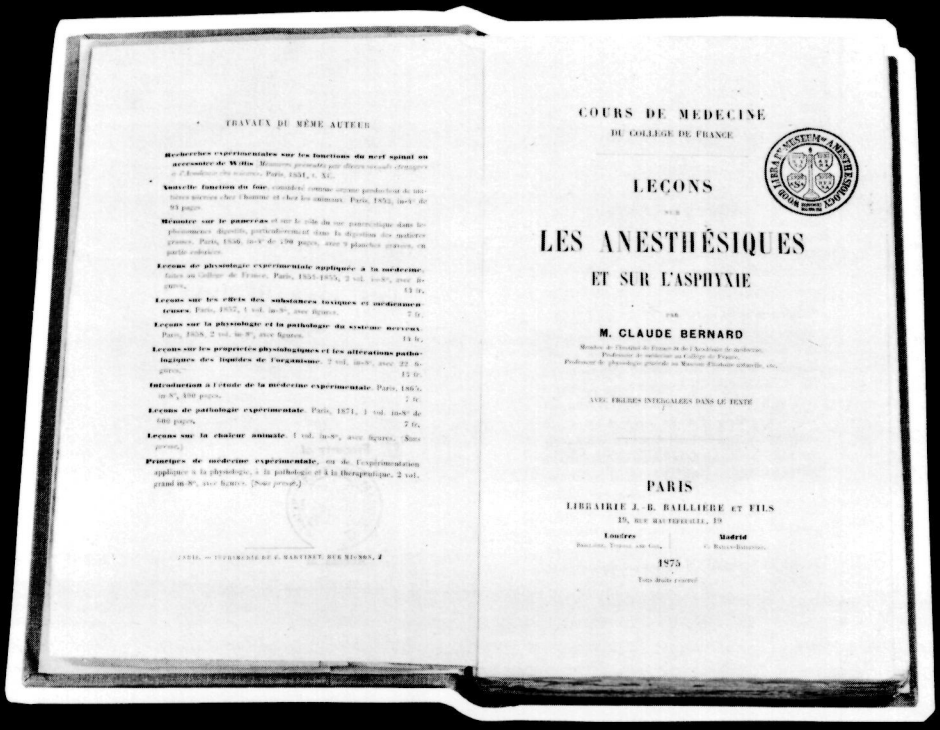
# The History of Anesthesiology

Reprint Series: Part Seven



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LEÇONS

SUR

# LES ANESTHÉSIQUES

## ET SUR L'ASPHYXIE

PAR

**M. CLAUDE BERNARD**

Membre de l'Institut de France et de l'Académie de médecine,
Professeur de médecine au Collège de France,
Professeur de physiologie générale au Muséum d'histoire naturelle, etc.

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## PHYSICAL THEORIES OF ANAESTHETIC ACTION

The mechanism by which anaesthetics reversibly abolish consciousness has challenged scientists ever since the discovery of anaesthesia. Anaesthetics exhibit a wide variety of chemical structures including the inert gases such as xenon. What physical properties are shared by this diverse group of agents? Originally Claude Bernard speculated on a bio-chemical alteration in cells while Paul Bert demonstrated the dependence of nitrous oxide analgesia on alterations in barometric pressure. At the turn of the century Meyer and Overton independently drew attention to the correlation between anaesthetic potency and lipid solubility, suggesting that anaesthesia results when any agent reaches a given concentration in a neural membrane. Later, Ferguson made a more general thermodynamic statement of this theory. But how does anaesthesia result from this? Mullins suggested that anaesthetics might occlude spaces in membranes, reducing their permeability, and that anaesthetics might have different solubilities in various neural membranes. Recent theories account for the fact that high pressures reverse anaesthesia by postulating that anaesthetics expand membranes. The development of these theories based on *in vivo* data forms a conceptual link with modern molecular level studies of anaesthetic membrane interactions.

By Keith W. Miller, Ph.D.

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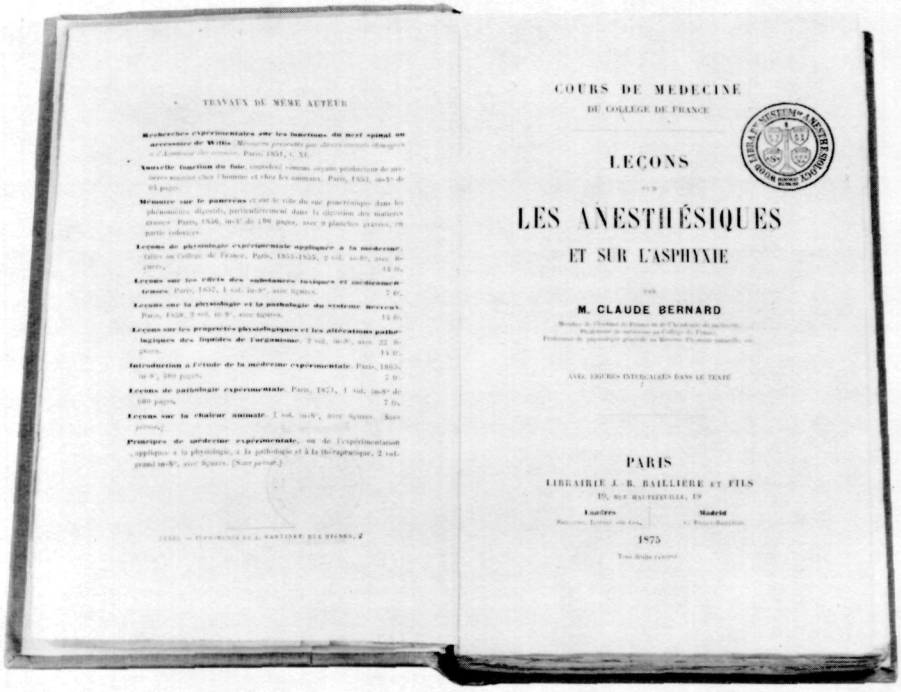
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PAUL BERT

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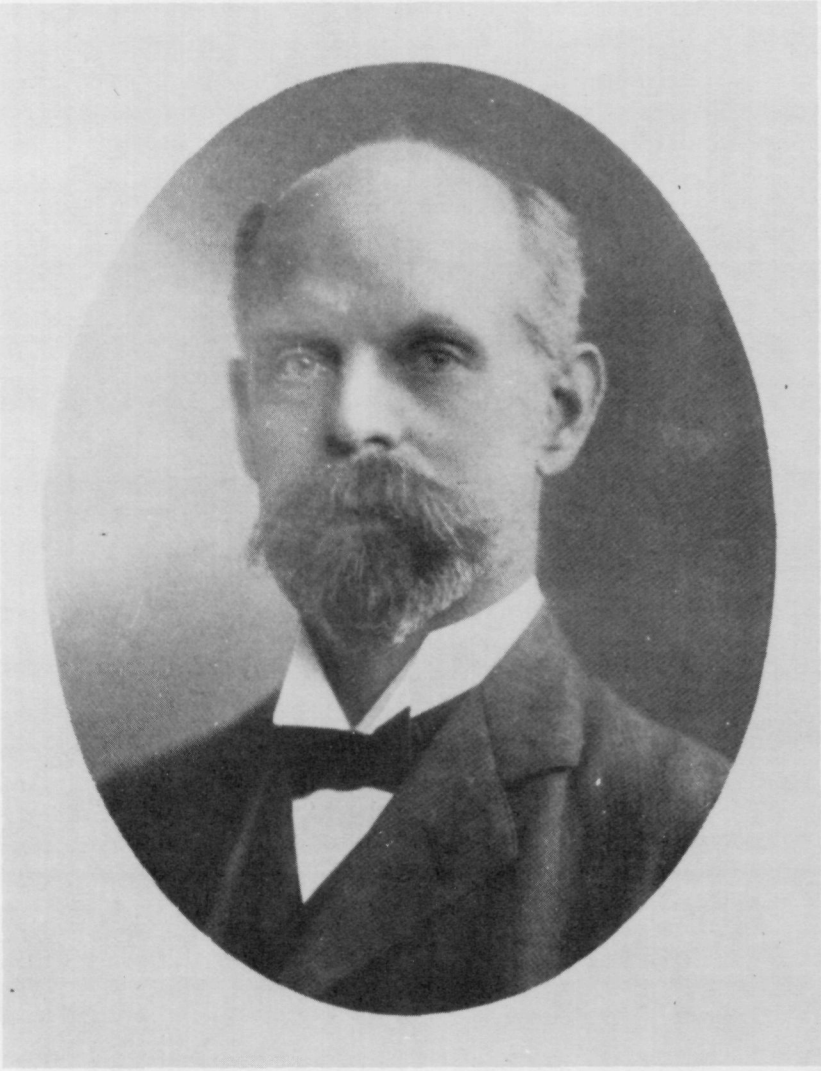
HANS HORST MEYER

1853–1939



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1906

# THE THEORY OF NARCOSIS *

HANS MEYER, M.D.,

Professor of Pharmacology, University of Vienna.

I ESTEEM it a high honor as well as a great pleasure to address you on this occasion and to have been asked to open the first course of lectures of the Harvey Society. The establishment of these lectures is additional evidence, if such be required, of the great interest displayed by American physicians in theoretical conceptions and in scientific research, and, in addition, of an earnest effort to encourage and diffuse such knowledge.

I look, however, on the invitation extended to me as evidence of your friendly and fraternal sentiments toward the whole body of German scientists, and I may be permitted on their behalf to offer you an expression of their cordial appreciation.

Following the suggestion of your president, I have selected as a theme for this evening's lecture a subject which, for a long time, has repeatedly attracted and interested not only pharmacologists, but biologists as well, namely, the relationship between the pharmacologic action of a drug and its recognized chemical or physical properties. The solution of this problem presents great difficulties. Even with a knowledge of the chemical and physical properties of an active substance it is yet impossible, without further knowledge, to determine which of these properties is responsible for the specific action on the animal organism. And this the more so, since we do not know the chemical point of attack in the organism, and hence can not know the nature of the chemical reactions which occur between poison and protoplasm. Only in one way can we reach a conclusion that admits of probability. If we find a large series of different

* Lecture delivered October 7th, 1905.

substances with different chemical and physical properties, which all possess identical or else very similar pharmacologic actions, then we can fix on the chemical and physical properties common to all and on which the common pharmacologic action naturally depends. So, for example, out of a mass of keys different yet all able to open the same lock we can determine which part of each key is the essential one, what form common to all fits the lock. And it is hardly necessary to point out that from this we can obtain an insight into the construction of the lock itself. In our case this means an insight into the chemical organization of protoplasm.

The first experiment in this direction was made by two English investigators—Crum-Brown and Fraser. They discovered the notable fact that practically all the ammonium bases, that is, organic bases in which the pentavalent nitrogen is connected with four valencies to carbon, exercise the same pharmacologic action, regardless of other differences in their constitution and nature; the action in this case being the same as that of curare, a paralysis of motor nerves. This definite relationship has been confirmed by many investigators, but the explanation for it is still lacking. It appears to me possible that the strongly basic character of all these ammonium bases is the chief factor. They are much more strongly basic than alkaloids and even sodium and potassium, and the few exceptions, such as betain, antipyrin and others which do not possess this strongly basic character, are also without the curare-like action.

Another series of experiments along this same direction, in connection with the action of the neutral alkali salts, has been conducted by Hofmeister. He has shown that all the effects of these salts, including their laxative and diuretic actions, may be explained by their physical properties, their diffusibility and osmotic strength.

This brings us to a third especially large group of substances whose actions are all identical in principle. I refer to those substances which are commonly designated anesthetics. To this group belong bodies quite distinct from each other chemically;

alcohols, aldehydes, ketones, esters, ethers and numberless others. They all possess the common action of depressing the central nervous system. Wherein is the relationship? On which of their common properties is their narcotizing action dependent? The chemical composition of the nervous system itself gives the first clue for an understanding. It differs from all other tissues in its richness in fat-like constituents, and on the basis of this peculiarity Bibra and Harles attempted many years ago to explain the action of anesthetics. They found that the anesthetics dissolved ordinary fat, and, as a result of a quantitative estimation of the fat content of the brain of a normal and narcotized animal, assumed that the anesthetics directly removed fat-like substances from the brain. But these conclusions could not be confirmed. From another standpoint, however, Hermann arrived at a similar opinion of the action of narcotics. Hermann had discovered the presence of lecithin in the red blood cells, and since the anesthetics, such as ether, chloroform, etc., dissolve the blood cells, he explained this by their power of dissolving lecithin, and pointed out the parallelism between this process and the narcosis of the central nervous system. In both of these hypotheses there appeared to me to be an element of truth, and in order to establish this and define its character I myself instituted a series of experiments.

I started with the following assumption: If fat-solubility is indeed a necessary condition for narcotic action it is to be expected that all indifferent, fat-soluble substances must act as narcotics if they can enter the cells, and that, on the other hand, if by any circumstance they lose their fat-soluble property, then they must also become inactive. I have tested this assumption by investigating a series of substances made by combining components which in themselves had no narcotic action, but whose combinations were soluble in fat. As examples may be mentioned the amides of organic acids. These amides are neutral compounds which are soluble in fat and which all possess the typical narcotic action, with, however, one single exception, carbamide, and this particular one is insoluble in

fat. Another group (comparable to the amides) is composed of condensation products of glycerin; the chlorhydrins, acetins and glycerin-ether. These also are soluble in fat and act as narcotics. All these substances, however, are very readily split up by hydrolysis into components insoluble in fat and on such decomposition lose immediately their narcotic power.[1]

Now, if from these and similar observations the conclusion can be drawn that the solubility of an anesthetic in fat is certainly one of the conditions for narcosis, the further question presents itself whether this condition is an essential one and whether it can be utilized as a measure of narcotic power. Were this the case, a quantitative relationship between narcotic power and solubility in fat must exist. But it is obvious that other factors must influence the action of narcotics in the animal body, for their affinity for the watery components of the body, as well as that for the fat-like constituents, must be considered. According to their relative solubility in the fat-like and non-fat-like constituents of the body they will distribute themselves between those constituents. So, for example, such substances as are very little soluble in water will dissolve for the most part in the fat-like constituents.

Richet had previously made the observation that the anesthetics which are little soluble in water possess a marked narcotic action, and he regarded this relationship as a general law. As a matter of fact, however, from this single relationship no general law can be deduced, for substances such as alcohol and chloral, which are both equally soluble in water, possess very different narcotic powers. The proper expression of the law must, therefore, be that the distribution relationship, the so-called distribution coefficient, of the narcotics between fatty and watery solutions, is the determining factor of narcotic action. To test the correctness of this hypothetical law I have determined the strength of action of a large number of different narcotic poisons by estimating the smallest molecular concen-

[1] The amides yield fatty acids and ammonia salts, the glycerin derivatives, glycerin and acetic or hydrochloric acids.

tration of their solutions which was sufficient to induce narcosis of small fish and tadpoles placed in them. A second series of experiments was then carried out with the same substances for the purpose of determining their distribution coefficient between water and fat. A mixture of water and oil was used to estimate the relative quantities passing into these two substances. The comparison between the distribution coefficient so obtained and the narcotic strength of the narcotics did, as a matter of fact, yield the expected result, as a glance at the following table reveals:

TABLE SHOWING THE RELATIONSHIP BETWEEN THE DISTRIBUTION COEFFICIENTS F/W AND THE CONCENTRATIONS, EXPRESSED IN GRAM MOLECULES, OF SOLUTIONS EXERTING EQUAL NARCOTIC ACTION.

| | F/W | Concentration |
|---|---|---|
| Trional | 4.4 | 0.0013 |
| Tetranal | 4.0 | 0.0018 |
| Butychloral | 1.6 | 0.002 |
| Sulphonal | 1.1 | 0.006? |
| Bromalhydrate | 0.7 | 0.002 |
| Benzamid | 0.6 | 0.002 |
| Triacetin | 0.3 | 0.010 |
| Diacetin | 0.23 | 0.015 |
| Chloralhydrate | 0.22 | 0.025 |
| Æthylurethane | 0.14 | 0.025 |
| Monoacetin | 0.06? | 0.02? |
| Methylurethane | 0.04 | 0.4 |
| Æthyl alcohol | 0.03 | 0.5 |

With an increase in the distribution coefficient there occurs an almost parallel increase in the narcotic strength, that is, decrease in the molecular concentration necessary for narcosis. The few departures from the general rule which occur in the table can be explained by the naturally inexact method of estimating the narcotic power.

A further proof of the correctness of the view described may be offered. It is known that the solubility of most substances in water and fat changes in a different way with variations in temperature. The distribution coefficient is also variable according to temperature. It must be expected, then, that the narcotic strength as well will vary with changes in

temperature. And this is the fact. I examined six substances, of which with higher temperature three gave higher and three lower distribution coefficients. And it was found that in exact accordance with the rise or fall of the distribution coefficient so the narcotic strength rose or fell, so that tadpoles which were just narcotized by a certain chloralhydrate solution at 30 degrees C. were aroused and quite active on cooling to 3 degrees, and on subsequently warming to from 25 to 30 degrees again passed into narcosis. From this the direct dependence of narcosis on the physical relationship of the narcotic to the fat-like substances, the lipoids of the body, and the watery constituents seems to be definitely proved.

As a result of all these studies we arrive at the following explanation of narcosis: The narcotizing substance enters into a loose physico-chemical 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. It also becomes evident that the narcosis immediately disappears as soon as the loose, reversible combination, which is dependent on the solution tension, breaks up. It follows further that substances chemically absolutely indifferent, as the volatile saturated hydrocarbons, can act as narcotics.

Quite in opposition to this idea, it has been frequently put forward and accepted that the breaking up of the narcotics, with a chemical action of definite atomic groups thus set free, as the ethyl group, for instance, is responsible for the narcosis. But even in the case of sulphonal and its related sulphones, from which this idea originates, it can be shown that the action is induced by the entire unchanged molecule, and that the lack of activity of certain sulphones is due not, as is generally believed, to their not being broken up, but to a low distribution coefficient.

This simple theory also explains the fact that all structures capable of stimulation, not only the cells of the nervous system, but all others, and all plant cells as well, are depressed by the narcotic members of this series, for in all living cells lecithin,

a lipoid body, is to be found. And, indeed, the establishment of the fact that the effect on the lipoids by narcotics, such as ether and chloroform, is such as to immediately inhibit the vital processes of the cell, shows us that these lipoids are among the constituents essential to the life of the cell. Moreover, by establishing this fact it seems to me that the general biologic significance of the theory becomes apparent.

That many narcotics induce not pure narcosis alone, but often show other distinct actions, as, for example, the occurrence of convulsions, which quite overshadow any narcosis present, is easily to be understood when one remembers that the narcotics may possess an affinity not only for the cell lipoids but for other cell constituents as well, and through some union with these, concomitant effects quite different from narcosis may be induced. This occurs, for instance, in the case of the phenols, whose narcotic action is thrown into the background by the appearance of clonic spinal convulsions.

No attempt is made to explain every type of narcosis by means of the theory presented here. It is very probable that some other disturbances in chemical equilibrium can occur in the cell and inhibit the performance of its function and that substances such as morphin are narcotic through their relationship to other points of attack than the "alcohol lipoids"; and most probably the same can be said concerning the very remarkable narcosis from magnesium salts, lately discovered by Meltzer.

I desire to add in conclusion that shortly after I had published my theory of alcohol narcosis the physiologist Overton published experiments which, carried out independently of mine and from a different point of view, in fact with somewhat different methods, brought him to an identical conclusion, *i.e.*, to a similar theory of narcosis, so that he has confirmed my work and accepted the formulation of my theory literally. I take this as a strong and gratifying argument for the correctness of our assumption.

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# SOME PHYSICAL MECHANISMS IN NARCOSIS[1]

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*Received January 8, 1954*

CONTENTS



## I. INTRODUCTION

In 1920, Miller (42) offered an explanation of how the addition of 2 per cent sodium chloride to aqueous solutions of phenol increased the toxicity of the phenol for bacteria. His explanation was that the sodium chloride raised the chemical potential of the phenol in solution and hence, effectively, the escaping tendency of phenol toward bacteria. Unfortunately there was no generalization of this suggestion to include phenomena other than toxicity, and it remained for Ferguson (16) to show that the use of thermodynamic indices (chemical potential; activity) was helpful in predicting the aqueous concentrations of various substances that were necessary for toxicity and for narcosis. As will be seen later, the suggestion of Ferguson did not contribute any new information to the understanding of narcosis, but it did free the discussion of such phenomena from the artificiality of the Meyer–Overton hypothesis by showing that the partition coefficient, the vapor pressure of narcotics in solution, and various solubility relationships of narcotics are all derivable in principle from the thermodynamic activity. There is no argument that the concept of partition coefficients is im-

[1] This study has been aided by a grant from the Research Laboratories of Eli Lilly and Company.

portant in a discussion of mechanisms for narcosis; the difficulty has been that none of the substances selected as models of the phase to which narcotics are supposed to be partitioned were entirely satisfactory. The suggestion that, since at equilibrium the activity must be the same in all phases, one should measure activities in an accessible phase (water solution) has been most successful in interpreting data of many sorts. Ferguson (17) has emphasized that if the Meyer–Overton hypothesis is correct, the use of thermodynamic indices to express concentrations merely reinforces the hypothesis; on the other hand, the use of such indices does not require any particular mechanism for narcosis.

This review will be concerned mainly with the examination of data on narcosis for a variety of organisms and with the interpretation of the data in physicochemical terms. No attempt has been made to collect all the papers in this field, because several excellent reviews have appeared recently. Among these may be cited those of Butler (7) on general anesthesia, of Toman (55) on peripheral nerve, of Harris (25) on anesthetics, and of McElroy (40) on the biochemical aspects of narcosis. In addition, a recent symposium, "Mécanisme de la Narcose" (1951), may be consulted for summaries of the views of the various participants.

## II. NARCOSIS AND DEPOLARIZATION

Before proceeding further, it will be useful to define some of the terms to be used during this discussion. One may define narcosis generally as any *reversible* decrease in physiological function induced by physical or chemical agents. Such a definition admits of many subdivisions, such as narcosis of cell division or narcosis of the central nervous system (general anesthesia). In contrast, toxicity might be defined as any irreversible decrease in physiological function. Most substances that are narcotics can be shown to be capable of producing both narcosis and toxicity, depending upon the concentration used in the case of chemical agents and upon some measure of intensity in the case of physical agents.

From studies of nerve and muscle, it is apparent that narcosis can be produced with or without depolarization (3, 12, 30), so that an understanding of the process of depolarization is of some relevance to narcosis. From an analysis of the rate of depolarization in frog nerve as a function of the concentration of the depolarizing agent Mullins and Gaffey (45) suggested that such agents act by altering the interfacial tension between the fiber and its environment. Such an alteration might facilitate the removal of material from the membrane, and depolarization would occur when this rate exceeded the synthetic capacity of the fiber to replace the lost material. If such an analysis is correct, one can cause depolarization in three ways: first, by interfering in some way with the oxidative metabolism of the synthetic center producing new membrane; second, by removing membrane faster than it can be replaced; and third, by abolishing the potassium-ion gradient across the membrane and hence the potential. Oxygen lack and cyanide are agents that work on the first mechanism, while ether ($>0.5\ M$) and alcohols, as well as a variety of other non-reactive chemical

agents, cause depolarization by the second mechanism. The following discussion will deal mainly with agents which do not produce depolarization at concentrations which are narcotic, since there are very few agents which depolarize before they produce narcosis. The term "membrane" is used here to connote a phase in the cell other than aqueous. There is no cytogeographic specification in the term.

### III. THERMODYNAMIC PRINCIPLES

Reference has been made to concentration without specifying just what sort of concentration this should be. The thermodynamic measure of concentration is one where an equal number of molecules are "effective" at any given time. Thus 1 *mM* butanol and 14.3 *mM* ethanol have the same "effective" concentration. The cause for this difference in aqueous concentrations is merely that the solvent water has interacted with so many more of the ethanol molecules that they are no longer "effective." One judges the thermodynamic activity, as this "effective" concentration is termed, by the ratio of $p_{nar}/p^0$, where $p_{nar}$ is the partial pressure of the narcotic in a solution that just causes narcosis and $p^0$ is the vapor pressure of the pure liquid. If one employs as a solvent a substance relatively similar to the narcotic, e.g., hexane as a solvent for pentane, the interaction of the narcotic molecules with the solvent will be at a minimum and Raoult's law will hold in that $p_{nar}/p^0 \cong A_{nar} \cong X_{nar}$, that is, the activity of the narcotic will equal its mole fraction, where $X$ is the number of moles of narcotic divided by the total number of moles of narcotic plus solvent. If the phase in a cell in which the narcotic acts is such that Raoult's law is followed, then one may expect that narcotic thresholds expressed as $A_{nar}$ will be more constant than thresholds expressed as aqueous concentrations. In the case of water, this sub-

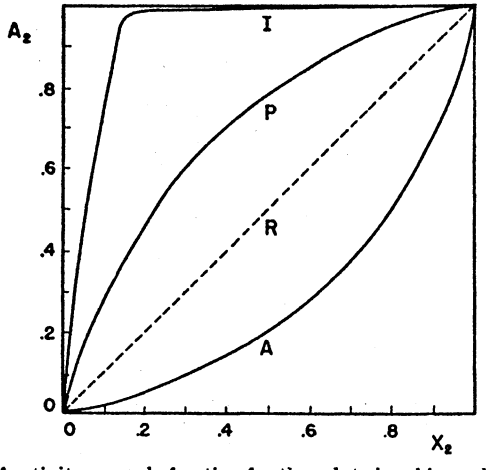



FIG. 1. A plot of activity *vs.* mole fraction for the solute in a binary liquid mixture. The curves are for (A) a substance that associates with the solvent, (R) a substance that follows Raoult's law, (P) a polar solute, and (I) an incompletely miscible solute.

stance is so different in physical constitution from all the common narcotics that marked deviations from Raoult's law are the rule. Figure 1 is a plot of a number of examples of the behavior of solutions of various substances in water. In discussing binary solutions it is customary to use $A_2,X_2$ to refer to the solute and a subscript 1 to refer to the solvent. The letter R on the diagram refers to a solute that obeys Raoult's law in solution; the letter A is used to designate a solute that associates with water molecules and hence has less than ideal activity at a given mole fraction. An example of such behavior might be aqueous solutions of glycerol. The letter P designates the behavior of a polar substance such as 1-propanol, as here hydrogen bonding competes with the tendency of the paraffin chain to be forced out of solution. As a result, 1-propanol shows moderate positive deviations from Raoult's law. The letter I represents a substance such as ethyl ether, which shows only limited miscibility with the solvent. These deviations from Raoult's law are expressed as an activity coefficient $\gamma = A/X$; the more positive the deviation the greater the value of the coefficient $\gamma$. For dilute solutions (low mole fractions) the limiting slope of the curve is taken to obtain the activity coefficient at infinite dilution ($\gamma_\infty$) (see reference 4). If physiological data at high mole fractions are being considered, the appropriate value of the activity coefficient at any given mole fraction can be read off the curve and used in calculations. Since studies in narcosis usually are concerned with mole fractions less than 0.1, $\gamma_\infty$ is usually the more useful value. If vapor pressure–composition data are not available for a given substance, and if the substance is relatively insoluble, $\gamma_\infty$ can in many cases be approximated in the following way: In a saturated aqueous solution a given liquid must have the same activity in the solution as in the pure liquid, e.g., $A = 1.0$ in both cases. Since very insoluble substances do not in general interact with water molecules, curve I of figure 1 is almost a straight line right up to saturation. The activity coefficient can therefore be computed by taking the reciprocal of the mole fraction of solute in a saturated solution. Ethyl ether is soluble in water to the extent of about 1.0 mole/liter or 0.0185 $X$; since at this value $A$ must equal 1.0, $\gamma = A/X = 1/0.0185 = 54$. A value determined by Butler (5, 6) from vapor pressure data is 69. For chloroform, $X$ in a saturated solution is 0.0015 and $1/X$ is 663, *vs.* 817 as found by Butler. In both these cases, the deviations found are referable to some interaction between water and solute molecules.

## IV. GASES AS NARCOTICS

There are only a rather limited number of substances that have vapor pressures greater than 1 atm. at room temperature; hence the pharmacological properties of most gases are well established. If the chemically reactive gases such as nitrogen dioxide, chlorine, etc. are excluded from consideration, the inert gases may be divided into (*1*) pure paraffins and their substitution products and (*2*) inorganic gases. A sufficiently large number of substances in both these categories has been investigated to allow one to state that there is no inherent difference in the narcosis produced by a gas representing one or the other class. Since the suggestion of Ferguson (16) has focussed attention on the thermody-

namic properties of narcotics, it is worthwhile examining some of the relationships that ought to hold for these substances.

Although the solubility of gases in liquids varies over a considerable range, it is in principle predictable from the vapor pressure of the gas. Recalling Raoult's law, the solubility of a gas should be proportional to its partial pressure.

$$p_2 = p^0 X_2$$

If we choose to take methane as an example, its vapor pressure (or more strictly its fugacity) at 20°C. is 289 atm. (extrapolated), so that its solubility at a pressure of 1 atm. should be $\frac{1}{289} = 0.00346$ mole fraction. This value is within a few per cent accurate for the value of the solubility of methane in non-polar solvents whose internal attractive forces are low, such as hexane (0.0031). In general, no great error will be made if the solubilities of gases are computed in this manner for non-polar solvents. It is only in water that one may expect anomalous solubility values. It should be mentioned that when pressures of gases greater than a few atmospheres are considered, some correction for the deviation of the gases from ideal gas laws must be employed. The function fugacity (36) is a correction for this non-ideality and is defined such that $f/p$ approaches 1 as $p$ approaches zero. Such fugacity corrections as necessary can be obtained from tables and applied to the calculations being made.

If narcosis can be demonstrated to take place at constant thermodynamic activity of the narcotizing molecules, this means that since

$$A_{nar} = f_{nar}/f^0 \cong p_{nar}/p^0$$

where $A_{nar}$ is the thermodynamic activity for narcosis, $f_{nar}$ is the fugacity for narcosis, $f^0$ is the fugacity of the narcotic in some standard state, and the values of $p$ are the respective partial pressures for narcosis and for the pure narcotic, a gas or any other substance ought to be narcotizing at some constant fraction of its own vapor pressure. The standard state for narcosis can be considered, most conveniently, as the pure liquid narcotic. We could have a standard state for gases (e.g., $f = 1$ atm.), but then it would not be possible to compare values of the activity calculated in this way with the values for liquids, which are, as a class, the most important and varied of narcotics.

In table 1 are tabulated a variety of values for the thermodynamic activities necessary to cause narcosis, mostly in mice, but other values are also given. If the paraffins are considered first, the values of $A_{nar}$ can in no sense be considered constant, but they rise in a regular way as one ascends the paraffin series. Pentane and higher paraffin homologs are liquids at room temperature, but these substances have been included here to show the continuity in the series. From propane on, the value of $A_{nar}$ could be reasonably approximated by merely dividing the molal volume $V_m$ of the substance by 1000. In the ethylenic series, the values of $A_{nar}$ are only about half those required in the paraffin series and the values of $V_m$ are smaller. Acetylene is little different from ethylene, in spite of a definitely smaller $V_m$, but the degree of anesthesia obtainable from acetylene is greater, hence the figures may not be comparable. The cycloparaffins resemble

TABLE 1

*Narcosis by gases*

| SUBSTANCE | $p^0$ AT 37°C. | $p_{nar}$ | $A_{nar}$ | $V_m$[a] | OBSERVER |
|---|---|---|---|---|---|
| | *atm.* | *atm.* | | *ml./mole* | |
| $CH_4$ | 310 | 3.7 | 0.012 | 39 | Meyer and Hemmi (43) |
| $C_2H_6$ | 50 | 1.3 | 0.025[b] | 55 | Ferguson (17) |
| $C_3H_8$ | 13 | 0.9 | 0.07 | 75 | Seevers and Waters (50) |
| $C_4H_{10}$ | 3 | 0.2 | 0.08 | 97 | Seevers and Waters (50) |
| $C_5H_{12}$ | | | 0.11 | 116 | Führer (18) |
| $C_6H_{14}$ | | | 0.13 | 132 | Führer (18) |
| $C_7H_{16}$ | | | 0.15 | 147 | Führer (18) |
| $C_8H_{18}$ | | | 0.20 | 164 | Führer (18) |
| $C_2H_4$ | 57 | 0.8 | 0.014 | 50 | Meyer and Hemmi (43) |
| $C_3H_6$ | (14)[c] | 0.4[d] | 0.029 | 69 | Seevers and Waters (50) |
| 1-$C_4H_8$ | (5)[c] | 0.2[d] | 0.04 | (90)[c] | Seevers and Waters (50) |
| $C_2H_2$ | 41 | 0.65 | 0.016 | 42 | Meyer and Hemmi (43) |
| Cyclopropane | 12 | 0.35[d] | 0.029 | 62 | Seevers and Waters (50) |
| Methylcyclopropane | (2.7)[c] | 0.15[d] | 0.056 | (80)[c] | Seevers and Waters (50) |
| $CH_3Cl$ | 8 | 0.065 | 0.01 | 56 | Meyer and Hemmi (43) |
| $C_2H_5Cl$ | 3 | 0.07 | 0.02 | 73 | Seevers and Waters (50) |
| $CHCl_3$ | 0.42 | 0.015[d] | 0.03 | 81 | Goodman and Gilman (22) |
| $(CH_3)_2O$ | 8.1 | 0.12 | 0.02 | 60 | Meyer and Hemmi (43) |
| $(C_2H_5)_2O$ | 1.1 | 0.038[d] | 0.03 | 105 | Goodman and Gilman (22) |
| He | (2300)[c] | 54[e] | 0.02 | 32 | Marshall (39) |
| A | 860[f] | 41[e] | 0.048[g] | | Marshall (39) |
| Xe | 83 | 0.7 | 0.01 | 43 | Lawrence *et al.* (33) |
| $N_2$ | (1300)[c] | 10[e] | 0.008 | 35 | Marshall (39) |
| $N_2$ | 1030[f] | 54[e] | 0.05[g] | 35 | Marshall (39) |
| $N_2O$ | 44 | 1 | 0.02 | 36 | Meyer and Hemmi (43) |
| $SF_6$ | (25)[c] | 2 | 0.08 | 76 | Carpenter (10) |

[a] Molal volume at the boiling point in the case of liquids above their critical temperature; otherwise at 20°C.

[b] Grain weevil.

[c] Extrapolated.

[d] Mean values for man and laboratory animals.

[e] Fugacity.

[f] $p^0$ at 20°C.

[g] Frog.

the ethylene series more than they do the paraffins, both with respect to the values of $A_{nar}$ and with respect to $V_m$; the halogen substitution products similarly follow the rule that $V_m$ seems to influence $A_{nar}$. Ether and chloroform have been included in this table for purposes of comparison. Chloroform fits into the proper position in the table (e.g., its $A_{nar}$ is about what one would expect from

its $V_m$), but ether has an $A_{nar}$ far lower than one would predict on the basis of its $V_m$. For the inorganic gases, the values of $A_{nar}$ are all very low, in keeping with the very small $V_m$ values of these substances. The results with the rare gases are especially interesting because of the certainty that there can be no chemical interaction between these molecules and the organism. The case of sulfur hexafluoride is an interesting one, because this inert and non-toxic substance has only about half the $p^0$ of nitrous oxide and might therefore be expected to be a good anesthetic, with more potency than nitrous oxide. Its large $V_m$, however, requires a much higher value of $A_{nar}$ and provides an independent confirmation of the suggestion made here regarding the importance of the molal volume in determining the threshold for narcosis. The value of $A_{nar}$ for helium cannot be considered as very accurate, because of the very considerable uncertainty of $p^0$, involving as this does an extrapolation over far too great a temperature range.

The importance of these data is in stressing the advantage of their analysis by thermodynamic methods. The partial pressures required for narcosis by various gases vary from 0.065 to 54 atm. or by a factor of about 800, while the value of $A_{nar}$ varies only from 0.01 to 0.08 or by a factor of 8. To summarize the results with gases, the introduction of the idea of thermodynamic activity gives, as a first approximation, a constancy of the data from substance to substance. If due allowance is made for $V_m$, it is possible to regularize further the experimental data.

## V. SOME PHYSICAL PROPERTIES OF LIQUIDS

The alcohols represent as a class the substances most used for the study of narcosis. The choice, while perhaps convenient from an experimental point of view, could not have been more unfortunate from a theoretical viewpoint because of the extremely complex behavior of the alcohols in solution. Some idea of the behavior of organic liquids generally may be had by considering their solubilities in a highly polar solvent, water. In figure 2 (left) the solubilities of a variety of organic substances in water are plotted against the number of carbon atoms in the molecule. The solubility is for the pure liquid narcotic in water, so that in the case of gases, the solubility is computed at the vapor pressure of the liquid at 20°C. Certain features of interest are that, as a first approximation, there is no difference in the water solubility of the ketones, acids, alcohols, and ethers. The solubilities of triple-, double-, and single-bonded hydrocarbons fall in regular order, and the addition of chlorine to a hydrocarbon further depresses its solubility. On the other hand, one might expect from a comparison of solubilities that the alcohols and acids, which are so polar, ought to be more soluble. Solubilities have not been compared at constant fugacity; thus ether makes up for its low polarity by having a high vapor pressure. On the right in figure 2 is a plot of solubility at unit vapor pressure for the same substances. The various compounds fall in the order of their polarity: thus, acids are the most soluble, then alcohols, ketones, and ethers. The 1,1-dichloroparaffins are more soluble than the monochloroparaffins, and the hydrocarbons are the least water-soluble.

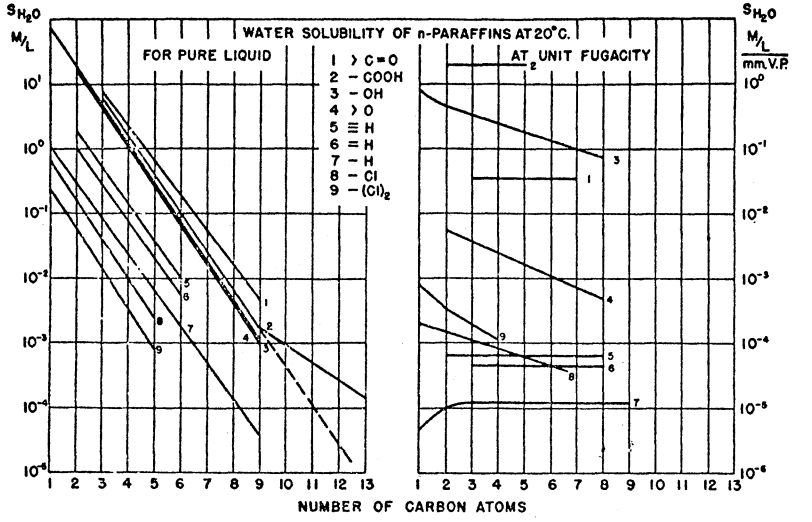



FIG. 2. The water solubility of various organic compounds is plotted against chain length. Values are for normal paraffin compounds. For curve 9 the values are for the 1,1-dichloro compound. Values for solubilities have been obtained from various sources, especially the *Handbook of Chemistry and Physics* (1949). Values for 1-pentyne were determined in this laboratory. On the left the solubility is given for the pure liquid in moles per liter. The values on the right are the solubility, in moles per liter, divided by the vapor pressure of the pure liquid.

Another significant fact illustrated by the right-hand curve of figure 4 is that while the solubility of the paraffins in water at unit fugacity is independent of molecular volume (or number of carbon atoms), the alcohols show an appreciable decrease in solubility. This decrease is referable to the greater and greater difficulty of fitting the paraffinic fraction of molecules of larger and larger volume into the structure of a highly associated solvent. For the acids, the situation is more complex, involving as it does both dimerization and ionization, while the ketones, with a strong polar group and an "iso" structure of the paraffin chains, do not experience the same difficulties as do straight-chain alcohols. With the paraffins, the solubility is the limiting value which the alcohols presumably will approach as the number of carbon atoms increases. This effect will be considered further in connection with models of narcotic action.

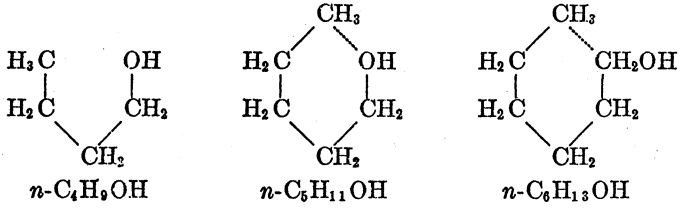
The change of activity of ethanol with mole fraction in a hydrocarbon such as heptane illustrates the behavior of polar:non-polar mixtures. Data are available on the activity coefficients for dilute solutions of the alcohols in benzene (6) and are shown in table 2. This behavior means that if one takes paraffins as a model of the phase to which narcotics are supposed to be partitioned, the alcohols as a class will require on the average about ten times as much mole fraction in the aqueous phase for a given mole fraction in the non-aqueous phase when compared with non-associated liquids. A further complication is the possible

TABLE 2

*Thermodynamic activity coefficients for solutions of alcohols in water and in benzene at 25°C. (Butler (5))*

| SUBSTANCE | $\gamma_{H_2O}$ | $\gamma_{C_6H_6}$ | PARTITION COEFFICIENTS | |
|---|---|---|---|---|
| | | | $\gamma_{H_2O}/\gamma_{C_6H_6}$ | $\gamma_{C_6H_6}/\gamma_{H_2O}$ |
| Methanol | 1.51 | 21.4 | 0.07 | 14.3 |
| Ethanol | 3.69 | 16.4 | 0.23 | 4.4 |
| 1-Propanol | 14.4 | 15.1 | 0.96 | 1.0 |
| 1-Butanol | 52.9 | 14.2 | 3.73 | 0.3 |

polymerization of the alcohols in non-polar solvents. A careful study of the volume changes when alcohols are mixed with non-polar solvents (53) shows that even at very low mole fractions the alcohols are associated to trimers; this change makes it possible for the alcohol molecules to pack more economically in some non-polar solvents. Further, these studies have shown that the step from 1-butanol to 1-pentanol can lead to the formation of a six-membered ring, with a further economy of packing of the alcohol molecules. Such an arrangement is shown below.




It is, therefore, not justifiable to consider aliphatic alcohols in paraffin as linear molecules, because trimerization and ring formation can alter the molecular structure to a very considerable extent. In benzene, the volume changes can best be accounted for by tetramer formation, and one can expect rather complex structural aggregates in a variety of other non-aqueous solvents.

The volume changes associated with various isomeric alcohols do not, by any means, follow the changes in volume for the normal alcohols. Thus the series methanol, ethanol, 2-propanol, 2-methyl-2-propanol corresponds to a series of methyl substitutions in methanol. The first substitution, methylmethanol (ethanol), corresponds to a large volume increase because of the unsymmetrical methyl group added. Further addition of methyl groups increases the molar volume very much less. 2-Methyl-2-propanol is thus a much more spherical molecule than 1-butanol and may be expected to fit more easily into a solvent of relatively spherical molecules or, conversely, to give a larger volume change when mixed with non-spherical molecules.

A recent development in organic chemistry is a series of paraffins where fluorine is completely substituted for hydrogen in the molecule. These perfluorocarbons are normal liquids, but while any given compound has about the same boiling

point and heat of vaporization per mole as its corresponding hydrocarbon, the molal volumes are almost twice as great. While the fluorocarbons will be completely miscible with most pure hydrocarbons at 25°C., they will be incompletely miscible with an only very slightly polar substance such as benzene. Even in solutions of the hydrocarbons, however, fluorocarbons will show very high activity coefficients.

Such substances as phenol and aniline may be expected to show abnormal behavior because, in addition to being associated by virtue of their polar groups, the benzene ring permits a much more efficient packing of the six carbon atoms than does an aliphatic structure. We may thus anticipate a smaller molal volume and a more compact liquid structure, one that will resist penetration by non-associating molecules.

The carboxylic acids are highly associated liquids up to $C_9$, but this association is developed to such a degree that two molecules unite to form a dimer. Acetic acid, for example, is dimerized in the vapor phase. The result of this strong association between two molecules in the pure liquid is to make the liquid approach the paraffins more and more as the chain length is increased. Carboxylic acids will not be dimerized in polar liquids such as water, but only in non-associated liquids (48).

## VI. THE CHEMICAL STABILITY OF NARCOTICS

It is obviously relevant to studies of narcosis to know whether the substance added as a narcotic is a stable molecule and one that is not transformed into another substance which then acts as a narcotic. Such a situation appears to have arisen in the case of chloral hydrate. Butler (7) has shown that the pharmacological behavior of this substance can be accounted for by a certain amount of transformation into trichloroethanol. Since chloral is very soluble, the apparent thermodynamic activities for its action, as noted by Gavaudan (21) are very low. When such activities are adjusted to the value for the relatively insoluble trichloroethanol, the activity for inhibition of mitosis, which Gavaudan found to be 0.00025 for *Triticum*, is in reality 0.02 (see reference 58).

Monohalogen substitution of methane results in compounds which are unstable in aqueous solution in the order Cl < Br < I, but even methyl chloride is rather unstable; the results of the hydrolysis would be a certain amount of methanol and halogen acid, which should not be highly toxic considering the small amount of material present for narcosis. The hydrolysis can also result in the methylation of sulfhydryl groups, with a consequent change in some essential structural elements of the cell. Lengthening the aliphatic chain stabilizes greatly the monohalogen substitution products of the paraffins, so that even ethyl chloride can be considered reasonably stable. Polyhalogen substitution of methane and other paraffins leads to much more stable molecules, with a maximum of stability being attained in the perhalogen structure such as carbon tetrachloride.

Carbon disulfide is an unusual substance, in that chronic poisoning with this material leads to permanent changes in the central nervous system, alterations

which commonly result in mental deterioration. Linderstrøm-Lang (38) cites the use of this substance in aqueous solution for a quantitative reaction with amino groups in proteins with the formation of a dithiocarbamate.

The carboxylic acids are metabolizable and are concerned in metabolic cycles. The lower members of this series cannot be introduced into the organism at high enough activities to produce narcosis without serious osmotic and pH disturbance. The higher acids are polar and have the possibility of interacting with proteins, of being metabolized, and of producing depolarization by virtue of their high surface activity (26).

The alcohols are capable of being metabolized, but this reaction does not occur at a rapid enough rate to interfere seriously with short-time experiments on isolated tissues. When the lower alcohols are delivered to the intact organism as vapors, it is not improbable that a non-equilibrium condition can be obtained. Such a situation is brought about by the very low vapor pressures of the alcohols in relation to their molecular volume and the relatively high aqueous mole fractions that must be obtained in the blood in order to bring about narcosis. Since metabolism is continually acting on the alcohol concentration in the blood, it is apparent that one can supply vapors with an $A_{nar}$ much higher than the values actually obtained in the tissues. The lower members of the aliphatic series of alcohols, methanol and ethanol, are depolarizing agents; they are capable of interacting with proteins to dehydrate and denature them in relatively high concentrations. The rate of oxidation of methanol by animals is greatly inferior to that of ethanol; and while the products of methanol oxidation (formaldehyde and formic acid) are toxic, it is questionable whether adequate concentrations of these substances are ever produced in the organism. The special toxicity of methanol to the retina might well be ascribed to its strong depolarizing action at concentrations well below the level for narcosis.

The paraffin hydrocarbons are not metabolized by organisms, other than a few specialized bacteria. The molecules are inert and do not react chemically with cell components. The introduction of a double or triple bond into a paraffin results in a greater water solubility because of the greater polarizability of this bond by the water molecules and a decrease in $V_m$. Such bonds are potentially oxidizable, but the molecules (at least for acetylene) appear to be inert. The unsaturated aliphatic hydrocarbons have a smaller molal volume, and a smaller partial molal volume in paraffinic solvents. Aromatic hydrocarbons are even more compact structures and, as expected, volumes are smaller for any given number of carbon atoms. This effect is also shown for cycloparaffins, each member having a considerably smaller volume than the corresponding straight-chain compound. A table of volume relationships for a number of such substances is given (table 3).

## VII. SOLIDS AS NARCOTICS

The thermodynamic properties of solutions of solids are in general similar to those of liquids. Because the standard state is defined as that of the pure liquid,

TABLE 3

*Molal volumes of six-carbon-atom compounds at 20°C.*

| SUBSTANCE | $V_m$ | $\Delta V_m$ | SUBSTANCE | $V_m$ | $\Delta V_m$ |
|---|---|---|---|---|---|
| | *ml./mole* | | | *ml./mole* | |
| *n*-Hexane | 132 | | 1-Hexanol | 125 | |
| | | 24 | | | 24 |
| Cyclohexane | 108 | | Cyclohexanol | 101 | |
| | | 19 | | | 13 |
| Benzene | 89 | | Phenol | 88 | |

it is necessary to melt a solid in order to have comparable standard states.[2] The activity of a saturated solution of a given solid in water is of course 1.0, which is the ratio $f_{soln}/f_{solid}$; this value then must be multiplied by $f_{solid}/f_{liquid}$, where $f_{liquid} = f^0$. The fugacity change on melting a solid is related to its heat of fusion and is given by

$$\log 1/A_S = \log f^0/f_S = \frac{\Delta H^F}{4.57} \frac{T_m - T}{T_m T}$$

where $A_S$ is the activity of the solid, $T_m$ is the temperature of the melting point of the solid, $T$ is the absolute temperature of the solution, and $\Delta H^F$ is the heat of fusion. Thus, the higher the heat of fusion and the higher the melting point of a solid, the more correction is necessary in data for thermodynamic activity. There are few data for the thermodynamic activity of solid non-ionic substances in solution, but there is no reason why the approximate values for activities cannot be obtained by assuming Henry's law to hold and obtaining activities from solubility data. A certain amount of caution, however, should be used in attempting to determine the activities of highly soluble substances using this relationship.

From solubility data Gavaudan has calculated the activities relative to the solid of a number of compounds used by Levan and Ostergren (35) as inhibitors of mitosis. The agreement in values is not very good, and it is possible to show that some improvement can be made by correcting these calculations to an activity with the liquid as a standard state. The second column of table 4 gives the values of activity calculated by Gavaudan, and the fifth column the corrected value of activity for the inhibition of mitosis. Unfortunately there are not enough values for the heats of fusion of compounds available to allow computation of corrected values for the last two members of the series. For those compounds so corrected, the ratio $A_{nar}/V_m$ is approximately constant.

[2] That a substance is a solid when its $V_m$ is 70–85 ml./mole is proof that the molecule is highly polar and can be expected to show anomalies in its physiological action. Even in the case of a large molecule such as morphine, the statement is frequently made that this substance is an exception to the hypothesis of lipid solubility (25). Such an interpretation is the result of using the wrong "oil" in partition coefficient measurements. The *Merck Index* gives the solubility of morphine in water as 0.2 g./liter and in amyl alcohol as 8.8 g./liter. The solubility of morphine in ether is about that in water.

TABLE 4

*Thermodynamic activities isoinhibitory for mitosis in Allium*

| SUBSTANCE | $A_1$ | MELTING POINT | $\Delta H^F$ | $A_i$ | $V_m$ |
|---|---|---|---|---|---|
| | | °C. | *kcal./mole* | | *ml./mole* |
| α-Naphthylamine | 1.0 | 50 | 3.2 | 0.57 | 130 |
| Naphthalene | 0.24 | 80 | 4.4 | 0.075 | 108 |
| α-Methylnaphthalene | 0.10 | −22 | | 0.10 | 142 |
| α-Chloronaphthalene | 0.08 | Liquid | | 0.08 | 135 |
| α-Bromonaphthalene | 0.10 | 6 | | 0.10 | 138 |
| α-Iodonaphthalene | 0.12 | Liquid | | 0.12 | 149 |
| β-Methylnaphthalene | 0.50 | 35 | | | 142 |
| β-Chloronaphthalene | 0.50 | 55 | | | 148 |

* From Levan and Ostergren (35), as calculated by Gavaudan (21).

The case of α-naphthylamine may be recognized as that of a polar substance, and hence not comparable to the rest of the compounds in the series.

A further point of interest is the data assembled by Gavaudan as to compounds which act at very low values of thermodynamic activity. Such substances, acting on *Triticum* as inhibitors of mitosis, are: phenol, 0.005; chloral hydrate, 0.00025; diphenylamine, 0.02; hexanitrodiphenylamine, 0.0004; and colchicine, 0.001. These values of activity are to be compared with values of 0.10–0.30 for normal paraffins and chloroparaffins. Such low values of activity suggest some sort of chemical reaction between the narcotic molecules and the cell, but the possibility cannot be ruled out that, at least in the case of phenol and the phenylamines, a reaction similar to depolarization has taken place. Staveley, Jeffes, and Moy (52) have assembled data showing the increase in the solubility of water in benzene brought about by the addition of a third substance. The figures, expressed as increase in mole fraction of water in benzene per unit mole fraction of substance added, are as follows: phenol, 200; aniline, 40; methanol, 80; ethanol, 125; nitrobenzene, 9; chloroform, 0 (all values are $\times 10^{-3}$). If the cell membrane could be similarly affected by such agents, we would expect a depolarization to result. In this connection Davies (13) has shown that dinitrophenol in a concentration of $10^{-4}$ *M* strongly depolarizes the cerebral cortex. The activity at this concentration is about 0.003 and hence very much lower than the usual levels for physiological effects. In spite of the well-known use of dinitrophenol as an agent for uncoupling phosphorylations, further investigation is necessary to establish whether this substance is acting in a specific or a non-specific fashion.

## VIII. THE EFFECTS OF PRESSURE ON NARCOSIS

In a most interesting study of the effects of hydrostatic pressure on the luminescence intensity of *P. phosphoreum*, Johnson and Eyring (27) showed that the depression caused by a number of narcotics could be reversed by applied hydrostatic pressures. According to their interpretation, the narcotics caused a reversible denaturation of enzymes responsible for luminescence, with a volume increase. Other substances were shown to be non-pressure-sensitive and were

considered to act through a mechanism not involving a volume change. It is possible to account for the observed phenomena in another way, namely, to consider the change in mole fraction to be expected in an aqueous and in a non-aqueous phase, both containing a narcotic such as ethanol, when pressure is applied to the system. The equation representing the change to be expected is

$$\left[\frac{\partial(\ln X_{nar}/X_{H_2O})}{\partial P}\right]_T = \frac{\bar{V}_{nar} - \bar{V}_{H_2O}}{RT}$$

where $X_{nar}$ and $X_{H_2O}$ are the mole fractions of narcotic in the non-aqueous and aqueous phases, $V$ is the partial molal volume $(\partial V_m/\partial/N_1)_{N_2}$, and $N$ is the number of moles of a component. Thus if we take values for the partial molal volume of ethanol in water and in a non-aqueous solvent (heptane), it is possible to compute the expected change in mole fraction in heptane as pressure is applied. The actual values for ethanol in water and heptane are 51 ml./mole (29) and 66 ml./mole (53), and this gives a change in mole fraction about 50 per cent of what would be required to conform with the results of Johnson and Eyring. It is entirely possible, however, that the $\bar{V}$ of ethanol in the membrane is actually much larger than the value observed in heptane; hence one cannot rule out the explanation that narcosis is relieved by pressure simply because pressure tends to force a narcotic out of the membrane. On the other hand, substances that show no difference in $\bar{V}$ in aqueous and non-aqueous solvents cannot be expected to be influenced by pressure.

## IX. THE BEHAVIOR OF NARCOTICS IN NON-AQUEOUS SOLVENTS

Since studies on gases have indicated that $A_{nar}$ somehow appears to be related to the $V_m$ of the narcotic, it becomes necessary to consider whether or not the effects observed can be explained by some non-ideality in the interaction between successively larger narcotic molecules and the cell. For reasons that will appear later, the discussion will be concerned with a liquid, since this is the standard state selected for narcosis. The results are, however, equally applicable to gases.

The problem of calculating the activity coefficients of the components of a binary liquid mixture has never been satisfactorily solved. Some idea of the difficulties involved can be obtained from a discussion by Robinson and Gilliland (49). The most successful treatments have been semiempirical at best. In order to use a theoretical treatment, some idea of the complexities of the liquid state must be considered.

To simplify the discussion, it will be convenient to consider two types of liquids (normal and associated) and two types of solutions (ideal and non-ideal). The physics of solutions recognizes several other classes of liquids and solutions, but it appears that mechanisms involved in narcosis can be developed from a consideration of only the above types. Normal liquids may be considered to be the hydrocarbons and most of their substitution products not otherwise classified below. Associated liquids are the alcohols, both aliphatic and aromatic, the carboxylic acids, the amines, water, and some nitroparaffins. Ideal solutions can be formed by mixtures of two normal liquids provided there is no great disparity

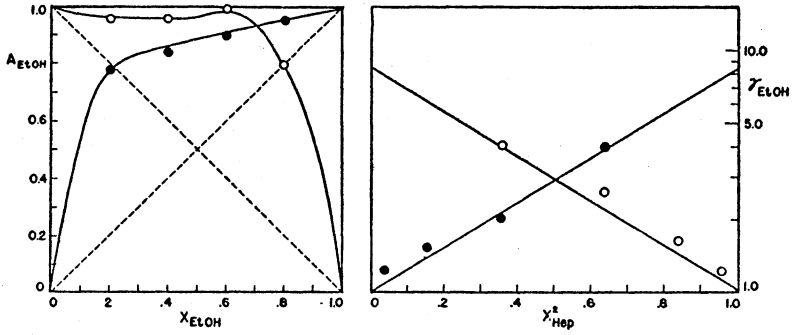



FIG. 3. On the left is a plot of activity *vs.* mole fraction for ethanol in heptane at 30°C. (54). On the right the activity coefficient of ethanol is plotted on a logarithmic scale against the mole fraction (squared) of heptane. Solid circles are data for ethanol; open circles are for heptane.

in molecular volumes, and between two associated liquids of the same type e.g., two alcohols, or two amines etc., again providing there is approximate equality of molal volumes.

Non-ideal solutions can be of a variety of sorts and are indeed to be regarded as the rule rather than the exception. The data in figure 3 show the behavior of two liquids, one normal (heptane) and one associated (alcohol), when they are mixed in varying proportions. A model of what is happening may be had by considering that ethanol, a highly associated liquid, is an isomer of dimethyl ether, a gas, since both have the same formula, $C_2H_6O$. The difference in vapor pressure between these two substances is referable to the fact that while the ether is a relatively normal liquid, the whole of the ethanol liquid structure is bound together by hydrogen bonds. As one dilutes ethanol in water these bonds are broken, but new hydrogen bonds with water form. When ethanol is diluted with heptane, hydrogen bonds cannot be formed, and in dilute solutions one has highly volatile unassociated ethanol molecules. At high mole fractions of ethanol, hydrogen bonding is acting to bring the whole of that liquid into as tight a structure as possible, and the heptane molecules are, to a greater and greater extent, forced out of the liquid and into the vapor. This, of course, leads to a high activity coefficient for heptane at high mole fractions of ethanol. It can be shown (see 23) that the data for certain types of deviations from Raoult's law can be represented by the function

$$\ln \gamma_2 = wX_1^2/RT$$

where $w$ is a constant. This is shown on the right-hand diagram of figure 3.

The problem at hand is one of calculating the activity coefficients that narcotics may be expected to show as one ascends an homologous series. One approach to this problem is as follows (23): While in dilute solutions Raoult's law is valid for the solvent, the term for the solute (narcotic) should be written

as in equation 1, where $w$ is the interchange energy or the energy necessary to exchange a molecule of solvent for one of solute.

$$p_{nar}/p^0 = X_{nar}\, e^{w/kT} \tag{1}$$

Such an equation comes about because, while the solvent molecules in dilute solutions are entirely surrounded by one another, the solute molecules are entirely surrounded by solvent. Since the activity coefficient is defined by equation 2, equation 1 can be rearranged to yield equation 3. Recalling the data on

$$\frac{p_{nar}/p^0}{X_{nar}} = \gamma_{nar} \tag{2}$$

$$\ln \gamma_{nar} = w/kT \tag{3}$$

gases, we could arbitrarily require $w$ to be a function of $V_m$ and obtain the relationship that log $A_{nar}$ is related to $V_m$.

To avoid purely empirical approaches to the problem of the calculation of activity coefficients, one may use the method of Hildebrand (24), who has been able to relate $w$ to the difference between the $\Delta H^v/V_m$ of the solvent and the solute and the $V_m$ of the solute. The quantity $(\Delta H^v/V_m)^{1/2}$ is called the solubility parameter of the liquid and is designated by $\delta$. Another, perhaps more descriptive, name for $\delta^2$ is the cohesive energy density of the liquid. For dilute solutions,

$$w = V_m(\delta_1 - \delta_2)^2$$

and this term is the heat of mixing. If the molecules of solvent and solute are considerably different in volume, the mixture cannot show an ideal entropy of mixing, and a further correction of the activity coefficient will be necessary. This correction is much smaller than the heat of mixing term and, for simplicity, ideality deviations in entropy have been neglected. It is to be emphasized that Hildebrand intended the use of solubility parameters to apply to mixtures of relatively non-polar molecules, and extrapolation to polar hydrogen-bonding molecules has been performed because there does not appear to be a more satisfactory way of obtaining activity coefficients.

In order to estimate activity coefficients for narcotic molecules in the membrane, the Hildebrand expression is substituted into equation 3 and moles are employed rather than molecules. The expression then becomes

$$\ln \gamma_{nar} = V_m(\delta_{nar} - \delta_{mem})^2/RT \tag{4}$$

where $\delta_{mem}$ = the membrane solubility parameter, or cohesive energy density. In future discussions the terms "narcotic" and "membrane" will be used to designate solute and solvent.

In general, the heats of vaporization of the alcohols are available only at the boiling point, so that there is the choice between obtaining these values for the less volatile alcohols at high temperatures and attempting the extrapolation to room temperature, or of extrapolating from values of $\delta$ for the lower alcohols.

TABLE 5

*Solubility parameters ($\delta$) of narcotics* at 25°C.*

| SUBSTANCE | $\delta$ | $V_m$ | SUBSTANCE | $\delta$ | $V_m$ |
|---|---|---|---|---|---|
| | | *ml./mole* | | | *ml./mole* |
| Pentane | 7.0 | 116 | Methanol | 14.6 | 48 |
| Hexane | 7.3 | 132 | Ethanol | 13.5 | 66 |
| Heptane | 7.5 | 147 | 1-Propanol | 12.5 | 82 |
| Octane | 7.6 | 164 | 1-Butanol | 11.0 | 98 |
| Ether | 7.4 | 105 | 1-Pentanol | (10.5) | 114 |
| Chloroform | 9.3 | 81 | 1-Hexanol | (10.0) | 131 |
| Cyclohexane | 8.2 | 109 | 1-Heptanol | (9.5) | |
| Ethyl chloride | 8.5 | 73 | 1-Octanol | (9.0) | 162 |

* The author is indebted to Professor J. H. Hildebrand for pointing out that methanol and phosphorus have the same $\delta$ and yet phosphorus is not very soluble in methanol. The factors responsible are the differences in the nature of the intramolecular forces of these two substances.

Solubility parameters can be calculated from activity coefficient *vs.* mole fraction data. For the data of Butler on the activity coefficients of the alcohols in benzene, we calculate a $\delta$ for methanol of 15.5. For the higher alcohols the $\delta$ values are about 1.0 unit higher than those given above. The partition coefficients (11) of methanol and ethanol partitioned to 1-butanol and 1-octanol can be obtained by calculation, using the above values of $\delta$.

The values of $V_m$ for the alcohols, as given in table 5, are those for the partial molal volume of the alcohols in heptane. These values were used in the computations to be presented later in this paper. Actually, the volumes of the alcohols in the pure liquid appear to give a better fit with some of the data.

The latter course has been followed in the belief that the values so obtained are more reliable. Table 5 gives values for $\delta$ obtained in this manner. The first four alcohols and the substances on the left-hand side of the table have values calculated directly. It is now possible to calculate the activity coefficients of molecules in the membrane by assigning an arbitrary, but constant, value for the solubility parameter of the membrane and taking the values of $\delta$ and $V_m$ for the various alcohols. The results of such calculations are shown in figure 4. Depending upon the choice of $\delta_{mem}$, the curve for activity coefficient *vs.* chain length rises more or less steeply as one ascends an homologous series. With the paraffins, the change in $\delta$ with chain length is much smaller than with the alcohols, but the low $\delta$ of the paraffins will result in high activity coefficients where $\delta_{mem}$ = 10–13. In general, as the $CH_2$ chain length is increased, the alcohols and the paraffins can be expected to approach a common value for $\delta$.

## X. NARCOSIS AT CONSTANT VOLUME FRACTION

From a knowledge of the thermodynamic activity necessary for narcosis, as measured in the aqueous phase, and from the computed activity coefficient of the narcotic in the membrane, the mole fraction of the narcotic in the membrane ($X_{nar}$) can be calculated. The question then arises as to just what property of the narcotic molecule is responsible for narcosis. On theoretical grounds it does not seem reasonable that a certain mole fraction of any inert substance in the mem-

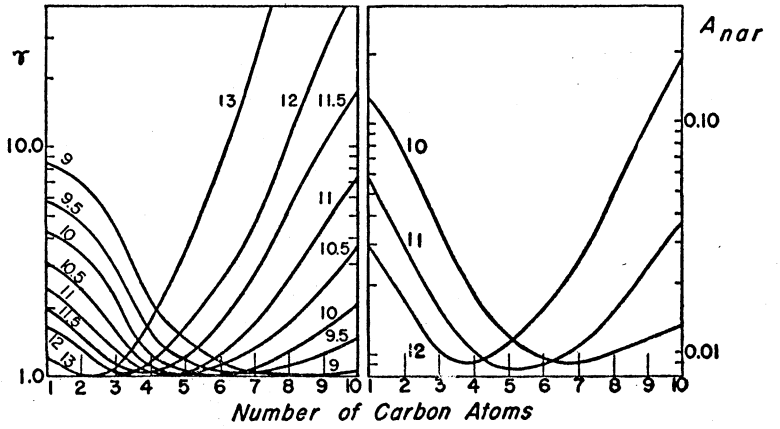



FIG. 4. On the left the activity coefficients for the homologous series of normal alcohols calculated from equation 4 are plotted *vs.* the number of carbon atoms in the chain. The numbers on the various curves designate various values of the solubility parameter of the solvent (membrane).

On the right the activity threshold for narcosis to be expected in an homologous series of normal alcohols is plotted *vs.* chain length. The curves are calculated on the basis that the mole fraction for narcosis is 0.01 for the lowest member of an homologous series where $\gamma = 1.0$. For $\delta = 12$ this was at 1-propanol; for $\delta = 11$, at 1-butanol; etc.

brane will cause narcosis, but rather that a certain *volume fraction* of any inert substance will cause narcosis. In the physical chemistry of solutions, Raoult's law is a definition of ideality,[3] valid only when the molecular volumes of the two components of a solution are equal. Otherwise, the volume fraction $\phi_1 = X_1V_1/X_1V_1 + X_2V_2$, where $X$ is the mole fraction and $V$ the molal volume, is preferable. If the molecular volume of a membrane molecule and the mole fraction of the membrane are considered to be very much greater than those of the narcotic in the membrane, e.g., a dilute solution, then $X_{nar}V_m$ will be proportional to the volume fraction of the narcotic in the membrane. This principle can then be stated as: equal degrees of narcosis with equal volume fractions of narcotic in the membrane, or $X_{nar}V_m = k$. Proof of the validity of this volume fraction rule can only come from the demonstration that for experimental data $X_{nar}V_m$ is, empirically, a better constant than is $X_{nar}$ alone. The problem is complicated by the fact that $X_{nar}$ depends upon computed values of the activity coefficient. Values of $A_{nar}$ can be predicted, for higher members of an homologous series, by computing $X_{nar}$ when $\gamma_{nar} = 1$ and multiplying this value by the $\gamma_{nar}$ calculated for higher members of the series. A plot of such computed values of $A_{nar}$ is shown in figure 4 (right). It appears, then, that one cannot expect to observe narcosis at equal values of $A_{nar}$ nor of $A_{nar}V_m$, but rather that the $A_{nar}$ will be higher the larger the $V_m$ of the narcotizing molecule and the greater the difference in solubility parameter between narcotic and membrane. If $A'_{nar}$ is the activity

[3] For a discussion of the theoretical basis for Raoult's law, see E. A. Guggenheim (Trans. Faraday Soc. **32**, 151 (1936)).

for narcosis by a higher member of an homologous series, and $A_{nar}$ is the observed activity for narcosis by a lower member of an homologous series, these two activities will be related by equation 5.

$$A'_{nar} = A_{nar} \frac{V_m e^{-V_m(\delta_{nar}-\delta_{mem})^2/RT}}{V'_m e^{-V'_m(\delta'_{nar}-\delta_{mem})^2/RT}} \tag{5}$$

If $\delta_{nar}$ is constant throughout an homologous series, as is approximately the case for the normal paraffins, then equation 5 becomes an expression containing only a volume term for the narcotic.

$$A'_{nar} = A_{nar} \frac{V_m e^{-KV_m}}{V'_m e^{-KV'_m}} \tag{6}$$

## XI. LIQUIDS AS NARCOTICS

Pharmacological experience with the perfluorocarbons presents some of the most convincing evidence that the molecular volume and solubility parameter are of great importance in predicting the action of narcotics. We have, in some unpublished experiments, exposed mice to oxygen saturated with the vapor of pentane, $C_5H_{12}$ ($V_m = 116$, $\delta = 7.0$), and also with perfluoropentane, $C_5F_{12}$ ($V_m = 183$, $\delta = 5.5$). The vapor pressures are, respectively, 450 mm. and 500 mm. at 20°C. With pentane, narcosis ensued in about 15 sec., while with the perfluoro analog no physiological effects were evident after an exposure of 1 hr. A similar inertness of perfluoroethyl ether (9) has been noted (see also reference 34).

One of the most interesting questions raised in connection with the theory of narcosis has been why the values of $A_{nar}$ for synaptic and non-synaptic transmission in ganglia were different from alcohol to alcohol. In figure 5 are plotted the data of Posternak and Larrabee (see 4). It is apparent that for synaptic transmission the product $A_{nar}V_m$ is more constant than $A_{nar}$ alone. This constancy requires that the values of $\gamma_{nar}$ in the membrane be constant up to $C_8$ at least. Ether and chloroform also fit on the curve very well. For non-synaptic transmission, the required values of $A_{nar}$ rise with increase in $V_m$ and ether and chloroform do not fit on the curve. Here the activity coefficients are increasing with increase in chain length. These data can be reconciled if one assigns $\delta_{mem} = 11.5$ to the non-synaptic membrane and $\delta_{mem} = 10$ to the synaptic membrane. Larrabee and Posternak (31) have noted that what they term "selective action," that is, the narcosis of synaptic transmission without the narcosis of non-synaptic transmission, appears to be related to the molecular weight of the narcotic molecule, and that large molecules are more selective. Stated according to our analysis, small molecules used in studies of narcosis tend to be more polar (higher $\delta$) than large molecules, and molecules with high $\delta$ values are more easily accommodated in a membrane of higher solubility parameter. Similarly, chloroform and ether have $\delta$ values of 9.3 and 7.5, and will show high activity coefficients in membranes of high cohesive energy density. (Calculated values of $A_{nar}V_m$ for fibers: $CHCl_3 = 10$, ether $= 30$.)

If some data on peripheral nerve narcosis are now considered, it will be of

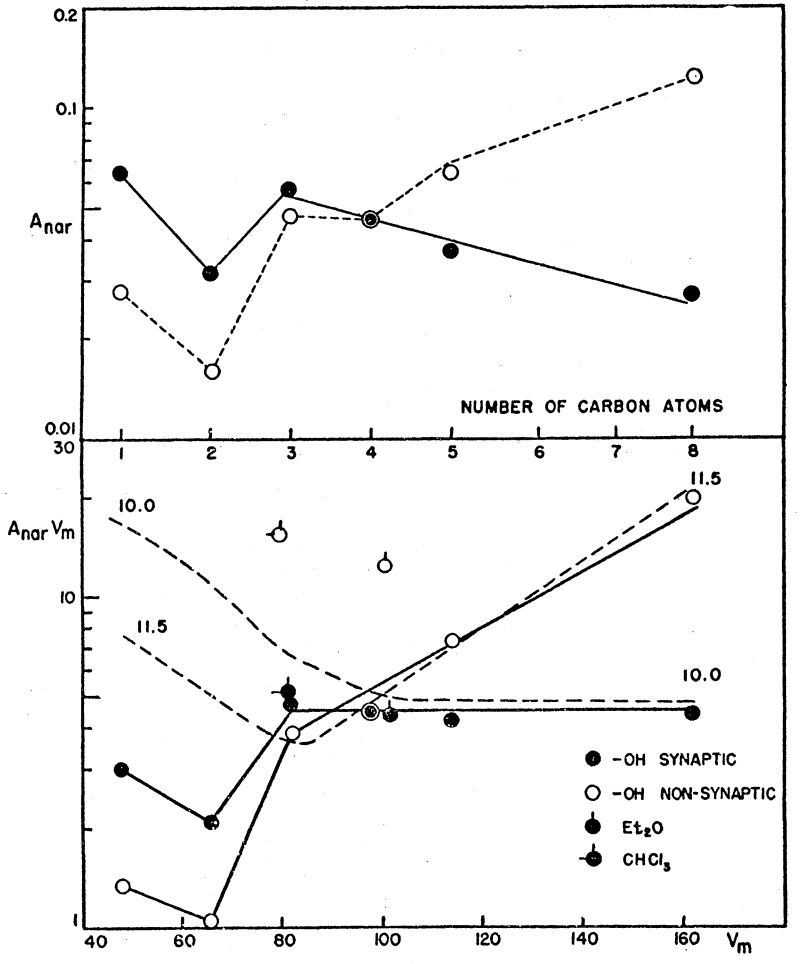



FIG. 5. The top chart is a plot of the activity threshold for the narcosis of synaptic (solid circles) and non-synaptic fibers (open circles) of sympathetic ganglia by the alcohols (4).

In the lower chart the same data are presented as the activity threshold for narcosis times the molal volume (ml./mole) of the narcotic for synaptic and non-synaptic fibers. The dotted lines are the calculated values of $A_{nar}V_m$ for membrane solubility parameters of 10 (synaptic) and 11.5 (non-synaptic).

interest to see whether one can use the same parameter as in the non-synaptic fibers already considered. In the lower half of figure 6 are plotted data of Laget, Posternak, and Mangold (30) on the narcosis of frog sciatic nerve by alcohols. The calculated values of $A_{nar}V_m$ fit well on a curve for $\delta_{mem} = 11.5$, with the exception of methanol where the calculated value is somewhat too high. One

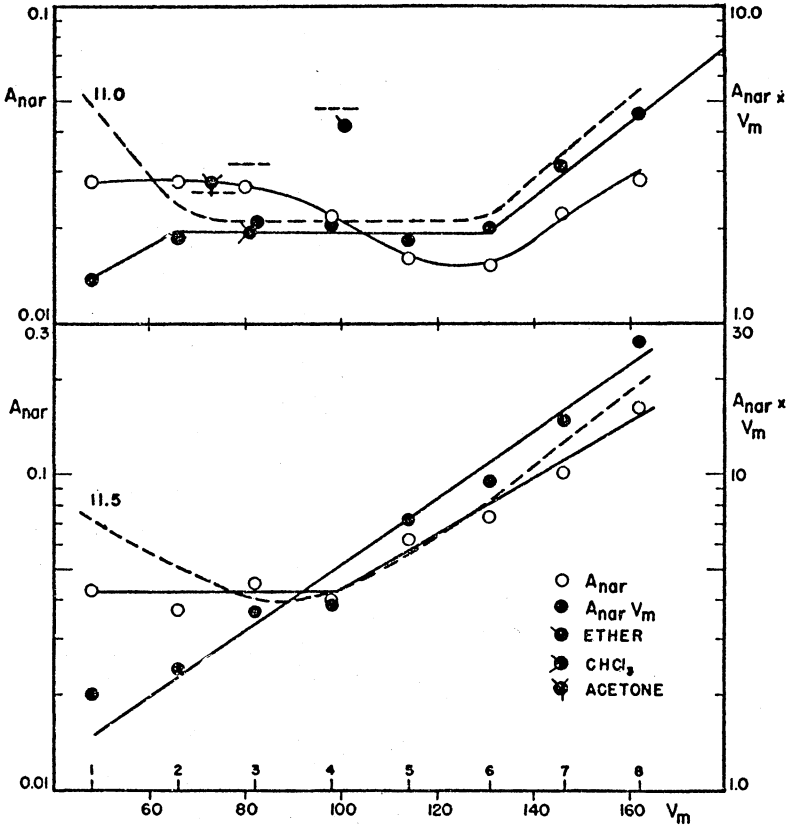



FIG. 6. The upper graph is the threshold for the reversible narcosis of tadpoles by the alcohols (56): open circles, $A_{nar}$; solid circles, $A_{nar}V_m$. The dotted line indicates the calculated values of $A_{nar}V_m$ with a membrane solubility parameter of 11.

The lower graph represents the activity of various alcohols necessary to block conduction in frog sciatic nerve (30). Open circles, $A_{nar}$; solid circles, $A_{nar}V_m$.

can expect that as one deals with narcotics with greater and greater differences in $\delta$ from the membrane, the accuracy of the calculation will become less and less. In addition to this, as the authors have shown, methanol, and to a much smaller extent, ethanol, are depolarizing agents at concentrations below those necessary for narcosis; hence it is difficult to predict what effect this subsidiary action would have in determining the narcotic threshold.

In the central nervous system one may expect to find a membrane somewhat different from that of either the synapse or the nerve fiber. The upper plot in figure 6 is based on the data of Vernon (56) on the reversible suppression of spontaneous movements in tadpoles. From a consideration of the values of $A_{nar}$ one might conclude that, allowing for experimental error, the data indicate

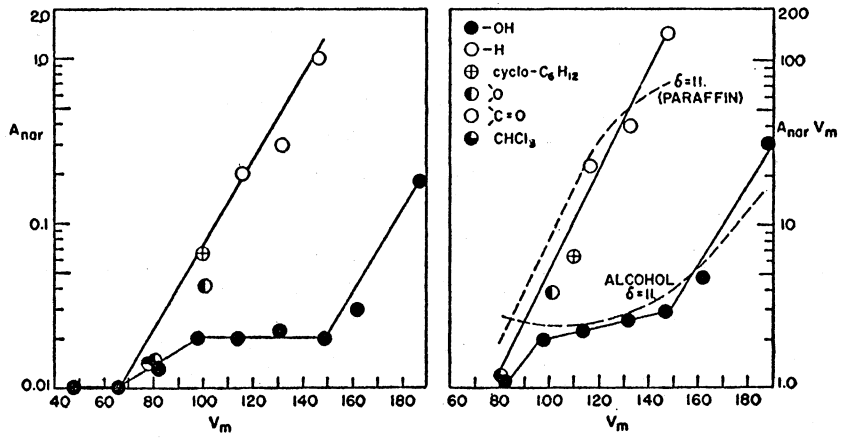



FIG. 7. On the left, a plot of the thermodynamic activity for reversible suppression of reflex activity in *Gobio* (19). On the right, values of $A_{nar}V_m$ for the same data are plotted. The dotted lines represent calculated values of $A_{nar}V_m$ for a membrane solubility parameter of 11.

a constancy of narcosis at a mean value of $A_{nar}$ of 0.02. If, however, the product $A_{nar}V_m$ is considered, the constancy of the data for ethanol through 1-hexanol is improved. If $\delta_{mem} = 11$ is assigned to the structure being acted on by the narcotic, the curve is a reasonable fit for the data, again excepting methanol because of its strong depolarizing power. Calculated values of $A_{nar}V_m$ for acetone, ether, and chloroform are indicated on the diagram by dotted lines; these too show good agreement except for the chloroform point.

A further test of the validity of using solubility parameters in narcosis is to see whether a single membrane parameter can predict values of $A_{nar}$ when two homologous series of narcotics of very different internal pressures are used. Such a study is that of Gary-Bobo and Lindenberg (19), as shown in figure 7. The test organism was the fish *Gobio*, and the index of the effectiveness of the narcotic was the suppression of reflex activity. If a $\delta_{mem}$ value of 11 is selected, the two series of data are in reasonable agreement. It cannot be expected that the agreement in these cases can be as good as with a single series of compounds, especially for $\delta$ values far from those of the membrane.

By far the most extensive investigation into the thresholds at which various different types of narcotics act has been that of Ferguson (17). Working with the grain weevil, and supplying the various narcotics as vapors, Ferguson obtained the results shown in figure 8 (left). The most conspicuous difference between these data and others that have been considered here is the reversed position of the alcohols and the paraffins. It is impossible to assign a membrane solubility parameter somewhere between those of the alcohols and the paraffins, as was done with the data of Gary-Bobo and Lindenberg, and still predict the shapes of the two curves. The difficulty in reconciling these extensive data underlines the inadequacy of the theoretical treatment for obtaining activity coefficients of

polar mixtures. Van Arkel (1) has analyzed the problem and has shown that the $\Delta H^v/V_m$ of polar substances yields not the internal pressure of the liquid, but this value plus a contribution referable to the orienting forces of the dipoles. Thus, even when there is equality of the values of $\Delta H^v/V_m$, it is possible that the heat of mixing is greater than zero, for two substances with different dipole moments. When one component of a mixture is polar, and the other non-polar, an ideal solution is impossible, as there will always remain the orientation factor of the polar component to make the heat of mixing positive. It is to be hoped that in the near future physicochemical investigations can be made to provide adequate methods for handling the data in narcosis. Lacking this treatment, we can only conclude that the *apparent* $\delta$ for the structure being acted on by the narcotic is very low, about 7.5. Such a value suffices to bring the alcohols into rough agreement with the rest of the aliphatic substances when $A_{nar}V_m$ is plotted against $V_m$. An attempt has been made to achieve separation between the dispersion ($\delta$) and the orientation forces in the membrane in the following way: The data of Butler (6) giving the activity coefficients of the alcohols in dilute solution in benzene were used to evaluate the polar:non-polar interaction term. When the $A_{nar}$ values of the alcohols were divided by the activity coefficients so obtained, and then multiplied by $V_m$, the results fitted very well on the curve for all other narcotic agents (see the right-hand plot of figure 8). If these activity

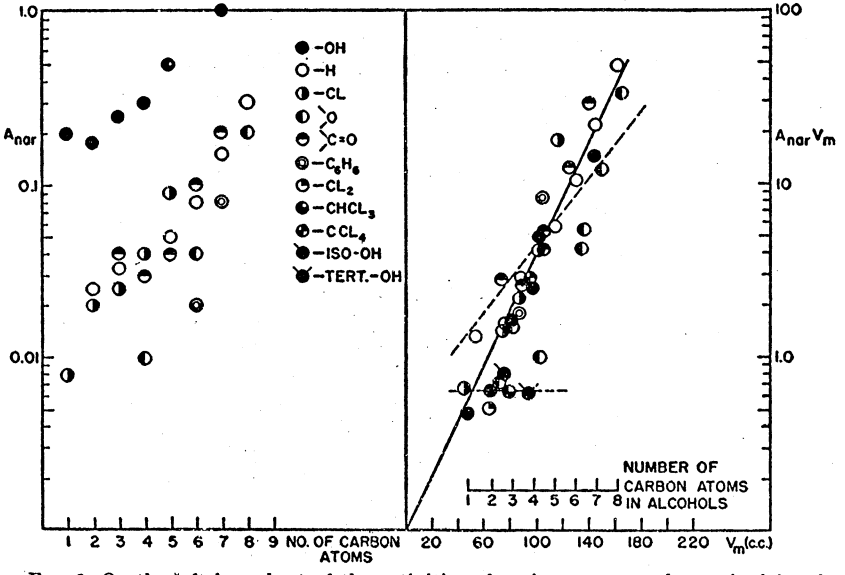



FIG. 8. On the left is a chart of the activities of various compounds required for the narcosis of the grain weevil (17). Values for the nitroparaffins have been omitted from the plot. On the right, the data are plotted as $A_{nar}V_m$. Values of $A_{nar}$ for the alcohols have been divided by the activity coefficients of the alcohols in benzene solution. The dotted line indicates a membrane solubility parameter of 11 (for the paraffins, sloping dotted line; for the chloroparaffins, horizontal dotted line).

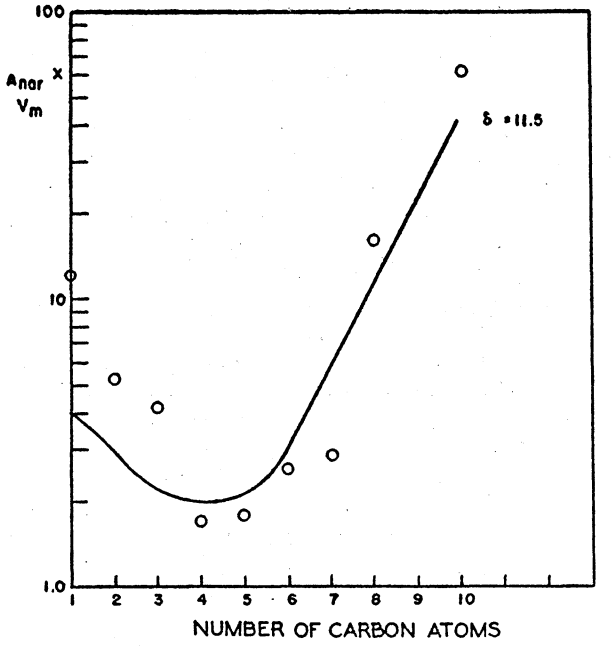



FIG. 9. The product $A_{nar}V_m$ for the rejection threshold of blowflies for the alcohols is plotted against the number of carbon atoms in the alcohol (15). The line drawn represents a membrane solubility parameter of 11.5 for the affected structure. The alcohols were delivered as vapors and affected olfaction.

coefficients had been the correct values, the resulting curve should have been a line parallel to the $V_m$ axis. What has been accomplished by this correction is to make the points equivalent to the paraffins. If a $\delta$ value of 11 is now selected for the membrane and the paraffins are considered to have a constant $\delta = 7$, the resulting curve is a rough approximation of the observed data. For the cluster of points at the low end of the curve, inspection reveals that the $\delta$ value of these compounds (mostly chloroparaffins) is about 9.5. The line for this particular calculated value of $A_{nar}V_m$ has been drawn in.

The situation for the grain weevil is not the common one in so far as data presently available on narcosis are concerned. For frogs and mammalian nerve, the situation with respect to the alcohols and the paraffins is just the reverse, in that the paraffins require much higher values of $A_{nar}$ than do the alcohols. Hence the affected structure in the grain weevil, while polar, appears to have a different sort of polarity from those previously treated.[4]

[4] Ferguson (17) cites the data of Weese (57) on the narcosis of mice by alcohol vapors in support of the contention that $A_{nar}$ for the alcohols both in the grain weevil and in mice is about 0.2 for ethanol. Since the activity coefficient for ethanol in water is about 3, this means that the mole fraction of ethanol in water (or in blood) = 0.2/3 = 0.07 $X$ or 3.8 $M$, an incredible figure! Miles (41) gives 0.08 $M$ as a figure for deep anesthesia in man. This is 0.0016

The alcohols have been shown by Dethier and Yost (15) to be rejected by the blowfly (*Phormia*) at certain threshold concentrations. Whether or not this process is a receptor narcosis or a receptor stimulation remains to be demonstrated, but the data obtained are plotted in figure 9 and an analysis of the activity coefficients to be expected shows that the curve can be fitted by a membrane solubility parameter of 11.5. The fit for methanol is not good, and indeed the general shape of the curve obtained suggests that the vapor of the first three alcohols may not be in equilibrium with the organism.

## XII. MEMBRANE COHESIVE ENERGY DENSITY

The fact that the apparent membrane solubility parameter, or cohesive energy density, is equal to that of 1-butanol, for example, requires some sort of interpretation. This does not mean that the membrane is necessarily similar to butanol, but it does indicate that the membrane is not at all similar to olive oil, nor does it seem likely that the membrane is similar to oleyl alcohol or other long-chain alcohols. While solubility parameters for these substances are not available, a value of about 8.0 does not appear to be too unlikely. To achieve $\delta$ values as high as 11 with an alcohol, the hydrogen-bonding part of the molecule must be increased to the same extent as the paraffinic chain. One way of doing this is to consider the membrane molecule as being composed of a paraffinic part and of a water-soluble part. In view of the popularity of lipoproteins as units of the plasma membrane, the possibility exists that the strong cohesive forces holding the membrane together come from hydrogen bonding in the aqueous portion of the molecule. Another way of achieving high cohesive energy density is to construct the membrane molecule of methylene groups and hydroxyl groups in the ratio of perhaps 4:1 (as in butanol).

If molecules from two different membranes are compared, and it is assumed that the molecules are chemically similar and that both contain an equal number of hydrogen-bonding groups, the molecule with the larger molal volume will have the lower $\delta$. Thus it is possible to interpret the smaller $\delta$ values of the central nervous system as meaning larger membrane molecules, larger spaces between the molecules, and a more permeable and more labile structure. This interpretation is not at variance with what is known about differences between the central nervous system and nerves.

## XIII. PERMEABILITY OF THE MEMBRANE TO NARCOTICS

The analysis presented in the last three sections is not meant to imply that as one ascends an homologous series there is a decrease in the ability of higher and higher members of the series to penetrate the cell membrane. This point appears to be of sufficient importance for us to contrast the permeability of the membrane for a particular molecule and the *acceptance* by the membrane of the same molecule. This acceptance implies an ability of the molecule to stay fixed

$\chi$ or $A_{nar} = 0.0048$. The high figures of Weese must certainly be due to a non-equilibrium condition. Indeed, Loewy and von der Heide (37) have shown that absorption of methanol vapor by rats is a slow process, taking many hours before an equilibrium is attained.

in the membrane. Other things being equal, whether a molecule penetrates into the cell interior will depend upon the probability that a large enough free space in the membrane will occur to permit the passage, while the membrane acceptance for a molecule depends upon its having a cohesive energy density and volume appropriate to the space in the membrane that is to be occupied. Membrane acceptance

$$\alpha = 1/\gamma_{nar} = X_{nar}/A_{nar}$$

might thus be defined as the reciprocal of the activity coefficient of the narcotic in the membrane. Since narcotic action is judged by measuring $A_{nar}$ in an aqueous phase, equal degrees of narcosis can be expected when $A_{nar}V_m\alpha = k$, a constant. The membrane acceptance is, of course, related to the ideal partition coefficient of the narcotic, $P$, which may be defined as the ratio of the activity coefficient at infinite dilution for the narcotic in water divided by that in the membrane:

$$P = \gamma_{H_2O}/\gamma_{nar} = X_{nar}/X_{H_2O}$$

Since

$$A_{nar}V_m\alpha = X_{H_2O}V_mP$$

the relationship between the membrane acceptance and the partition coefficient is $\alpha = P/\gamma_{H_2O}$.

## XIV. THE MECHANISM FOR CUT-OFF

Because there have been so many explanations proposed for the various phenomena in narcosis, it is worthwhile to examine some of these suggestions and to compare them with the treatment already employed. Meyer (44) has suggested that a certain molal concentration of narcotic molecules in the lipid phase of cells is necessary for narcosis, but no definitive explanation as to what this concentration was supposed to affect was offered. Gavaudan, Dodé, and Poussel (20) considered that the required concentration of Meyer exercised a certain "constraint" on the rest of the molecules of the membrane. This "constraint" was supposed to make the structure more rigid, and hence narcotized. The explanation of "cut-off," or the cessation of physiological activity above a certain chain length in an homologous series of compounds, was that because of the decrease in the solubility of the larger molecules in the lipid phase to which they were partitioned, a point was reached where these molecules were no longer soluble. Ferguson (17) has pointed out that, contrary to the requirements of the Gavaudan theory, large aliphatic molecules do not become more and more insoluble in lipids of the olive oil type, but rather the reverse. He has proposed instead, as an explanation for cut-off, the ingenious idea that as molecules become larger and larger in size, they become more and more ideal in their interaction with the membrane, until finally there is no distinguishing the membrane from the narcotic molecules. There do appear, however, to be some difficulties with this formulation. If the mixing of large paraffin molecules with the membrane were ideal, one would expect that instead of membrane molecules being dis-

placed in favor of narcotic molecules, the volume of the whole system would simply increase and the membrane would be thicker. Thus the discussion again reduces to a consideration of the forces that hold a liquid together, or its cohesive energy density. The displacement of polar molecules from a liquid by non-polar molecules, or *vice versa*, is not likely at low mole fractions for two different reasons, as considered previously. The phenomenon of cut-off, it is suggested, occurs as a result of large difference in cohesive energy density or solubility parameter between membrane and narcotic, and as a result of increases in $V_m$. The perfluorocarbons with $\delta$ values of about 5.5 will all be without influence on a membrane of $\delta = 10.5$, both because $\Delta\delta^2 = 25$ and because $V_m = 200$ or $\gamma_{nar} = 4000$ (25°C.).

## XV. THE EFFECTS OF MOLECULAR SHAPE

Consideration has not so far been given to the effects of molecular shape on narcosis. Ferguson (17) has commented that the more spherical molecules seem to be better narcotics than do the more linear. He cites several examples for the grain weevil, particularly the substitution series of methanol, ethanol, 2-propanol, and 2-methyl-2-propanol, with activities of 0.2, 0.18, 0.15, and 0.08. Also available from his data are chloromethane, dichloromethane, trichloromethane, and tetrachloromethane, where the first three substances have $A_{nar} = 0.008$ and the fourth 0.03. Although figures for the solubility parameters of all these substances are not available, the much lower boiling point of 2-methyl-2-propanol suggests that the decrease in internal pressure, rather than the shape of the molecule, may be the determining factor in this series. In the chloromethane series, tetrachloromethane has a very different $\delta$ from the others and is, in addition, non-polar.

The case of ethyl ether is one which will require some special treatment. Its low solubility parameter of 7.4 would suggest that somewhat higher than normal values for $A_{nar}$ would be required in most cases, whereas actually the reverse is true. In Ferguson's data, the ethers (except for thiophene and furan) show consistently lower values of $A_{nar}$ at any given $V_m$ than does any other series of compounds. An explanation of this phenomenon may be found in the observation that ether and benzene show practically ideal behavior when mixed, in spite of a difference in $\delta$ of almost 2.0. Hildebrand has suggested that this may be due to the slight volume contraction observed in the mixing of these compounds. Substances that can cause a contraction in volume of the membrane might well be considered as being able to cause narcosis at lower values of $A_{nar}V_m$ because part of the volume occlusion necessary for narcosis has been achieved by a rearrangement of membrane molecules. It will not be possible to deal with such effects as volume changes in detail until a more satisfactory physical treatment of the subject has been made.

## XVI. NARCOSIS AT CONSTANT THERMODYNAMIC ACTIVITY

From an analysis of a variety of data, Brink and Posternak (4) suggested that the empirical rule of "equal degrees of narcosis at equal thermodynamic activi-

ties" could be applied with certain reservations. Brink quite correctly interpreted this rule as an indication that narcotic molecules fit into the cellular structures upon which they act in the same way as they fit into their own pure liquids and further suggested that the rule was limited in its applicability. The author has examined some of the reasons for its limitations. The Brink rule implies a constant activity coefficient of the narcotic in the cell if one interprets the rule as meaning that a constant mole fraction of narcotic is partitioned to the cell at constant activity. A good fit with experimental data is obtained for propanol–octanol, when $\delta_{mem}$ is relatively low. This is so because increases in the activity coefficient of the narcotic (hence decreases in mole fraction at constant activity) are approximately compensated for by increases in molal volume. For very small and for very large molecules, the equal activity rule will not be valid for different reasons. Small molecules can be expected to show activity coefficients in the membrane close to unity (unless $\delta$ values deviate greatly from $\delta_{mem}$), but the small value of $V_m$ means that $X_{nar}$ and hence $A_{nar}$ will have to be high. Large molecules can be expected to show very much higher activity coefficients in the membrane. In this case, the larger values of $V_m$ do not compensate for the increases in $\gamma_{nar}$, and $A_{nar}$ will increase.

## XVII. MODELS OF NARCOTIC ACTION

Another way of looking at the problem of the interaction of narcotics with the membrane is shown in figure 10. On the right is a lattice of membrane mole-

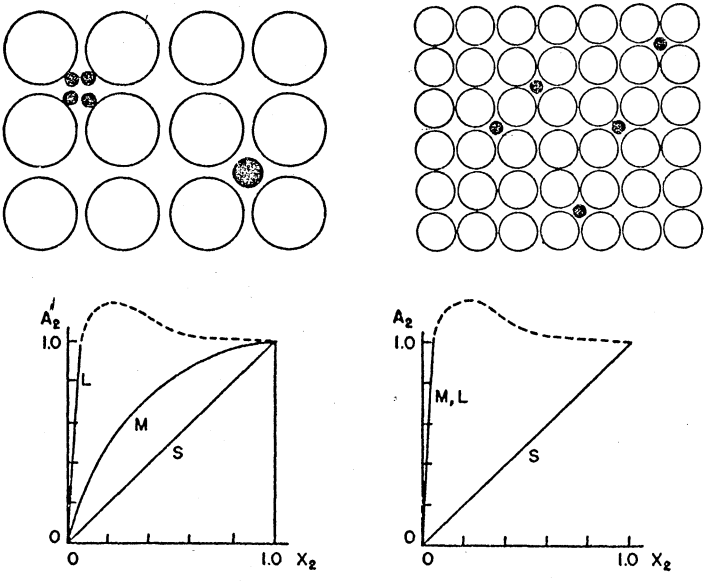



FIG. 10. The diagrams illustrate lattices of membrane molecules (open circles) into which narcotic molecules (solid circles) are fitted. A plot of activity *vs.* mole fraction for the narcotic molecules is given in each case for small, medium, and large molecules.

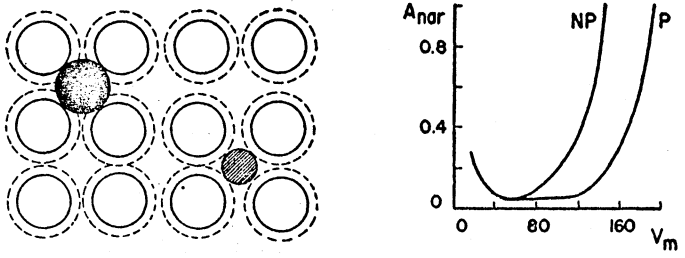



FIG. 11. Left: A lattice of membrane molecules occupied by a paraffin (hatched circle) and by an alcohol (solid circle). The dotted lines around the membrane molecules indicate the maximum space available to non-polar molecules.

Right: A plot of the activity required for narcosis as a function of the molal volume of (P) polar and (NP) non-polar molecules.

cules; some of the free space has been filled in with small narcotic molecules. If the molecules are small enough, they will fit into the lattice easily and occlude a certain amount of free space. If they are too small, the occlusion will not be as effective. The curve "S" (lower right) is intended to demonstrate that molecules of the sort shown in the diagram will follow Raoult's law, while medium-sized and large molecules will both show very great deviations from this law (curves M and L). The fact that the membrane can accommodate molecules larger than the average size of the free space is referable to the fact that the lattice is in thermal motion and random deformations occasionally result in the creation of free space much larger than the average. On the left is a diagram with larger membrane molecules and hence larger free spaces. Small molecules will show ideal behavior but will have to be packed in aggregates to occlude the free space; medium-sized molecules will show only slight deviations from Raoult's law; only with very large molecules will the deviations be extreme.

To account for the differences between polar and non-polar narcotics, a modification of this diagram is shown in figure 11. The non-polar molecules are able to fit into a very much smaller free space than are the polar molecules. Such an arrangement permits the alcohols to be physiologically effective at much larger values of $V_m$ than are the paraffins. In addition, the paraffins do not occlude free space as efficiently as do the alcohols, so that it is quite possible that the product $X_{nar}V_m$ may have to be larger than for polar molecules. The matter of molecular shape thus appears to be less important than the matter of polarity of the molecule, because polarity confers upon the molecule the possibility of entering into a larger effective free space in the membrane and thus occluding more efficiently.

The question arises as to what disturbance at the cellular level is occasioned by the occlusion of free space in the membrane. Brink has shown that narcotics can depress respiration in frog sciatic nerve, but only at levels of $A_{nar}$ far above those required for changes in threshold. A similar observation on sympathetic ganglia is that of Larrabee, Ramos, and Bulbring (32). At the same time, it can be demonstrated that narcotized nerves are just as susceptible to depolarization

by potassium chloride as are normal fibers. Such an observation would suggest that there is not enough volume occlusion to prevent the entry of potassium ion. There remains the possibility that narcosis is due to the inability of sodium ion to pass the membrane once a certain amount of volume has been occluded. This suggestion receives support from the observation of Davson (14) that in the erythrocyte narcotics can be shown to depress permeability to sodium ion but not to potassium ion.[5] From a more general viewpoint, the mechanism mentioned above has the possibility of depressing cell function by preventing the passage in either direction of any particular molecule or ion, depending only upon the degree of volume occlusion occasioned by the narcotic. From a theoretical point of view, one would like to know what substance is first impeded in its transit across the membrane, but unfortunately there is no easy experimental approach to this problem. In the case of peripheral nerve, conduction can continue for long periods of time without oxygen, without glucose, without phosphorylation (azide block), without potassium ion, and sometimes without calcium ion, but function does require sodium ion or some other cation of equivalent properties. The entry of sodium ion into fibers represents as early an event in the time course of excitation as it has been possible to verify experimentally (28). When it becomes possible to determine what other ions move, in response to a stimulus, to produce the membrane changes which permit movement of sodium ion, it will be plausible to assign narcosis to a mechanism earlier in time than inward movement of sodium ion.

In the case of the alcohols, the mole fraction required in the membrane for narcosis ($X_{nar}$) appears to be about 0.01 for both the synaptic and non-synaptic membranes, when $V_m$ for the narcotic is 100 ml./mole. Hence $X_{nar}V_m$ is 1 ml./mole, or approximately the magnitude of the "free" volume in hydrogen-bonded liquids (24). If the $V_m$ of the membrane is taken arbitrarily as 300 ml./mole, then the volume fraction for narcosis $\phi_{nar} = 0.003$. While $X_{nar}$ is independent of any assumption of membrane $V_m$, the value of $\phi_{nar}$ does not require any statement of the $V_m$ of the narcotic.

In the narcosis of non-excitable tissues values of $A_{nar}$ are usually much higher than for the nervous system. For the inhibition of mitosis in plant tissue chloroform requires an $A_{nar} = 0.1$ (21), as compared with 0.03 for general anestheisa. There is no reason to believe that sodium ion is involved in mitosis, and such a result can be interpreted as interference with the transfer of other substances across the membrane.

## XVIII. NON-NARCOTIC EFFECTS OF FOREIGN MOLECULES

So far consideration has been given to the occlusion of free space in the membrane occasioned by the introduction of chemical substances; the interesting question arises as to what the physiological effect might be if a given substance,

[5] Some confusion in the results of the treatment of erythrocytes with alcohols and urethans has arisen owing to a failure to distinguish between depolarization and narcosis (see 46, 47). It is not surprising that 1-butanol at an activity of 0.4 causes an increase in cation permeability.

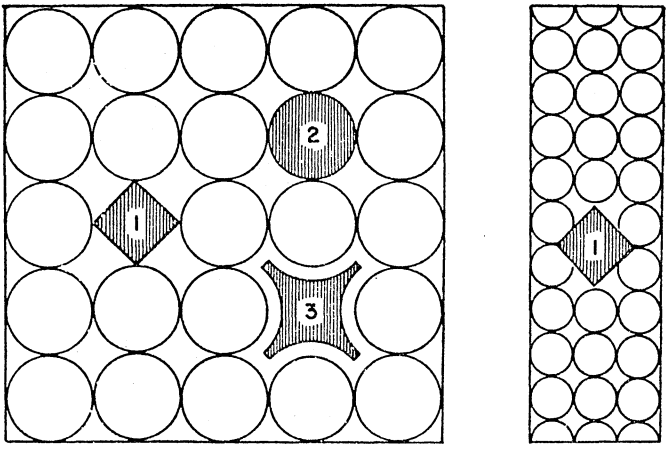



FIG. 12. Left: A membrane lattice into which has been introduced several foreign molecules which have, with respect to the membrane, (*1*) anticomplementary shape (convulsant), (*2*) the same shape (inert), (*3*) complementary shape (narcotic). These molecules have $V_m$ and $\delta$ comparable to those of the membrane. The diagram is intended to show an increase in the interstitial space created by the anticomplementary shape.

Right: Molecule of anticomplementary shape introduced into a lattice of molecules that are smaller in $V_m$ than the foreign molecule. The diagram is intended to show that there is little or no change in the interstitial space.

instead of occluding space, actually increased the amount of free space. Such an effect could come about in a variety of ways, but only one of these will be discussed. If one considers the diagram (see figure 12) for the membrane previously shown and gives the membrane dynamic characteristics such that its molecules are occasionally lost to the outside and replaced from within, it is possible for a very large foreign molecule (of dimensions comparable to those of the membrane molecules) to replace a membrane molecule. This is the suggestion of Ferguson (17) mentioned earlier. Providing the shapes of the membrane molecule and the new molecule are sufficiently different, more free space is created than existed previously. Thus one might expect that the action of large molecules, with the proper characteristics to stay fixed in the membrane (solubility parameters comparable to those of the membrane), in creating a membrane "leak" would be excitants rather than narcotics. There are a number of such molecules, e.g., cocaine, strychnine, and camphor, that are termed convulsants and might be considered to cause their effects by the mechanism mentioned above. Butler (8) has raised the question as to why $\gamma$-hexachlorocyclohexane is a convulsant and yet does not differ greatly in its solubility relationships from its $\delta$-stereoisomer, which is a depressant. The critical difference between these compounds, it is suggested, is their very different molecular shapes.[6]

[6] That $V_m$ is also of great importance is suggested by the data of Slater and Leary (51), who found that 2,2-diethyl-1,3-propanediol is a narcotic, while its higher homolog, 2,2-diethyl-1,4-butanediol, is a convulsant. This latter substance, minus one hydroxyl group,

A further observation with the convulsants is that they can be antagonized by anesthetics. These small molecules can readily fill in the extra volume created by the convulsant. On the other hand, if a molecule has the same $V_m$ and $\delta$ characteristics as the membrane and in addition the same shape, we can expect physiological inertness in this substance, while if the shape of the molecule is complementary to the membrane, narcosis could result. In the case of peripheral nerve, central convulsants either have no effect or act as blocking agents. It may be suggested that this action is due to the fact either that only part of the molecule can be accommodated in the membrane because a free volume large enough to accommodate such a molecule is unlikely to occur by chance, or that the membrane molecules, being smaller, can more easily fill in around the large molecule.[7]

## XIX. SOME REMARKS ON GENERAL ANESTHESIA

This subject has been reviewed most thoroughly and critically by Butler (8), especially with respect to the theory of metabolic inhibition as a cause of anesthesia. A few remarks will be made here concerning the reasons why certain chemical substances are practical anesthetics (in the sense of their acceptance in clinical practice) while others are not. If one considers first the gases, these are ethylene, nitrous oxide, and cyclopropane. Both ethylene and nitrous oxide suffer from the disadvantage that the vapor pressures of the pure liquid substances are too high; hence at atmospheric pressure the anesthesia that they produce is too weak. If one can assume unit activity coefficients in the membrane for these small molecules, the product $A_{nar}V_m$ for both substances is 0.7. Cyclopropane, on the other hand, is an appreciably larger molecule, and the liquid has a much lower vapor pressure. This makes possible a greater narcotic potency. At the same time, although a $\delta$ value for this substance is not available, it can be estimated as about 7.5 at room temperature, by extrapolation from values for other cycloparaffins. Such a value will give the molecule a reasonable selectivity of action if it is assumed that non-central nervous system structures have high $\delta$ values as compared with the central nervous system. One of the problems with the gases is that it is difficult to extrapolate $\delta$ values from the boiling point of the pure liquid with any accuracy. In general, however, the $\delta$ values of gases will be low, and it is suggested that this is the reason why gases are satisfactory general anesthetics. The question why cyclopropane, rather than

is an isomeric octyl alcohol, which would certainly be narcotic. The suggestion is strong that the extra hydroxyl radical permits an attachment of the molecule in the membrane, and that the distance from hydroxyl 1 to hydroxyl 4 is critical. The shape of both molecules is rectangular in the plane of the hydroxyl group, rather than spherical or cylindrical.

[7] Small molecules can, at least in theory, be convulsants in peripheral structures and narcotic in structures of the central nervous system because of the differences in structure of these membranes. The evidence from studies of the threshold of single nerve fibers (*Sepia*) after the application of narcotics (2) suggests that at low mole fractions the narcotic decreases before finally increasing the threshold. The mechanism for such a change may well be the creation of membrane "leaks" by a few molecules. This effect is not ordinarily observed in nerve, presumably because the decrease in threshold of some fibers is compensated for by the increase in threshold of others.

propane or propene, is a practical anesthetic may be answered by the fact that the much larger $V_m$ of propane (hence lower $\delta$) requires too high a partial pressure of this substance, and that propene, with about the same $V_m$ and vapor pressure as cyclopropane, has a chemical instability as a result of its double bond and is, in addition, rather difficult to purify. The liquid anesthetics, chloroform, ether, and ethyl chloride, have solubility parameters of 9.3, 7.4, and 8.5 and volumes of 81, 105, and 73 ml./mole. We might anticipate that the difficulty with chloroform would be that its high solubility parameter would permit too much of this narcotic to be partitioned to non-central nervous system structures at levels of $A_{nar}$ necessary for anesthesia. In general, all halogen substitution products of methane and ethane have $\delta$ values of from 8.5 to 9.5 and values of $V_m$ of 70–80 ml./mole. Perhaps the difficulties experienced clinically with such substances can be referred to the lack of what might be termed selective action on the central nervous system. Ether, on the other hand, has both a large $V_m$ and a low $\delta$ (= 7.4), so that if we assume membrane solubility parameters of 12 for tissues other than the central nervous system and 11 for the central nervous system, then the activity coefficients for chloroform for central nervous tissues = 1.8, and for peripheral tissues = 3.0. This is a selectivity ratio of 1.7. For ether, the central nervous tissue $\gamma$ = 6.4 and the peripheral tissue $\gamma$ = 50, or a selectivity ratio of 7.8.[8] The search for an "ideal" general anesthetic has been intensive, and it is by no means certain that considerable improvement cannot be made. The attractiveness of halogenated paraffins has been their lack of combustibility, but the high solubility parameters of these substances make them unlikely candidates for further development. Since the low solubility parameters of the fluorocarbons lead to physiological inactivity, perhaps some fluorochloroparaffin might have both the proper $\delta$ and $V_m$ and still be stable and non-inflammable.

## XX. CONCLUSIONS

Narcosis by chemically inert molecules appears to take place when a constant fraction of the total volume of some non-aqueous phase in the cell is occupied by narcotic molecules. If the narcotic behaves ideally in this non-aqueous phase, the thermodynamic activity of the narcotic multiplied by its molal volume is a constant, about 1 ml./mole. Higher values of thermodynamic activity for narcosis as one ascends an homologous series are, it is suggested, referable to an increase in the activity coefficient of the narcotic in the non-aqueous phase, corresponding to an increased difficulty of inserting larger and larger molecules into this phase.

A consideration of the data from a variety of studies of narcosis enables one to infer something about the structure of the non-aqueous phase to which narcotics are partitioned. This phase resembles a highly polar liquid. If the mole-

[8] Since the brain can hardly be considered homogeneous in its interaction with narcotics, it is possible to make an anesthetic too selective in its action by lowering the $\delta$ of the narcotic to the point where only the most sensitive cells are affected. Thus the excitatory effects of the higher hydrocarbons may correspond to a narcosis of only parts of the cerebral cortex. The "standard sequence of anesthetic depression" (25) cannot proceed beyond the first stages because the membrane acceptance of other regions of the brain is too low.

cules comprising this phase are considered to be reasonably large, then the liquid must be held together by forces of hydrogen bonding. In the case of the nervous system it is possible to show that while in peripheral nerve the phase has a highly polar structure, in the central nervous system it has a considerably less polar structure, and hence one that can accommodate less polar molecules more easily.

The possibility of creating lattice defects in the non-aqueous phase by inserting molecules equivalent in size and polarity to the non-aqueous phase but different in shape makes possible an explanation of the physiological action of convulsants. The two effects, narcosis and convulsant action, operate independently of each other in that convulsants will overcome the effects of narcotics and *vice versa*.

The experimental evidence presently available is inadequate for the support of any definitive mechanism for the action of narcotics in causing narcosis. In the case of excitable structures, however, it becomes less and less likely that a proposal of enzymatic inhibition can be supported by experimental data if by enzyme one means a protein structure made up solely of amino acids. Such an enzyme will have a structure totally unlike the phase to which narcotics can be shown to be partitioned. If the enzyme is modified by its being encased in a lipoidal envelope, it is conceivable that access of substrate to enzyme could be impeded by a narcotic. In this case the problem of whether narcosis is "physical" or "chemical" becomes one mainly of semantics.

The author is indebted to Dr. J. H. Hildebrand for several suggestions regarding the presentation of solubility theory and to Dr. F. Brink, Jr., for many suggestions relative to the presentation.

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MOLECULAR PHARMACOLOGY, 9, 131–143

# The Pressure Reversal of General Anesthesia and the Critical Volume Hypothesis

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SUMMARY

MILLER, K. W., PATON, W. D. M., SMITH, R. A., AND SMITH, E. B.: The pressure reversal of general anesthesia and the critical volume hypothesis. *Mol. Pharmacol.* **9**, 131–143 (1973).

The anesthetic potencies ($ED_{50}$) of four gaseous anesthetics and five liquid anesthetics were first determined in newts, using the abolition of righting reflex measured by the rolling response at 20°. The following results were obtained: $N_2O$, 0.69 atm; $N_2$, 21.5 atm; $SF_6$, 1.82 atm; $CF_4$, 11.0 atm; $CHCl_3$, 0.89 mM; butanol, 16.7 mM; pentobarbitone sodium, 0.85 mM; halothane, 0.39 mM; ether, 25 mM. The ability of elevated pressures to antagonize the effect of these anesthetics was then studied. For the liquid anesthetics, a graded response to pressure was observed and the reversibility of the antagonistic effect was demonstrated. Dose-response curves were obtained for the interaction of pressure with the gaseous anesthetics, and, from these, $ED_{50}$ values at various pressures have been interpolated. The data are used to compare the Meyer-Overton and the critical volume hypotheses; the latter not only is consistent with the data but also provides explanations for the antagonistic phenomenon and the lack of anesthetic effect for helium, neon, and hydrogen. The critical volume hypothesis is developed for three solvent model systems, from which estimates of the compressibility of the site of action are made.

## INTRODUCTION

In recent years attempts to infer the mode of action of general anesthetics from correlations of their relative potencies with their physical properties have led to the conclusion that a nonpolar site of action is more probable than an aqueous site (1–3). Whether such a site may be identified as being in the nonpolar region of membrane lipids, or as a hydrophobic region in either a protein or within a lipoprotein complex, is an open question, but the lack of structural specificity among anesthetics, recently re-emphasized by studies on the *d* and *l* forms of halothane (4), and the repeated and remarkable success of the classical Meyer-Overton olive oil correlation draw attention to the first of these areas.

The lipid solubility theories fall into two classes. The first, and earliest, of these has been formulated in modern form: "Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell. This concentration depends on the nature of the animal or cell but is independent of the

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narcotic" (5). The second class looks to the modification of the dimensions of cell membranes by anesthetics for a mechanism of anesthesia. The concept has been advanced in various forms by a number of authors, among whom Mullins has given the most detailed discussion (6). It may be stated in the form that anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical volume by the absorption of molecules of an inert substance. In this form we shall refer to it as the critical volume model.

These two hypotheses may be distinguished in principle by examining the correlation of relative potency with mole fraction or volume fraction solubility. In practice, while the two correlations lead to slightly different conclusions on the nature of the site of action, it has not proved possible to decide unequivocally between them, largely because the size range of anesthetic molecules is insufficient (1, 3). To date the best evidence for the critical volume model lies in the observation of the antagonism of general anesthesia by pressure (7), and it has been argued that this arises because the reduction in volume produced by an effective pressure balances the expansion which causes anesthesia. This conclusion is supported by calculations showing that anesthesia occurs when this expansion is of the order of 0.5%, while the pressures required to antagonize anesthesia are of a magnitude sufficient to oppose this degree of expansion (8). In the present paper the results of more detailed experiments on the pressure reversal of anesthesia are used to distinguish between the two versions of the lipid theory.

## METHODS

Experiments were carried out on Italian crested newts (*Triturus cristatus carnifex*) about 4½ inches long. Unless otherwise stated, all experiments were carried out at 20° ± 1°.

Abolition of the righting reflexes was used as an end point for anesthesia. To measure the anesthetic effect of the nongaseous anesthetics, concentrated standard solutions were prepared. These were diluted to the required concentration with oxygenated distilled water before being quickly transferred to sealed glass jars, each containing a single newt. After sufficient time had elapsed for equilibration the animals were tested for anesthesia by flipping them on their backs. Those that righted themselves within 10 sec scored 1 point; others scored zero. Each animal was tested at one dose five times and could achieve a score between zero and five out of five. The effect of the gaseous agents was measured by placing single animals in a small, cylindrical, stainless-steel pressure vessel containing soda lime and 1 atm of oxygen, adding the required pressure of anesthetic gas, and testing for anesthesia by rotating the chamber in a manner that has been previously described (8). These two methods of examining the righting reflexes have been shown to yield comparable results (9).

The scores achieved by all animals at each dose of anesthetic were summed and recorded as a percentage. The relation of logarithm of dose to the response was then analyzed by conventional probit techniques to yield the $ED_{50}$ and standard errors (3).

The ability of hydrostatic pressure to antagonize the anesthetics in aqueous solution was studied by completely filling the pressure vessel with the solution and raising the pressure in the absence of a gas phase. For the gaseous anesthetics the method was identical with that outlined above. Thus, after the anesthetic had been added, the pressure was raised with helium, which has previously been shown to be nonanesthetic (8). Pressures above cylinder pressure were achieved by means of an air-driven pressure booster pump. Responses were measured at each pressure, the pressure on each animal being raised in a stepwise manner. Compression was normally carried out over several minutes, and about 15 min were allowed before the response was measured. Unlike mice, these animals appeared insensitive to compression rate (8).

## RESULTS

The measurements of the effect of the gaseous anesthetics alone yielded the results presented in Table 1, together with

TABLE 1

*Effect of anaesthetics on righting reflexes of newts at 20°*

| Agent | Dose | No. of animals | Response | $ED_{50}$ [i.e., $P_{50}$ (atm)] ± SE |
|---|---|---|---|---|
| | *atm* | | % | |
| $N_2O$ | 0.476 | 11 | 73 | |
| | 0.612 | 11 | 65 | 0.687 ± 0.098 |
| | 0.749 | 11 | 40 | |
| $N_2$ | 20.41 | 10 | 50 | |
| | 23.82 | 15 | 43 | 21.5 ± 1.97 |
| | 27.22 | 10 | 26 | |
| | 34.02 | 10 | 6 | |
| $SF_6$ | 1.36 | 11 | 68 | |
| | 1.77 | 13 | 52 | |
| | 2.11 | 12 | 38 | 1.82 ± 0.243 |
| | 2.79 | 7 | 29 | |
| | 3.40 | 8 | 15 | |
| | 4.08 | 3 | 20 | |
| $CF_4$ | 8.58 | 9 | 76 | |
| | 9.53 | 9 | 58 | |
| | 11.15 | 11 | 44 | 11.0 ± 0.89 |
| | 12.51 | 9 | 49 | |
| | 14.55 | 9 | 22 | |
| $N_2O$ at 30° | 0.681 | 8 | 58 | |
| | 0.817 | 11 | 44 | 0.747 ± 0.079 |
| | 0.953 | 11 | 24 | |
| | 1.089 | 8 | 18 | |

the calculated $ED_{50}$ values and standard errors. For the liquid anesthetics the following $ED_{50}$ values, expressed as concentration in the bathing fluid, were obtained: $CHCl_3$, 0.89 ± 0.1 mM (SE); butanol, 16.7 ± 2.0 mM; pentobarbitone sodium, 0.85 ± 0.1 mM; halothane, 0.39 ± 0.05 mM; diethyl ether, 25 ± 2.5 mM.

Because of the long equilibration times (1–2 hr) required with the liquid anesthetics, and the difficulties of maintaining the oxygen level of the solutions in the pressure chambers, only qualitative results were sought for the interaction of pressure with these agents. Pressure reversal of anesthesia was successfully demonstrated in the presence of butanol (28 mM), ether (32 mM), halothane (1.15 mM), and sodium pentobarbitone (0.9 mM). Because of the absence of a gas phase, animals could be resuscitated after exposure to pressure (140–200 atm) without suffering from decompression sickness, and all such animals survived. The reversible nature of the effect could also be demonstrated by raising and lowering the pressure successively, the anesthetized animal being awakened by application of pressure and reanesthetized by release of pressure. In one experiment with sodium pentobarbitone a heavy overdose (1.61 mM) was administered to five newts to induce anesthesia rapidly. The rolling responses at 1, 68, 136, and 204 atm were then measured in rapid succession and found to be 4, 36, 56, and 60%, respectively, thus demonstrating the graded nature of the response to pressure even though equilibration was incomplete.

With the gaseous agents $N_2O$, $SF_6$, $CF_4$, and $N_2$, however, it was possible to obtain quantitative results for the pressure reversal of anesthesia. The results when the applied pressure was increased with helium are given in Table 2. The response vs. pressure curves obtained (see Fig. 1, for example) were analyzed by probit methods to yield the pressure at which the response was restored to 50% in each case (Table 3). [These curves were linear when the probit of the response was plotted with respect to pressure, but deviated significantly from linearity for log (pressure).]

This analysis assumes that the slope of the anesthetic dose-response relationship is not altered by pressure. The technical problems of adding small pressures of anesthetic in the presence of large pressures of helium, however, prevented a test of this assumption, although the remarkable constancy of the slope of the dose-response curve for anesthetics effective at pressures ranging from 0.7 to 22 atm (3) should be noted. In a previous publication (8) it was shown that with helium and neon above about 140 atm response falls off as a result of paralysis alone. With other gas mixtures paralysis was sometimes not observed until higher pressures (see Fig. 1). When paralysis was observed the data at that and higher pressures were excluded from the analysis. It was also found that with hydrogen no

TABLE 2

*Response of anesthetised newts at 20° to pressure*

The total pressure is composed of the stated pressure of anesthetic gas and the balance of helium.

| Anesthetic gas | Partial pressure | Response at total pressure $P_T$ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Anesthetic alone | 34 atm | 41 atm | 44 atm | 54 atm | 68 atm | 81 atm | 85 atm | 95 atm | 102 atm | 109 atm | 122 atm | 136 atm | 150 atm | 170 atm | 204 atm |
| | *atm* | % | % | % | % | % | % | % | % | % | % | % | % | % | | | |
| $N_2O$ | 1.09 | 10 (20)[a] | 35 (12) | | 46 (10) | | 94 (10) | | | | 100 (8) | | | 100 (8) | | | |
| | 1.36 | 0 (10) | 12 (10) | | | | 36 (10) | | 37 (6) | | 78 (10) | | | 88 (10) | | | 97 (6) |
| | 1.63 | 0 (12) | | | | | 12 (10) | | | | 52 (10) | | | 80 (10) | | 88 (10) | 92 (10) |
| | 1.36[b] | 0 (9) | | 33 (9) | | | 60 (9) | | | 84 (9) | | | 82 (9) | | | | |
| | 1.63[b] | 0 (9) | | | | | 49 (9) | | | 64 (9) | | | 73 (9) | | 71 (9) | | |
| $SF_6$ | 3.40 | 9 (11) | 29 (11) | | | | 55 (11) | | | | 64 (11) | | | 82 (9) | | | |
| | 4.83 | 0 (11) | | | | | 5 (8) | | | | 42 (10) | | | 70 (10) | | 84 (10) | 82 (10) |
| $CF_4$ | 23.4 | 0 (8) | | | | 17 (7) | | 30 (8) | | | | 58 (8) | | | 83 (8) | | |
| $N_2$ | 34.0 | 6 (10) | | | | | 47 (9) | | | | | | | 94 (10) | | | |
| | 68.0 | 2 (10) | | | | | | | | | | | | 20 (10) | | | 46 (10) |

[a] Numbers in parentheses represent number of animals.

[b] Experiments at 30°.

TABLE 3

*Relation of anesthetic dose in newts to pressure at which 50% response is obtained*

Experiments were performed at 20°, except where noted.

| Anesthetic gas | Pressure, $P_{50}$ | Total pressure giving 50% response, $P_T$ |
|---|---|---|
| | *atm* | *atm* |
| $N_2O$ | 0.68 | 0.68 |
| | 1.09 | 39.9 |
| | 1.36 | 85.1 |
| | 1.63 | 111.0 |
| | 0.75[a] | 0.75 |
| | 1.36[a] | 64.1 |
| | 1.63[a] | 84.0 |
| $SF_6$ | 1.82 | 1.82 |
| | 3.40 | 73.4 |
| | 4.83 | 124.9 |
| $CF_4$ | 11.0 | 11.0 |
| | 23.4 | 103.7 |
| $N_2$ | 21.5 | 21.5 |
| | 34.0 | 77.9 |
| | 68.0 | 208.4 |

[a] Experiments at 30°.

significant anesthesia was achieved at pressures up to 200 atm, and the onset of paralysis was delayed compared to helium and neon. Results for a group of nine animals were: 102 atm, 100%; 136 atm, 96%; 170 atm, 96%; 204 atm, 80% response.

*Comparison of Meyer-Overton and Critical Volume Models*

First we may test the two models using the potency data for newts, by comparing the correlations between relative potency and mole fraction or volume fraction solubility.

The critical volume model as formulated in the introduction to this paper assumes that anesthesia occurs at constant fractional volume expansion in some hydrophobic region. If we can treat this region as behaving as a bulk phase, we may write

$$E_a = \frac{\bar{V}_2 \cdot x_2 \cdot P_a}{V_m} \qquad (1)$$

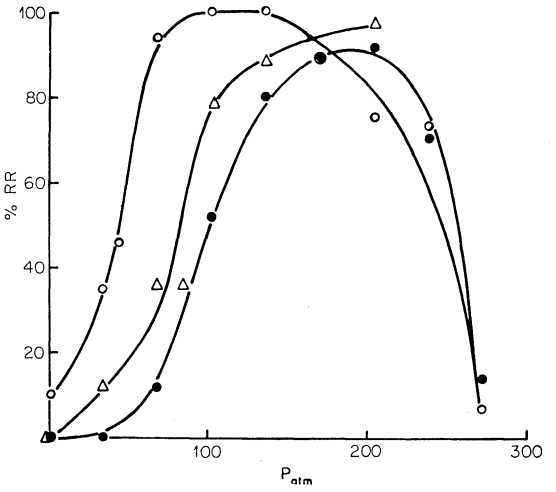


FIG. 1. *Rolling response (RR) of newts as a function of pressure after anesthetization with 1.09 atm (○), 1.36 atm (△), and 1.63 atm (●) of $N_2O$*

where $E_a$ is the fractional expansion, $\bar{V}_2$ is the partial molar volume of the anesthetic in the hydrophobic region, and $x_2$ is its corresponding mole fraction solubility. $V_m$ is the molar volume of the region and $P_a$ is the partial pressure of anesthetic. When $P_a = P_{50}$, the partial pressure required to anesthetize 50% of a group of animals ($ED_{50}$), the fractional expansion for all anesthetics should be constant. Thus a plot of log $P_{50}$ with respect to log $(\bar{V}_2 \cdot x_2)$ should yield a straight line of unit negative slope. The Meyer-Overton model, on the other hand, predicts a linear relation between log $P_{50}$ and log $x_2$.

These relations are examined for the data given above and in Table 1. Olive oil is used as a model of the site of action, for, although it is not a pure liquid, its composition is reasonably well controlled and its traditional use has furnished solubility data for a wide range of anesthetics. The solubility values used are from a recent compilation (10). Values for $\bar{V}_2$ are not readily available for such a wide class of solutes, but the volume of the anesthetic at the boiling point provides a reasonable approximation (6). Figure 2 demonstrates that the data are broadly consistent with either model.

The data on pressure antagonism provide the opportunity for a more rigorous test, as follows. The phenomenon of pressure rever-

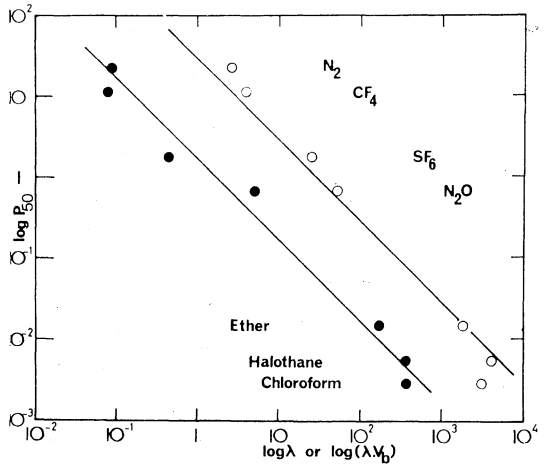


FIG. 2. *Correlation of relative potency of anesthetics for newts* (○) *with olive oil/$H_2O$ partition coefficient* (λ) *at 25°* (*Meyer-Overton hypothesis*) *and with expansion volume* (λ·$V_b$) (*Mullins hypothesis*) (●)

sal, on the Meyer-Overton model, would arise because anesthetics are believed to cause expansion at their site of action; a rise in pressure would therefore tend to force anesthetics out of solution at the site of action and change the partition between the active and other sites. The thermodynamic equation describing this effect is

$$\frac{\partial \ln (x/P_a)}{\partial P} = -\frac{\bar{V}_2}{RT} \qquad (2)$$

where $x$ is the mole fraction solubility per atmosphere partial pressure, $P_a$ is the partial pressure of the gas (or fugacity if gas imperfections are large), $\bar{V}_2$ is the partial molar volume of the gas, $R$ is the gas constant, and $T$ is the absolute temperature (11). If the $ED_{50}$ partial pressure is $P_{50}$ in the presence of 1 atm of oxygen and the solubility is $x_{50}$ at this pressure, while the $ED_{50}$ partial pressure is $P_a$ at a total ambient pressure $P_T$ when the solubility is $x_a$, the Meyer-Overton condition yields

$$P_{50} \cdot x_{50} = P_a \cdot x_a \qquad (3)$$

If we integrate Eq. 2 between $P_T$ and $P_{50}$ and eliminate $x_{50}/x_a$ between the two equations, we obtain

$$\frac{1}{\bar{V}_2} \cdot \ln \left(\frac{P_a}{P_{50}}\right) = \frac{1}{2RT} [P_T - P_{50}] \qquad (4)$$

Thus a plot of the left-hand site of Eq. 4 against $[P_T - P_{50}]$ should reduce the data for the four anesthetic gases in Table 3 to a single straight line. (Values of $\bar{V}_2$ for these four gases at the site of action have been approximated using the values for benzene.) Figure 3 clearly demonstrates that the Meyer-Overton prediction is imprecise. Thus the dependence of anesthetic concentration on pressure cannot be explained on the basis of the pressure dependence of solubility. Furthermore, it offers no explanation for the lack of anesthetic effect for helium, neon, and hydrogen.

The critical volume model offers a more direct explanation in terms of expansion and compression. Continuing the previous notation, the fractional expansion $E$ at a pressure $P$ of the anesthetic agent is given by

Fractional expansion

$$E = \frac{\bar{V}_2 x_2 P}{V_m} - \beta P = \left\{\frac{\bar{V}_2 x_2}{V_m} - \beta\right\} P \qquad (5)$$

where $\beta$ is the coefficient of isothermal compressibility. This is a more exact expression than Eq. 1, the first term representing expansion due to solution of gas and the second term the compression due to pressure on the fluid. For equal pharmacological

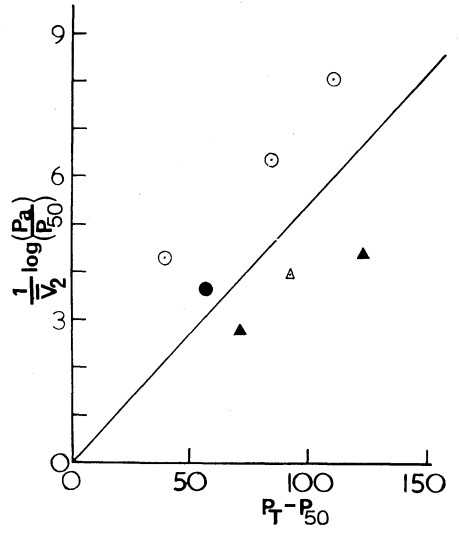


FIG. 3. *Test of Meyer-Overton model for pressure reversal*

⊙, $N_2O$; ●, $N_2$; △, $CF_4$; ▲, $SF_6$. See the text for experimental details.

effects the further expansion caused by the solution of more anesthetic when its pressure is raised from $P_{50}$ to $P_a$ may be equated to the compression caused by the antagonizing pressure over and above that of the anesthetic itself, i.e., $P_T - P_a$. The fractional compression of the system will be given by

$$\text{Fractional compression} = -\left\{\frac{\bar{V}_{He}\, x_{He}}{V_m} - \beta\right\}\{P_T - P_a\} \tag{6}$$

where $\bar{V}_{He}$ and $x_{He}$ are the partial molar volume and the mole fraction solubility of helium in the hydrophobic region. Therefore, at any combination of $P_a$ and $P_T$ that results in a 50% effect, we have

$$\left\{\frac{\bar{V}_2\, x_2}{V_m} - \beta\right\}\{P_a - P_{50}\} = -\left\{\frac{\bar{V}_{He}\, x_{He}}{V_m} - \beta\right\}\{P_T - P_a\} \tag{7}$$

for a given species and temperature. Dividing this by Eq. 5, we obtain

$$\frac{P_a}{P_{50}} = \left\{\frac{\beta}{E_{50}} - \frac{\bar{V}_{He}\, x_{He}}{E_{50}\, V_m}\right\} \cdot\{P_T - P_a\} + 1 \tag{8}$$

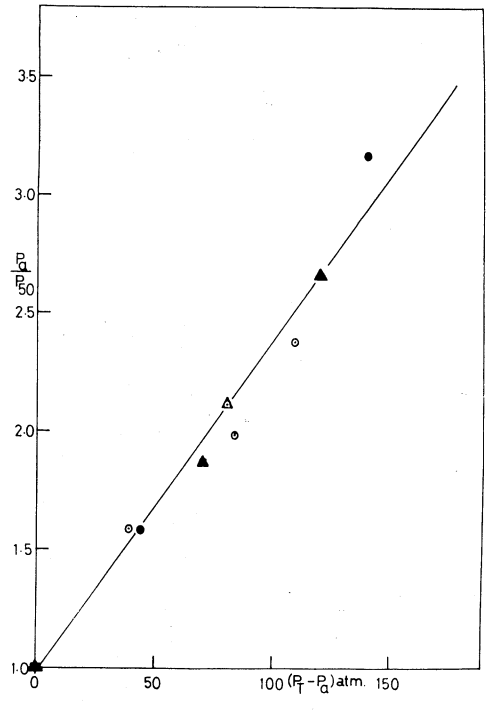


FIG. 4. *Test of critical volume model for pressure reversal*

⊙, $N_2O$; ●, $N_2$ ; △, $CF_4$ ; ▲, $SF_6$ . For experimental details, see the text.

Figure 4 shows a plot of $P_a/P_{50}$ against $P_T - P_a$ which demonstrates that the predictions of the critical volume model are consistent with the data. The slope contains the unknown physical parameters for the site of action $V_m$, $\beta$, $\bar{V}_2$, $x_2$, $\bar{V}_{He}$, and $x_{He}$ in addition to the measurable quantity $P_{50}$. The data for $N_2O$ at 30° also fit the correlation, suggesting that none of these unknown parameters is particularly sensitive to temperature.

*Calculations Based on Model Systems*

The above treatment neglects the effects of pressure on deviations from ideality of the gases (fugacity corrections) and, in the latter case, on the solubility of gases. While these effects are small they are by no means negligible, and the fit in Fig. 4 is better than might be expected from such an approximate treatment. A more complete treatment is desirable. To do this, however, some model system must be assumed, since the physical parameters for the actual site of action are not known. In what follows, the site is assumed to have the properties of olive oil, benzene, or carbon disulphide, and the effectiveness of the Meyer-Overton and critical volume approaches are compared in the light of the fuller analysis.

*Fugacity correction.* The behavior of all real, nonideal gases is given by

$$PV = RT + BP + CP^2 + \cdots$$

where $P$ is pressure, $V$ is volume, $T$ is absolute temperature, $R$ is the gas constant, and $B$ and $C$ are the second and third virial coefficients. Thus for an ideal gas $B = C = 0$. For our purposes $CP^2$ and higher terms may be neglected, and experimentally determined values of $B$ are obtained from a recent compilation (12) or, where not available, from the application of the principle of corresponding states (13). On the basis of this principle $B/V_c$ is considered as a universal function of $T/T_c$, where $V_c$ and

$T_c$ are the critical volume and critical temperature (14). The ratio of the fugacity, $P^*$, to the experimental pressure, $P$, is given by

$$RT \ln\left(\frac{P^*}{P}\right) = \int_0^P B\, dP \qquad (9)$$

In the case of a gas mixture the fugacity correction for component 1 at mole fraction $x_1$ is

$$RT \ln \frac{P^*}{P_1} = \int_0^{P_1} [x_1 B_{11} + x_2 B_{12}]\, dP \qquad (10)$$

where $B_{11}$ is the second virial coefficient for pure component 1 and $B_{12}$ is a term that arises from the interaction of the two components of the mixture.

Values for $B_{12}$ are often not available in the literature but may be estimated from the virial coefficients of the pure gases that comprise the mixture (13, 15).

The values of $B_{11}$, $B_{22}$, and $B_{12}$ used in making the corrections are summarized in Table 4. Comparison with experimental data, where available, indicates the errors involved in estimating $B_{12}$. The magnitude of the fugacity corrections for mixtures below 150 atm are small and do not exceed 10%.

*Variation of solubility with pressure.* The dependence of solubility on pressure has been given in Eq. 2. For exact calculations $P_a$ must be replaced by the appropriate fugacity. $\bar{V}_2$, values for which are given in Table 5, is virtually independent of pressure in the pressure range used in this work (11).

TABLE 4

*Second virial coefficients for fugacity corrections*

| Mixture components | | Pure component second virial coefficient (12) | | Estimated $B_{12}$ | Experimental $B_{12}$ | Reference |
|---|---|---|---|---|---|---|
| 1 | 2 | $B_{11}$ | $B_{22}$ | | | |
| He | $N_2O$ | 11.7 | −133 | 29 | | |
| He | $N_2$ | 11.7 | −4.5 | 27 | 21 | 16 |
| He | $CF_4$ | 11.7 | −88 | 34 | 26 | 15 |
| He | $SF_6$ | 11.7 | −280 | 38 | | |

TABLE 5

*Molar and partial molar volumes*

| Gas | Molar volume at boiling point (17) | $\overline{V}_2$ in benzene | Reference |
|---|---|---|---|
| | *ml* | *ml* | |
| He | 32 | 36[a] | 18 |
| Ne | 17 | 33[a] | 19 |
| $N_2$ | 35 | 53 | 20 |
| $N_2O$ | 36 | 47 | 20 |
| $CF_4$ | 54 | 82 | 21 |
| $SF_6$ | 76 | 97 | 21 |

[a] Estimated.

*Application of correction factors.* The expansion caused when a gas dissolves in a solvent is ideally proportional to $(\bar{V}_2 \cdot x_2 \cdot P_2)$ (Eq. 1). The deviations from this can be calculated as a correction term by using Eq. 2 with fugacity instead of partial pressure. There are two contributions to expansion in our case, one due to the narcotic gas, the other due to helium. The corrected expansions have been calculated for each gas independently of the other. The correction factor is such as always to reduce the expansion (or concentration, $x_2P_2$) below the ideal value. The size of the correction term increases with increasing size of the gas and with increasing pressure. At 100 atm it ranges from about 1.2 for nitrogen to 1.6 for sulfur hexafluoride. Using these corrections, the total solubility and expansion can be calculated for our data. The calculations have been carried out using three liquids as models of the site of action: olive oil, benzene, and carbon disulfide. We have recently shown that benzene and carbon disulfide are the best simple solvent models for the site of action of anesthetics in mice (3). Sources of solubility data were given in that paper. Partial molar volumes were taken to be those for benzene in all three solvents. Our conclusions are in fact independent of which of these solvents is considered. Much more data would be required to differentiate unequivocally between them, and when one considers the relatively crude nature of such a model for the active site this is not likely to be a rewarding pursuit.

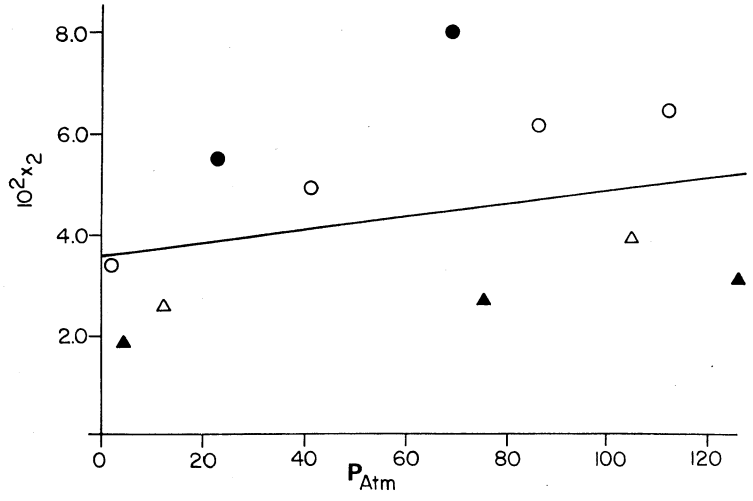


FIG. 5. *Mole fraction concentration ($x_2$) in olive oil of narcotic gas at $ED_{50}$ values at various pressures*
Correlation coefficient $r = 0.32$. ○, $N_2O$; ●, $N_2$; △, $CF_4$; ▲, $SF_6$.

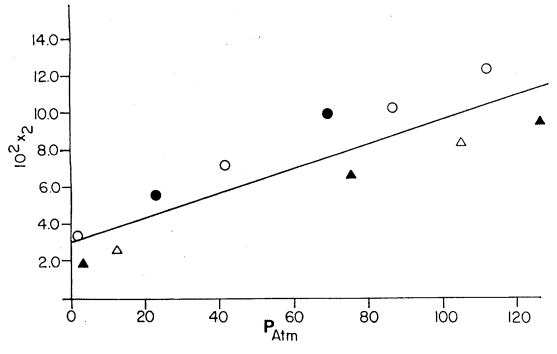


FIG. 6. *Mole fraction concentration ($x_2$) in olive oil of narcotic gas plus helium at $ED_{50}$ values at various pressures*
Correlation coefficient $r = 0.88$. ○, $N_2O$; ●, $N_2$; △, $CF_4$; ▲, $SF_6$.

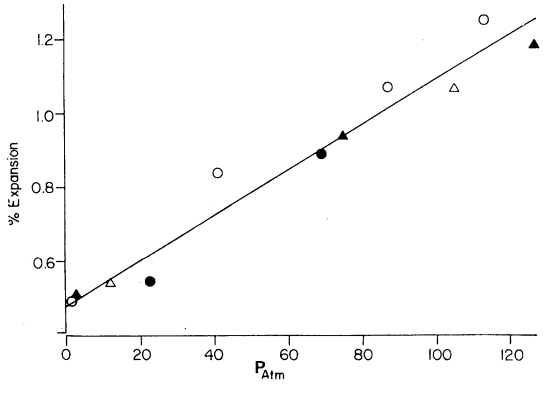


FIG. 7. *Percentage expansion of benzene caused by helium and narcotic gas at $ED_{50}$ values at various pressures*
Correlation coefficient $r = 0.98$. ○, $N_2O$; ●, $N_2$; △, $CF_4$; ▲, $SF_6$.

*Results of analysis.* First, on the classical Meyer-Overton hypothesis, the concentration of narcotic gases in the model solvent (for $ED_{50}$ values measured at various pressures) should be constant and independent of pressure. Figure 5 shows that this assumption produces a poor correlation, although the concentrations are not grossly pressure-dependent. If we assume that the helium is also contributing a subliminal anesthetic dose, the total inert gas concentration should be considered (Fig. 6). The data are now more strongly correlated, but the anesthetic concentration at the $ED_{50}$ has become pressure-dependent, and the model is incapable of offering an explanation for this without further ad hoc assumptions.

The critical volume model was tested by correlating the expansion due to the dissolution of gas with pressure. This should lead to a linear relation between expansion and pressure, where the slope of the line yields the compressibility of the site of action. The expansion caused by helium is included. Figure 7 illustrates the situation when benzene is chosen as the model solvent. This model is highly successful and yields an estimate of the compressibility of $6 \times 10^{-5}$ $atm^{-1}$. (The experimental value for benzene is $9 \times 10^{-5}$ at 25°.) Furthermore,

the model is consistent with helium and neon not being anesthetics. The expansion they cause when dissolving is smaller than the compression due to the effect of the pressure per se, so that the net effect is compression and the critical volume for anesthesia is never attained. With hydrogen, on the other hand, the two effects nearly balance and there is a very small net expansion. In newts this is insufficient for the critical volume to be achieved below pressures where other effects become important (approximately 140 atm). In mice, on the other hand, it appears that the critical volume may be achieved at 130 atm (22). If one accepts this figure, then either the hydrogen solubility is sufficiently enhanced at the site of action on going from 20° in newts to 37° in mice or the compressibility of the site in mice is less than that in newts. Calculations for mice, cited in Table 6 suggest that the former is the case.

The conclusions for the critical volume model are not strongly dependent on the choice of solvent. All three solvents give very good correlations, though the predicted degree of expansion at 1 atm (intercept) and the compressibility (slope) of the site of action vary slightly (Table 6). Such small variations between the models of the anesthetic site of action do not, however, seem significant. Indeed, what is striking is how successful the critical volume model is at providing a self-consistent explanation of the pressure dependence of the anesthetic potencies of inert gases.

TABLE 6

*Comparison of model solvents*

| Solvent | Expansion at $ED_{50}$ dose at 1 atm | $\beta$ values | Experimental $\beta$ values for pure solvent (23, 24) | Correlation coefficient, $r$ |
|---|---|---|---|---|
| | % | $10^{-5}$ $atm^{-1}$ | | |
| Newts | | | | |
| Olive oil | 0.20 | 3 | 6 | 0.96 |
| Benzene | 0.48 | 6 | 9 | 0.98 |
| Carbon disulfide | 0.29 | 4 | 7 | 0.94 |
| Mice (25) | | | | |
| Carbon disulfide | 0.58 | 3 | 7 | 0.99 |

Results of similar calculations using carbon disulfide as model solvent for the data recently obtained (25) for mice in nitrous oxide–helium mixtures are also shown in Table 6. [Using the degree of deviation of fluorinated anesthetics from relative potency data as the criterion for the correlation, the best fit was found to be with carbon disulfide for mice (3).] The higher expansion for an $ED_{50}$ dose at 1 atm reflects the higher value for the $ED_{50}$ (1.5 atm for mice, 0.7 atm for newts).

## DISCUSSION

The success of a nonpolar fluid, such as benzene, in serving as an analogue of the site of action of anesthetics suggests that this site too must be both nonpolar and fluid. There are three possible sites that may fulfill these criteria: (a) nonpolar sites within proteins, (b) the cell lipids, or (c) associations of lipid and protein.

Nonpolar (or hydrophobic) regions within proteins are common, but the need for "fluidity" suggests that only sites associated with a flexible or labile structure would be compatible with the critical volume model. Thus, whereas xenon and cyclopropane can occupy nonpolar sites in myoglobin, the rigidity of the protein prevents other anesthetics from interacting at this site (26). Although the inhibition of luminescence in luminous bacteria by anesthetics may be antagonized by pressure (27, 28), it is not yet clear whether this arises from direct interaction with the luciferase or not. There is thus at present no unequivocal evidence for the direct action of anesthetics at clinical doses on protein structure in a manner consistent with the relative potency data and the critical volume model. Although such interactions cannot be entirely ruled out, a relatively good case may, on the other hand, be made for interaction with membrane lipids.

Recent spectroscopic studies (29, 30) have revealed that the ends of the hydrocarbon chains remote from the polar head groups of lipids in bilayer membranes are in a highly fluid state, and it would not be too surprising if three-dimensional nonpolar fluids provide a good analogue of the be-

havior of such a region toward small, relatively nonpolar solutes such as general anesthetics. Such spectroscopic studies also show that the action of anesthetics, for example benzyl and other alcohols, is to increase the membrane "fluidity" (19, 31). Although these substances are hardly typical general anesthetics, equivalent effects have been demonstrated for clinical and inert gas anesthetics in the membranes of phospholipid vesicles (liposomes) by studying ion permeability (32). Again, monolayers of lipoprotein (33) and of phospholipid (34) are expanded by anesthetic but not by helium. The effects of anesthetics on liposome ion permeability are reversed by pressures comparable to those required in animals, and it has been suggested that the expansion, and consequent increase in freedom of molecular motion, within a lipid bilayer is involved in both processes (35). Recently reported work in which the effects of general anesthetics and of pressure on membrane "fluidity" were studied is consistent with this prediction (36).

Whether in a bilayer membrane the expansion would be isotropic (as in benzene) or anisotropic is not certain. Available evidence suggests the latter. Thus studies in which actual membrane concentrations of alcohols were determined revealed the anomalous result that only about one-third of the observed increase in area could be attributed to the dissolution of the drug molecules (37). We have calculated that this unaccountable increase in membrane area would result if the membrane decreased its thickness by about 10 nm while increasing its area at constant volume. [General anesthesia occurs at lower doses than these, and the effect would be smaller (38).] Such an effect seems quite plausible, for, as the polar head group area of the lipids increases, so does the "fluidity" of the hydrocarbon tails, which now move through a wider arc exhibiting even less anisotropy, as is observed spectroscopically (19, 31). At sufficiently high concentrations this effect would lead to lysis. A similar effect of decreasing thickness and increasing head group area has been discussed by Fettiplace, Andrews, and Haydon (39) with respect to nonpolar interactions between bilayers and proteins. With long-chain hydrocarbons, on the other hand, thickening at constant head group area may be occurring (39).

The mechanism by which this increase in "fluidity" and/or volume in the lipid leads to anesthesia is not clear. Is the action on the lipids themselves important, or is the disturbance in the lipid transmitted to some membrane protein? The small effects that anesthetic doses have on pure phospholipid membranes (e.g., $K^+$ permeability in liposomes increased by about 20% at clinical doses) and the variety of effects that anesthetics may produce in cells (40) seem to favor the latter hypothesis. Such a second-order involvement of lipids has been discussed in a recent review (41) and speculations on the role of anesthetics in a hypothetical lipid-protein interacting system for nerve propagation have been published (42). A suggestive experimental study employing spin-labeled fatty acids in sarcoplasmic vesicles demonstrated that the activity of calcium-dependent ATPase was directly related to membrane "fluidity" and that the enzymatic activity of a lipid-deficient membrane could be restored by the addition of oleic acid (43). If such effects can be shown to be more general, then the dependence of membrane protein function on membrane fluidity could provide a mechanism of action for anesthetics. It should be noted, however, that the latter experiments involved much greater changes in fluidity than would be produced by anesthetic doses alone.

If the interpretation of the pressure reversal of anesthesia in terms of expansion and compression of a membrane (and consequent changes in fluidity) is correct, several interesting corollaries may be noted. By addition of anesthetics and pressure it is possible to vary membrane fluidity and volume at constant temperature, and in this way anesthetics may be used as simple probes of the behavior of membranes. A knowledge of the dependence of the effective dose on pressure may provide an insight into the mechanism by which drugs act. The convulsions and hyperexcitability observed in primates at 60–80 atm in $O_2$-He breathing mixtures occur when membrane compression is of the order of 0.25%, and

it is interesting to note that the presence of anesthetic gases elevates the pressure threshold for convulsions (44).

## CONCLUSION

The critical volume hypothesis provides a much more self-consistent explanation of the relative potency of anesthetics and of the pressure reversal of anesthesia than does the classical Meyer-Overton lipid solubility hypothesis. The degree of self-consistency and the prediction of a realistic compressibility coefficient are most satisfactory. No other current hypothesis of the mode of action of anesthetics [e.g., the microtubule model (45)] is as successful. The critical volume model makes predictions that are accessible to direct experimental test; for example, the dimensions and "fluidity" of cell membranes under anesthetic and under pressure may be determined. The critical volume model, unlike the lipid solubility hypothesis, provides an indication of the mechanism of action of anesthetics.

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