $E_{(active)}$ and the light output is diminished. Above the optimal temperature pressure pushes $E_{(inactive)}$ to $E_{(active)}$ and increases the light intensity.

Eyring[18] proposes that at low temperature the enzymes are folded in water solution, their hydrophilic sites extending outside and covering hydrophobic sites inside. At higher temperature, this folded enzyme starts to unfold, as evidenced by large changes in entropy and enthalpy and by the increases in its volume. He postulated that molecules which are capable of forming hydrophobic bonds inhibit enzymes by unfolding the enzyme structure, thus acting as a type II inhibitor.

The present temperature data (fig. 4) formed a straight line when the $\log(I_1/I_2-1)(1+1/K)$ was plotted against the reciprocals of the absolute temperatures. Thus, the inhibition of firefly luminescence by these anesthetics conforms to type II, *i.e.*, the equilibrium constant between $E_{(inactive)}$ and $E_{(native)}$ is altered in favor of $E_{(inactive)}$. This implies that when anesthetic molecules attach to $E_{(native)}$, $E_{(native)}$ is transformed into $E_{(inactive)}$, a process analogous to thermal inactivation. This inhibition is accompanied by large changes in enthalpy (−80,000 to −89,900 cal/mol) and entropy (−277 to −320 entropy units). These findings indicate that the anesthetics induce unfolding of firefly luciferase. The magnitude of the changes of enthalpy and entropy supports the view that a major conformational change of the enzyme structure occurred.

In order to confirm hydrophobic binding of anesthetics to luciferase, the ED_{50}'s of anesthetic tensions were estimated from figure 2, and correlations with oil/gas partition coefficients and hydrate-dissociation pressures were examined by plotting anesthetic tensions

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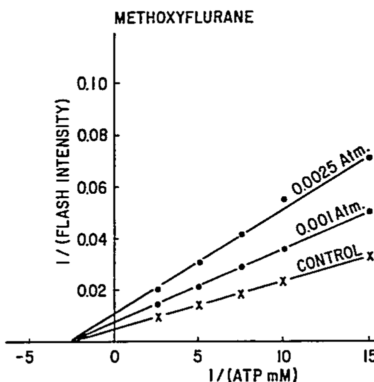

FIG. 6. Lineweaver-Burk plot of methoxyflurane inhibition. Flash was indueed at 21.5 C at various ATP concentrations. *Abscissa*: Reciprocal of ATP concentration in mM. *Ordinate*: Reciprocal of flash intensity. Methoxyflurane tensions are indicated in the figure. Noncompetitive inhibition is demonstrated.

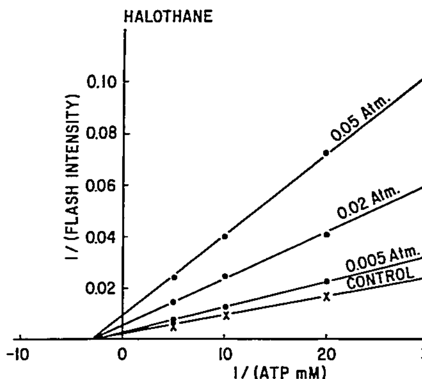

FIG. 7. Lineweaver-Burk plot of halothane inhibition. See legend to figure 6. Halothane tensions are indicated in the figure. Noncompetitive inhibition is demonstrated.

against these values (fig. 8). The results were very similar to those reported by Eger *et al.*,[19,20] obtained by plotting minimal alveolar concentrations of anesthetics necessary to induce anesthesia in dogs against oil/gas partition coefficients and hydrate-dissociation pressures. They found better correlation with oil/gas partition coefficients than with hydrate-dissociation pressures and concluded that the lipid phase may be the site of action of anesthetics. Miller *et al.*,[21,22] came to the same conclusion after determining ED_{50}'s of anesthetics with regard to the righting reflex in mice, and correlating these values with oil/gas partition coefficients. Figure 8 illustrates that correlation with oil/gas partition coefficients is better than correlation with hydrate-dissociation pressures. The correlation coefficient for oil/gas partition coefficients was 0.995 ($P < 0.01$) and that for hydrate-dissociation pressures, 0.902 ($P < 0.05$).

ATP, being a highly polarized molecule, should bind to hydrophilic sites of luciferase. The present demonstration of the noncompeti-

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tive nature of anesthetic inhibition implies that anesthetics do not attach to ATP binding sites, and is consistent with the unfolding theory.

If type II inhibition involves unfolding of luciferase analogous to the thermal inactivation process, the inhibition should be reversed by pressure. The antagonism between anesthetics and hydrostatic pressure was demonstrated by Johnson, Brown and Marsland[4]. In their study, inhibition of bacterial luminescence by chloroform, ethanol, diethyl ether, and ethyl carbamate was reversed by pressurizing the system to 150 to 300 atm. Johnson and Flagler[23] further demonstrated that tadpoles anesthetized with ethanol started swimming again when exposed to hydrostatic pressures of 150 to 350 atm. This observation was confirmed recently by Johnson (S. M.) and Miller[24] in a study of newts anesthetized with diethyl ether, butanol or nitrogen. The righting reflexes were regained when the ambient pressure was raised to 150 atm by hydrostatic pressure or helium. In a similar study, Lever *et al.*[25] found that mice as well as newts anesthetized with pentobarbital, diethyl ether, halothane, or nitrogen regained the rolling reflex when the system was pressurized to 100 to 200 atm. These authors calculated that anesthetics at clinical concentrations expand lipids about 0.4 per cent in volume.

These studies of pressure in tadpoles, newts and mice have demonstrated an analogy between the state of general anesthesia and the suppression of luminescence by anesthetics, indicating that the biological luminescent system is a suitable model for analysis of the actions of anesthetics. Some structure in the central nervous system vital to its function, presumably the cell membrane, may undergo the transition of folding and unfolding. The unfolded or expanded state may manifest itself as the anesthetized state.

Shanes[26] proposed a concept of membrane stabilization of excitable cells when the membrane potential becomes unresponsive to incoming stimuli without alteration of the resting potential. General and local anesthetics, central nervous system depressants, tranquilizers, and antihistaminics prevent the shift of membrane potential, thus making the cells less prone to depolarization. These agents are classified as membrane stabilizers.

Seeman[27,28] extended the concept of membrane stabilization from nerve cells to erythrocyte membrane and has shown that hypotonic hemolysis is preventable by a number of membrane stabilizers. Seeman and Roth further showed that general anesthetics suppressed hypotonic hemolysis of human erythrocytes concomitant with expansion of the erythrocyte membrane. They found that at clinical anesthetic tensions the erythrocyte membrane expands by about 0.4 per cent and surmised that similar expansion occurs at the nerve cell membrane because erythrocyte and synaptosome membranes are fluidized to the same extent at the same concentrations of lipid-soluble anesthetics. This hydrophobic expansion of cellular membrane has been advocated by these authors to be the mechanism of anesthesia. This measured value of the expansion of the erythrocyte membrane is identical to the value of lipid expansion calculated by Lever *et al.*[25]

Thus, membrane stabilization is not limited to nerve cells; analogies may be found in other cell membranes or in synthetic lipid monolayer film. Ueda[30] reported that the ADP-induced platelet aggregation is preventable by volatile anesthetics, presumably by their interaction with platelet cell membrane. The dose–response curve of platelet aggregation is very similar to that of the present study. Clements and Wilson[31] demonstrated that general anesthetics decreased surface tensions of monolayers of stearic acid, cholesterol and synthetic lecithin. They estimated that 1.6×10^{-11} mole of agent are adsorbed per square centimeter of the interface at clinical tensions. This corresponds to a decrease of 0.39 dyne/cm in the surface tension and increases the interfacial mass by slightly less than 1 per cent, a value not far from those reported for lipid expansion in the pressure antagonism study and for erythrocyte membrane expansion. They also reported that the surface tension of pure water did not respond to anesthetics, and concluded that this finding is not consistent with the idea that anesthetics interact solely with water.

Nervous activity is a complicated process

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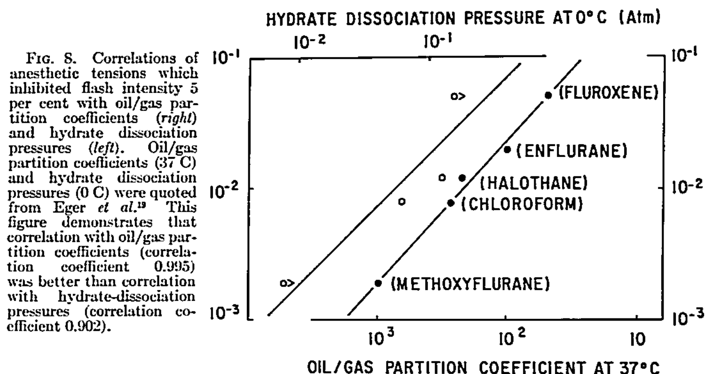

FIG. 8. Correlations of anesthetic tensions which inhibited flash intensity 5 per cent with oil/gas partition coefficients (*right*) and hydrate dissociation pressures (*left*). Oil/gas partition coefficients (37 C) and hydrate dissociation pressures (0 C) were quoted from Eger *et al.*[19] This figure demonstrates that correlation with oil/gas partition coefficients (correlation coefficient 0.995) was better than correlation with hydrate-dissociation pressures (correlation coefficient 0.902).

involving the excitation of a presynaptic membrane, release of a chemical transmitter, then the depolarization of a postsynaptic membrane. Together with the fact that the hydrophobic binding of anesthetics is rather nonspecific, the exact site on the nerve cell at which anesthetics interact with lipids is still impossible to define and is a subject of much speculation. In the present discussion let us consider some possibilities involving the postsynaptic event. The membrane potential is a bioelectrical phenomenon generated by 1) the unequal distribution of monovalent cations across the membrane and 2) the change in the conductance of these ions in the membrane. Therefore, two plausible explanations of anesthetic interaction may be postulated, involving either 1) the mechanism for the unequal distribution of sodium and potassium ions or 2) the structure of the cell membrane responsible for permeability to these cations.

In relation to the first possibility, sodium- and potassium-activated adenosine triphosphatase (Na + K ATPase) carries a special interest. This enzyme is located in the cell membrane, its activity is closely associated with the active translocation of Na^+ and K^+, and it contains about 40 to 50 per cent lipids. Although there is no direct evidence for the role of brain Na + K ATPase in the mechanism of action of central nervous system depressants, numerous membrane stabilizers suppress its activity.[32–41] Ueda and Mietani[36] reported that halothane and diethyl ether inhibited Na + K ATPase prepared from rat cerebrum. Trevor and Cummings[37] reported that Na + K ATPase activity of the lipoprotein fraction of rat cortex is suppressed by cyclopropane. Israel and Salazar[35] found that chloroform, diethyl ether, and acetone inhibited beef-brain Na + K ATPase. Ueda *et al.*[40] found that the inhibitory actions of some tranquilizers are stronger when the molecules are in the uncharged state, indicating hydrophobic binding to the enzyme. However, the concentrations of membrane stabilizers which inhibit enzymic activity are in excess of clinical concentrations.

The second possibility deals with the conformational change of the molecules of the cell membrane which may lead to a disturbance in the permeability of the cell membrane to the monovalent cations in an unknown fashion. The thermodynamic data of the present study show that a large ΔH is accompanied by a large ΔS and a small free-energy change, ΔG (mean 2,590 cal/mol). This simulates the melting of ice at 0 C, in which ΔH (1440 cal/mol) is opposed by ΔS (5.2 entropy units) and ΔG becomes zero. Our data indicate that the luciferase molecules become disordered (ΔS) by the association of the anesthetic molecules without much change in free energy. The major change is in the state

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of the enzyme. If similar changes occurred at the cell membrane, the molecules of the membrane structure would become disordered and expanded. This disorderedness of the molecules, together with the conformational changes, might modify the passage of the cations through the hypothetical channels in the cell membrane. Depolarization of the membrane would be suppressed and anesthesia ensue.

Finally, it may be argued that these findings in model systems result from the effects of properties other than those of the anesthetics, those of an organic solvent, for example. However, all lipid-soluble molecules, including rare gases and fluorinated carbons, appear to induce anesthesia when given at sufficiently high partial pressures. Miller *et al.*[22] reported that although C_3F_8 was a convulsant, its anesthetic effect was demonstrable when convulsion was prevented by the addition of N_2O. Only toxicity to the brain or other organs precludes clinical application of most of these compounds.

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APPENDIX

The theoretical basis for equations 5, 6 and 7 is detailed in a monograph by Johnson, Eyring, and Polissar.[42] A brief summary of the derivation of equations 6 and 7 is presented here.

The notations for the equations are:

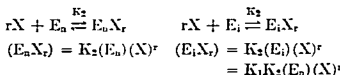
E_n = $E_{(native)}$
E_i = $E_{(inactive)}$
E_0 = total luciferase, $E_n + E_i$
L = luciferin
c = constant; proportionality constant times specific rate constant
K_1 = equilibrium constant for thermal inactivation

Type I Inhibition

An inhibitor combines with E_n and E_i indiscriminately. Subscript 2 (except I_2) refers to the events involving type I inhibition.

$$I_1 = c(L)(E_n)(ATP)$$

$$E_n \overset{K_1}{\rightleftharpoons} E_i \quad (E_i) = K_1(E_n)$$



$$rX + E_n \overset{K_2}{\rightleftharpoons} E_nX_r \qquad rX + E_i \overset{K_2}{\rightleftharpoons} E_iX_r$$

$$(E_nX_r) = K_2(E_n)(X)^r \quad (E_iX_r) = K_2(E_i)(X)^r = K_1K_2(E_n)(X)^r$$

$$(E_0) = (E_n) + (E_i) + (E_nX_r) + (E_iX_r) = (E_n) + K_1(E_n) + K_2(E_n)(X)^r + K_1K_2(E_n)(X)^r$$

$$E_n = \frac{E_0}{1 + K_1 + K_2(X)^r + K_1K_2(X)^r}$$

$$I_1 = \frac{c(L)(E_0)(ATP)}{1 + K_1}$$

$$I_2 = \frac{c(L)(E_0)(ATP)}{1 + K_1 + K_2(X)^r + K_1K_2(X)^r}$$

$$\frac{I_1}{I_2} = \frac{1 + K_1 + K_2(X)^r + K_1K_2(X)^r}{1 + K_1} = 1 + K_2(X)^r$$

$$\frac{I_1}{I_2} - 1 = K_2(X)^r = X^r e^{-\Delta H_2/RT} e^{\Delta S_2/R}$$

$$\ln\left(\frac{I_1}{I_2} - 1\right) = r\ln X - \Delta H_2/RT + \Delta S_2/R$$

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Type II Inhibition

Inhibitor combines with the enzyme in a manner that promotes thermal inactivation. As a result, inhibitor combines with E_i only. Subscript 3 is used for this reaction.

By treatment similar to the preceding equations:

$$E_0 = E_a + E_i + K_3(E_i)(U)^s$$

$$= E_a + K_1(E_a) + K_1K_3(E_a)(U)^s$$

$$E_a = \frac{E_0}{1 + K_1 + K_1K_3(U)^s}$$

$$I_1 = \frac{c(L)(E_0)(ATP)}{1 + K_1}$$

$$I_2 = \frac{c(L)(E_0)(ATP)}{1 + K_1 + K_1K_3(U)^s}$$

$$\frac{I_1}{I_2} = \frac{1 + K_1 + K_1K_3(U)^s}{1 + K_1} = 1 + \frac{K_1K_3(U)^s}{1 + K_1}$$

$$\frac{I_1}{I_2} - 1 = \frac{K_1K_3(U)^s}{1 + K_1}$$

$$\left(\frac{I_1}{I_2} - 1\right)\left(1 + \frac{1}{K_1}\right) = K_3(U)^s$$

$$= U^s e^{-\Delta H_3/RT} e^{\Delta S_3/R}$$

$$\ln\left(\frac{I_1}{I_2} - 1\right)\left(1 + \frac{1}{K_1}\right) = s\ln U - \Delta H_3/RT + \Delta S_3/R$$

Drugs and Their Actions

INTRAVENOUS CONTRAST MEDIA AND MORTALITY A nationwide survey of ten years' teaching-hospital experience covering 3.8 million excretory urograms revealed an overall death rate of 19 per million. Seventy-two per cent of surveyed radiologists employed intravenous pretesting with 0.5–1.0 ml contrast medium followed by 2–5 minute observation periods. Other tests, rarely used, were intradermal, subcutaneous, and ocular instillation of the contrast medium. There was no demonstrable reduction in mortality in pretested patients. Twenty-three deaths followed negative pretests, and two patients died consequent to reactions caused by the pretests. The intravenous pretest cannot be considered either safe or effective. Few patients who have serious reactions to the contrast media have histories of allergies. All radiologists performing excretory urography should be prepared to support the circulation and respiration in the face of an anaphylactic reaction. (*Fischer, H. W., and Doust, V. L.: An Evaluation of Pretesting in the Problems of Serious and Fatal Reactions to Excretory Urography, Radiology 103: 497–501, 1972.*)

POSTURAL HYPOTENSION The concurrent use of sympathomimetic amines or the consumption of tyramine-containing foodstuffs is contraindicated in patients treated with monoamine oxidase inhibitors, because the ensuing hypotension may be very severe. This undesirable combination, however, was found to be useful for the symptomatic treatment of patients suffering from idiopathic postural hypotension. Four patients who were receiving fludrocortisone therapy were given tranylcypromine in doses ranging from 30 to 80 mg daily while hydroxyamphetamine, amphetamine, or dextroamphetamine was administered with tyramine in the form of cheddar cheese or Chianti wine. Two of the patients showed improvement for nine and 26 months, respectively. Side-effects and/or progression of the basic disease processes forced discontinuation of therapy in three of the patients. (*Lewis, R. K., and others: Therapy of Idiopathic Postural Hypotension, Arch. Intern. Med. 129: 943–949, 1972.*) ABSTRACTER'S COMMENT: A most ingenious approach, utilizing a pharmacologic effect generally considered a complication of antihypertensive therapy to treat inappropriate regulation of systemic blood pressure.

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