THEORIES OF GENERAL ANESTHESIA

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Speculations concerning the nature of general anesthesia have appeared in large number over a period that now exceeds a full century without a satisfactory solution having yet been reached. In view of the long history and inconclusive status of the subject, there is scarcely anything in the physical or biological sciences that can be considered irrelevant. To attempt a review in the face of these circumstances would be rash were there any need to apologize for arbitrary selection of subject matter or for lack of competence in all the fields where it is called for. Comprehensive presentations of the historical development of the subject being already available, notably in the monograph of Winterstein (151) and the review of Henderson (68), it is not proposed to undertake here any systematic discussion of the older theories. Rather, the plan will be to review some of the more recent work that appears pertinent to the problem, consideration of the older theories being limited to those instances where the appearance of new experimental material permits a better evaluation or where an appraisal from a new point of view seems for any reason desirable. Only two aspects of the problem—not altogether unrelated aspects—will be examined in the present review: (a) the chemical and physical nature of the drugs that produce anesthesia, and (b) the mechanism through which alteration of cellular function is brought about by anesthetic drugs. No attempt has been made to treat even the recent litera-

ture comprehensively. Some of the theoretical ideas that have attracted the most attention in the past two decades have been intentionally disregarded. No doubt much of importance has been omitted through inadvertence. In this field it is not always the work that has been directed specifically toward this end that proves to have the most significant bearing on the problem. Systematic search of the literature is not feasible and bibliographic indices are of limited value. It is not the purpose of this review to furnish a complete catalog of the literature even in those aspects of the problem that are dealt with at some length.

THE SCOPE OF THEORIES OF ANESTHESIA

Before directing attention to any one specific theory, it is well to consider the more general question of the range of applicability of any theory. What range of biological effects are conceived as having a common mechanism? What chemical classes of drugs are conceived as acting through a common mechanism? General anesthesia, the subject of this review, is a clinical term, and it will be kept in mind that in the present connection it is a clinical phenomenon we are seeking to explain. However, the familiar anesthetic drugs also produce numerous effects other than the abolition of sensation in man. They depress reversibly various functions of many types of cells and organisms. How many of these phenomena are attributable to the same fundamental process? How many can profitably be studied with the view of elucidating the clinical phenomenon of general anesthesia? These questions present serious difficulties. There is no unanimity of opinion, and theorists have sometimes been inclined to evade any explicit answers.

Bernard (10) was the first to insist that all agents that depress the nerve cell (he included asphyxia and heat) do so by producing the same modification in the cell. He proceeded to the far-reaching generalization that the anesthetics depress every manifestation of life whatsoever. All these phenomena of depression he thought of as resulting from a single mechanism of action. Bernard's concept has had a profound influence on subsequent thought and has been incorporated without question into the structures of a number of theories. The viewpoint has been most ardently defended by Winterstein (151). Theorists who have adopted this premise have not hesitated to seek experimental support for their theories from the most diverse biological phenomena and even from studies of non-living systems.

Not all theorists have been willing to accept Bernard's simple view of a single, universal mechanism of narcosis. However, they have not found it easy to formulate the scope of their theories. The difficulties may be illustrated by an examination of H. H. Meyer's (113) thoughtful attempt to define the limitations of his lipide theory. Meyer limited his theory to the reversible inhibition of "exotropic irritability" of protoplasm. He did not believe that such inhibitions must all be attributable to the same mechanism. Different drugs might have different primary points of attack and might bring about the end result of depression through a variety of means. The lipide theory was to be applicable only to the "indifferent narcotics" or the "alcohol group," an admittedly ill-defined group of indifferent

fat-soluble organic compounds of which ethanol and chloroform were to be considered typical members. The farther removed a drug is from this group, the more its mechanism of action might be expected to deviate from that described in the theory. Two types of narcosis were specifically excluded from consideration under the theory: magnesium narcosis and asphyxial narcosis. The weakness of this formulation is not that it fails to claim universal applicability, for perhaps no theory should do so. Rather it is that it fails adequately to predict in what cases it is applicable. No criteria, either chemical or physiological, are stated by which it would be possible to decide whether a drug is to be thought of as acting through the mechanism described by the theory or through some other mechanism.

The concept of one all-inclusive phenomenon of "narcosis" does not rest on a very satisfactory logical basis. It must be remembered that Bernard's interpretation was derived from the most meager experimental evidence and that it was prompted by a philosophical desire for analogy and generalization. Many workers of a later era, likewise impatient to find unifying principles of general physiology, have been led into a disregard of the logical steps needed to establish the relationship of various phenomena. The position to be adopted here is that when the same drug produces two different effects or when two different drugs produce the same apparent effect, it is not justifiable without some other evidence to assume that the same mechanism is involved.

It is not easy to define the type of evidence that should be viewed as being necessary or sufficient. When a drug, such as ether, that anesthetizes man will, in concentrations of the same order of magnitude, also produce closely similar objective neurological effects in other mammalian species, it is almost beyond cavil that we are dealing with the same phenomenon in the different species and that experiments in the other species can advance the knowledge of human anesthesia. But when ether in a higher concentration inhibits the division of a sea urchin egg, it is by no means so obvious how this effect is related to the effect on the central nervous system of man. Although it may not be possible to establish unequivocally whether the effects of an anesthetic drug on the mammalian central nervous system and on some simpler system are produced through the same fundamental mechanism, it is feasible to obtain evidence that might strengthen the belief. If the effect on the simpler system were produced by all anesthetic drugs and only by anesthetic drugs and if there were a definite correlation between the effective concentrations of the agents for the two effects, this would be sufficient to suggest that the effects have a common basis. For reasons that will be discussed later, a study confined to a series of closely related chemical compounds, particularly a homologous series, would not be so convincing as would a study of compounds of diverse structures. Many of the "narcotic" drugs that have been included in studies on simple systems, or even on tadpoles, do not produce typical anesthetic effects in mammals. Bromal hydrate has even been included among the "narcotics" in some studies. This is a drug that is chemically so reactive under physiological conditions (25) that it cannot be conceived to have the same fundamental type of action as a drug such as ether on any living

cell. It is not contended that there is any effect produced by an anesthetic drug that can be considered irrelevant to the problem of anesthesia, whether it be on a simple form of life or even on a non-living system. The intention is to point out the unsatisfactory state of knowledge of the relationship between various effects of the anesthetics and to emphasize the need of caution in drawing far-reaching conclusions.

"Narcosis" is a term frequently used to designate the general depressant phenomena produced by drugs. There have appeared in the literature (68, 69) statements implying erroneously that in this particular technical sense the term was introduced by Bernard, who made a careful distinction between "narcosis," the general phenomenon, and "anesthesia," the special phenomenon. Actually Bernard used *anesthésie* in the general sense and *narcotique* in reference to the soporific properties of some of the opium alkaloids. "Narcosis" in English and cognate words in other modern languages have been employed in such a confusing variety of senses, both popular and technical, as to call for some discussion of usage. The word ναρκωτικός was used by Galen (59) to denominate a group of drugs, among which he listed opium. Opium being the drug having the most pronounced effects in this group, it was natural that "narcotic" properties should have come to be thought of as the properties of opium. Thus in the English language from the earliest appearance of the word until the present time, "narcotic" has usually been associated with opium-like properties. The effects of opium not being simple, it is understandable that the connotation of "narcotic" has not been clearly determined. For instance, to Chaucer (29) it was stupefaction; to the Congress of the United States (33), addiction. Vagueness and multiplicity of meanings are so firmly established in the usage of "narcosis" and "narcotic" in English as to render futile any insistence on rigid definitions, even for technical purposes. In the modern literature of general physiology, a "theory of narcosis" is usually a theory that seeks to explain a wide range of biological phenomena on a common basis. "Anesthesia" is then thought of as a special instance of the general phenomenon. Without any theoretical implications, the term "narcosis" is also sometimes applied to some specific depressant phenomenon where "anesthesia" would be objectionable on etymological grounds.

THE NATURE OF ANESTHETIC DRUGS

Since it has already been emphasized that the phenomenon forming the subject of this review is a clinical one, it should perhaps be pointed out that there is no intention of restricting the consideration of drugs to those in present-day clinical use as surgical anesthetics. Few drugs have the peculiar characteristics that make them relatively safe and convenient for practical clinical anesthesia. Yet there are many other drugs that are also capable of producing anesthesia. They may find application as hypnotics or be unsuitable for any therapeutic use, but there is no fundamental pharmacological basis for separating them from those agents actually used as anesthetics. It is true that there are some differences in the neurological effects produced by different anesthetics. Perhaps no two drugs give rise to precisely identical patterns of neurological derangement. At the present time, however, it is not clear to what extent these differences are indicative of fundamentally different mechanisms of action. This question therefore will not be emphasized here.

The very large number of chemical compounds capable of producing anesthesia, of itself one of the most striking characteristics of the pharmacological group, affords an excellent opportunity of learning what properties of a com-

pound are necessary and sufficient for anesthetic activity. Much thought has been directed to this type of study, and a number of theories or rules have been proposed in the attempt to furnish a basis for predicting the presence or the intensity of anesthetic activity. A theory of this type is not necessarily a theory of the actual mechanism of anesthetic action. However, it is evident that a knowledge of the nature of compounds producing a pharmacological effect may throw some light on the nature of the alteration in cellular function brought about by those compounds. A theory of drugs is a less ambitious sort of theory than a theory of mechanism and more susceptible to experimental confirmation or refutation. The continued study of drugs themselves still affords a profitable approach to the understanding of anesthetic action. Furthermore it is of utilitarian value in the search for new anesthetic and hypnotic agents.

(*a*) *Chemical properties of anesthetics*

When the number of substances known to have anesthetic properties was still small, there were numerous speculations as to the role played by the separate structural units of a molecule in its anesthetic action. It was even suggested that the disruption of a molecule *in vivo* would liberate fragments that were the effective agents. Hypotheses about the specific effects of certain groups actually led to the synthesis of some active drugs. Urethane and barbital, notably, were correctly predicted to be anesthetic because they combined favored chemical groupings, and only on that account were they tested as anesthetics. However, with the continuing growth of the list of anesthetics, it became increasingly clear that there is no specific structure necessary for anesthetic activity. Although terms such as "hypnophore group" have found their way into the literature even into the twentieth century, lack of structural specificity can now be considered the outstanding characteristic of the anesthetics.

Neurological effects that have been termed "narcotic" are even produced by the inert gases, argon (8), krypton (100) and xenon (100, 101),—argon at elevated pressures and krypton and xenon at atmospheric pressure. Similar effects are produced by nitrogen at high pressures (6, 8, 28, 115). The observations on these gases, however, are scanty and in some cases conflicting and it is not altogether clear from the published records that typical anesthetic effects can be produced. If the inert gases can be considered true anesthetics, the action of these spherically symmetrical atoms without any permanent dipoles furnishes the most conclusive demonstration that anesthesia need not depend on the effect of any specific structural grouping.

There is nothing that can be called in any sense a general or comprehensive theory relating structure and activity. Any prediction of the activity of a new compound from its structure will be based either on analogy from closely related compounds or on predictions of its physical properties. Yet conjectures as to chemical structures that will result in anesthetic activity are not limited to historical curiosities. Even now, of course, any synthesis having as its objective the creation of an anesthetic drug must be inspired by some sort of tentative hypothesis relating structure and activity. Such hypotheses formulated principally to

suggest a synthesis are usually not publicly announced and need not be based on any large amount of data or have any defensible logical structure. When a very large number of closely related compounds have been studied, as is the case with the barbituric acids, it becomes possible to make generalizations that allow predictions of some degree of reliability. But even in a field as extensively explored as the barbituric acids, there can be no complete confidence in a prediction. A compound closely related structurally to the familiar anesthetic members of the group may prove to be a convulsant. Moreover, some of the generalizations that have been published in regard to the barbituric acids were not justified by the data on which they were based, and with the study of larger numbers of compounds have been found not to be applicable. Generalizations have often had the unfortunate effect of discouraging explorations that might have been profitable.

Höber (73) would exclude barbituric acids and alkaloids from the category of "narcotics" on the basis that they interact with protoplasm as weak acids or bases, whereas narcotics do not enter into a chemical reaction with cell components. Although it is quite conceivable that barbituric acids may have a different mechanism of action from some of the non-ionizable anesthetics, it is not apparent why the property of partial ionization should of itself necessarily indicate a different mechanism of action. Those barbituric acids that are anesthetic are only ionized to a moderate degree at physiological hydrogen ion concentrations and it would seem likely that it is the non-ionized molecule that is responsible for anesthetic action. In the inhibition of division of sea urchin eggs by barbituric acids, Clowes, Keltch and Krahl (30) have presented evidence suggesting that the undissociated molecule is the effective form. Furthermore, some of the 1,3,5,5-tetraalkyl barbituric acids, which are incapable of losing a proton, are anesthetic and there is no evidence to indicate that they act through a different mechanism from closely related compounds that are capable of ionization.

Although it is not possible to find any specific structural feature necessary for anesthetic activity, it is perhaps possible to indicate some structural features incompatible with anesthetic activity. Active anesthetics have not been found among those organic compounds that are ionized to a very large extent at physiological hydrogen ion concentrations, and the presence of a group conferring a high degree of ionization can be taken as an indication of inactivity. Also very few compounds having high solubility in water are anesthetic. If in an active anesthetic a group is introduced that, by formation of hydrogen bonds with water, increases solubility, the anesthetic activity will be reduced or abolished. Metabolic reactions leading to a strongly ionized or highly soluble product are important in terminating the pharmacological action of a number of anesthetics (*e.g.*, hydrolysis of esters, conjugation of alcohols with glucuronic acid, production of carboxyl, hydroxyl or keto groups in some barbituric acids). From structure alone predictions of lack of anesthetic activity can often be made with considerable confidence, with much more confidence that can any prediction of the presence of anesthetic activity.

(b) *Physical properties of anesthetics*

The most notable features of the anesthetic group are the chemical diversity of compounds producing qualitatively similar effects and the great differences among these compounds with respect to their effective doses (a range of the order of a thousandfold). In the absence of any evidence of a chemical structure essential for anesthetic activity, attention has long been directed toward the discovery of physical properties that are common to the anesthetics and that could account for quantitative differences in activity. The physical properties of anesthetics will be discussed here from two points of view: first, with regard to the validity of empirical rules relating properties to activity; and second, with regard to the theoretical implications of these rules.

(i) *Empirical rules*

Since we are concerned here not only with the presence or absence of anesthetic activity but with quantitative differences in activity, some comment on the difficulties of quantitative comparison is in order. These difficulties are particularly serious in the estimation of activity in mammals, the activity of most interest in the present review. Concentrations of the drugs in the brain would probably give the most valid basis for comparison, but these data are available for very few anesthetics. The best index of activity usually obtainable is the total amount of drug in the body required to produce anesthesia. This quantity is known if the anesthetic is given intravenously, or in some cases it can be arrived at by chemical analysis. If the drug is given by a route where absorption is incomplete or is slow relative to the rate of inactivation, the activity may be seriously underestimated. Even when the drug is introduced by intravenous injection or by inhalation, the total dose may be misleading as an index of inherent activity. If the drug enters the brain more rapidly than other bulkier tissues, as is apparently the case with some anesthetics, this initially uneven distribution will lead to an exaggerated estimate of activity. The aforementioned difficulties are inherent even in comparisons performed in the most carefully controlled manner with the most uniform technic. But in order to arrive at a judgment of a theory, it is desirable to bring into consideration as large a number of drugs as possible, and this necessitates the use of reports from many sources. Different experimental conditions, different technics, different species of animals, and frequently faulty statistical presentation of doses introduce more serious obstacles to legitimate comparison. Unsatisfactory as such material may be, it is not altogether lacking in usefulness. Since anesthetics in general do not differ greatly in their effects in different species of mammals, comparisons even between experiments on different species are not entirely inadmissible. Comparative data based on surveys of the literature are obviously of limited quantitative significance and are to be accepted with reservations. They may nevertheless be found of some value in testing a theoretical concept.

In attempting to find correlations between anesthetic activity and physical properties, it is essential to examine large numbers of compounds representative of a broad range of chemical structures. Limitation to a group of closely related compounds, especially to a homologous series, can result in a very misleading

study. This is a principle that has been stressed by Meyer and Hemmi (114). The relationships prevailing within a homologous series can be well illustrated by the normal aliphatic alcohols, a series that has been extensively investigated, both physically and pharmacologically.[1] In passing up the series there are progressive changes in various physical properties. The vapor pressure of the pure liquid, the distribution coefficient between cotton seed oil and water, the concentration depressing the surface tension of water by a given amount, and the water solubility of the higher members all change by approximately the same proportion with each successive addition of a methylene group. The aforementioned physical constants are all measures of a distribution of the alcohol between two phases, and the geometric progression has its source in the fact that the work required to transfer a mole of the alcohol from one phase to the other increases by about the same amount with each additional methylene group. The properties are all interrelated, yet they are influenced in different ways by the specific structural features of the molecule. For instance, the lowering of the surface tension of water is dependent upon the interaction of the alcoholic hydroxyl group with water. In the paraffin hydrocarbons surface activity is no longer present, but a geometric progression in water solubility and vapor pressure in ascending the series is still found. In the series of alcohols the anesthetic doses, as well as the concentrations required to produce a number of other biological effects, decrease approximately in geometric proportion as the number of carbon atoms increases (within certain limits). This proportion moreover is about the same as that by which the various physical constants listed above are altered. Thus within the series of aliphatic alcohols, anesthetic activity shows almost equally close correlation with vapor pressure, oil/water distribution coefficient, surface activity, and water solubility. Only by extending the study to compounds of different structural types will it be found that some of these correlations approach universal validity much more nearly than do others. In the paraffin hydrocarbons, despite their lack of surface activity, anesthetic activity is still present and increases geometrically within the series as in the alcohols (52). The striking correlation between anesthetic activity and surface activity in the alcohols, which was first pointed out by Traube (144), is thus shown to be dependent upon a special structural feature that is not essential to produce anesthesia. This illustrates the fact that in a homologous series a physical property may be quite closely correlated with a biological effect without this correlation having any general applicability and without its being directly concerned with the mechanism of action. A similar line of reasoning will show the hazards of drawing conclusions concerning two different biological effects produced by the same drugs within a homologous series. Assume, for example, two hypothetical biological effects produced by the alcohols, one dependent upon concentration of the alcohol at an interface, the other upon solution of the alcohol in a homogeneous phase. In ascending the series of alcohols the activity in both of these effects will be found to increase in the same proportion. Yet it would evidently by erroneous to accept this as evi-

[1] Compilations of physical and pharmacological data on the alcohols are to be found in von Oettingen's monograph (147) and Ferguson's paper (46).

dence either of a causal relationship between the two biological effects or of their production by a common mechanism.

Probably the best known and most extensively investigated correlation is that between anesthetic activity and the distribution clefficient between olive oil and water, the correlation that formed the basis of the lipide theories of H. H. Meyer and of Overton. No attempt will be made here to review the voluminous literature bearing on this subject. It should be mentioned that the determination of this coefficient is sometimes technically difficult and that many of the published values are probably seriously in error. This particular distribution coefficient was never considered by the early advocates of the lipide theory to be of any special significance. Olive oil was chosen simply as a readily available substance that might serve as a model of the lipides of the brain or of the cell membrane. It was realized that these lipides differ in their properties from olive oil, and the adherents of the lipide theory were inclined to explain any discrepancies in correlation on this basis. Knowledge of the chemical composition of the plasma membrane is incomplete and investigation of the solvent properties of those substances thought to be important components, the phosphatides, cerebrosides and sterols, is technically difficult. Meyer and Hemmi (114) proposed oleyl alcohol as being a better model than the fats for the cellular lipides involved in narcosis. They found that the distribution coefficient between oleyl alcohol and water showed a closer correlation with effectiveness in tadpoles than the olive oil/water coefficient. Collander (31) has investigated the distribution of numerous compounds between water and various organic solvents. It was found that the solvent properties of organic substances depend upon two major factors, *viz.* (a) their polarity (hydrophilia) and (b) their acidity or basicity. Collander points out that the solvent properties of the plasma membrane cannot be deduced from its assumed chemical composition. In the membrane the lipide molecules are oriented in layers with the hydrocarbon chains parallel. These layers will be about as hydrophobic as a hydrocarbon and in this respect will differ entirely from the same lipides in a bulk phase where there is random orientation. It would seem doubtful then whether there would be any theoretical advantage in studying distribution coefficients with solvents that are chemically more closely related to the membrane lipides than is olive oil.

If a sufficient range of chemical structures are represented in the comparison, anesthetic activity—at least so far as it can be evaluated in mammals—fails to show anything approaching a perfect correspondence with the oil/water distribution coefficient. It must be conceded, however, that when anesthetic doses are multiplied by distribution coefficients, the products do not vary over nearly as wide a range as the doses themselves. For the barbituric acids (distribution coefficients determined on the acid forms) this product lies in a considerably lower range than for such drugs as ether, paraldehyde and chloroform. A conspicuous deviation from the Meyer-Overton rule, in fact for a long time the most troublesome exception to any proposed rule relating physical properties and anesthetic properties, is found in the case of chloral hydrate. This compound has a lower oil/water distribution coefficient than most other drugs of comparable activity.

It now appears clear that the anesthetic effects of chloral hydrate are attributable in large part at least to trichloroethanol, chloral hydrate being rapidly reduced to the alcohol in all tissues (24, 26). The physical properties of trichloroethanol are more consistent with those of other typical anesthetics of comparable activity than are those of chloral hydrate. Whether chloral hydrate itself has any anesthetic activity whatever is not known, and this would be difficult to ascertain. So far as theoretical considerations are concerned, chloral hydrate can be removed from the list of anesthetics, and its exceptional physical properties cannot be regarded as evidence against any theory.

The "narcotic" effects of nitrogen, argon, krypton and xenon have been attributed to their lipide solubility (6, 8, 100, 115). Because of the unique chemical features of the inert gases, they occupy a position of especial theoretical interest. If a theory can offer a unifying interpretation of the action of the inert gases and of the organic anesthetics, this must be viewed as an impressive accomplishment. Unfortunately, the data at hand scarcely permit a comparison of these gases with the organic compounds. In most experiments with the gases, the effects did not reach the stage of full anesthesia, and it is not obvious that they are even qualitatively of the same nature as the effects of organic anesthetics. In the group of inert gases, xenon and perhaps krypton have greater "narcotic" activity than argon, and helium fails to show any activity. This order of activity is compatible with the lipide theory, the fat/water distribution coefficient increasing with atomic number in this group. Other gases such as nitrogen and hydrogen, which are presumably entirely unreactive under physiological conditions, can perhaps be profitably included with the inert gases for the purposes of the present consideration. Nitrogen, which has a distribution coefficient about equal to that of argon, has somewhat less effect on the central nervous system. Although far from satisfactory, perhaps the most appropriate comparison to disclose the relationship to the organic compounds is that of nitrogen with ether, these two substances having nearly equal distribution coefficients between olive oil and water. At 10 atmospheres, where nitrogen produces definite effects but not anesthesia, the total amount of nitrogen in the body at equilibrium, calculated from data at atmospheric pressure (7), will be about 0.007 moles per kgm. This is about half the amount of ether in the body at the stage of light anesthesia. These data are not inconsistent with the theory that the lipide/water distribution determines the effectiveness of both substances. Hydrogen, on the other hand, is without effect at pressures where nitrogen has pronounced effects (28). The fat/water solubility ratio of hydrogen is somewhat less than that of nitrogen. Yet the difference in this ratio as well as in the separate solubilities of the gases in water and in oils (100) are hardly sufficient to account for the difference in activity between hydrogen and nitrogen. This consideration led Case and Haldane (28) to doubt that lipide solubility is the factor responsible for the effects of nitrogen and the inert gases.

Ferguson (46) introduced a new point of view in the study of the physical properties of anesthetics. He based his reasoning on the premise that narcotic action (and certain other drug actions) depend upon a physical mechanism,

which is governed by the equilibrium existing between the concentration of the drug in the external phase and its concentration in the affected phase. If an equilibrium exists, the thermodynamic potential of the drug must be the same in all phases in equilibrium. If this potential can be measured in any phase in equilibrium with the affected phase, the potential in the latter is known, even though the site and the nature of this phase be unknown. Ferguson proposed accordingly that drugs could be most logically compared on the basis of their potentials in an accessible phase rather than on the basis of their concentrations there.

The thermodynamic potential used by Ferguson is the partial molar free energy, given by the equation,

$$\bar{F} = F_0 + RT \ln a,$$

where F_0 is the molar free energy in a standard state and a is the activity. In this study the pure substance was chosen as the standard state. The activity of the pure substance is thus defined as unity. In a vapor phase the activity is approximately the ratio of the partial pressure of the vapor to the saturated vapor pressure of the substance at that temperature. In a solution of a substance of low solubility, the activity is approximately the ratio of the concentration to the concentration of a saturated solution. The activity coefficient of a substance is defined as the ratio of the activity to the molar fraction.

Ferguson compiled from the literature numerous data on "narcotic" as well as bactericidal and insecticidal effects and calculated the thermodynamic activities of the effective concentrations of the drugs. Of particular interest in connection with the subject of this review is the series of compounds administered by inhalation to mice by Meyer and Hopff (115). This series includes drugs of diverse chemical structure, the effective concentrations of which vary over a very large range. The thermodynamic activities, as calculated by Ferguson, vary over a much narrower range. Ferguson concludes that if a particular pharmacological effect on some one organism depends upon a "physical" mechanism (as he assumes "narcosis" to do), the thermodynamic activities of the drugs producing this effect will lie in a narrow range, such variation as there is in this range presumably being due to secondary effects depending on the chemical structure of the drug. Any major deviation from this range of activity is indicative of a "chemical" rather than a "physical" mechanism of action.

The concept of equal narcotic effect at equal thermodynamic activities has again received emphasis from Brink and Posternak (16), who have presented further examples of experimental data in support of the rule.

If the thermodynamic activity of a drug in an aqueous phase of an animal, say the plasma, is approximately the same as that of an equal concentration in water, and if furthermore the concentrations of anesthetics in this phase are approximately proportional to the average concentrations in the body as a whole, then Ferguson's theory leads to the prediction that the total dose of a drug required to anesthetize is proportional to its water solubility. This prediction is recognizable as being essentially identical with a generalization long celebrated

as "Richet's rule."[2] The assumptions mentioned above are of course not strictly justified, but it is likely that with many anesthetics of the familiar types they would not introduce errors of a very large order of magnitude. A crude test of the concept is thus available in a survey of the anesthetic doses and solubilities of large numbers of compounds where chemical analyses do not afford more direct information. Only discrepancies of a large order could of course be considered of any significance.

In a homologous series or even among closely related compounds an inverse relationship between potency and water solubility is often evident. Attention has been called to this in the cases of a number of chemical groups, *e.g.*, the paraffin hydrocarbons (52) and the ethers (32). When the consideration is extended to more diverse types, it cannot be claimed that the ratios of dose to water solubility lie in a very narrow range. Yet this ratio does show less extreme variability than the doses themselves, and it is a striking fact that among the more active anesthetics (anesthetic dose less than 0.01 mole per kgm.) there are very few having high solubility in water (greater than 1 mole per l.). (The solubilities of barbituric acid derivatives are here taken as those of the acid forms.) Among common drugs the most outstanding exceptions are chloral hydrate and ethyl carbamate, which are considerably more soluble than most other agents of comparable anesthetic potency.[3] As mentioned above, it is justifiable to remove chloral hydrate from the anomalous group since there is no reason to believe that it is itself an anesthetic. Aside from any question of theoretical interest, the relation of activity to water solubility is a matter of some practical importance. High potency not being associated with high water solubility, the total anesthetic dose is generally soluble only in quite a large volume of water. This is a consideration sufficient of itself to render most classes of anesthetics unsuitable for practical use by the intravenous route.

A crucial test of all theories seeking to predict anesthetic activity from physical properties is afforded in the comparison of optical antipodes. All properties of an antipodal pair measured in a symmetrical environment are identical. If anesthetic activity is a function solely of any such property or any combination of such properties, the pair should evidently be equally potent anesthetics. Despite the theoretical opportunities offered by the study, relatively little attention has been given to optically isomeric anesthetics. Studies have been reported on the enantiomorphic forms of three derivatives of barbituric acid (75, 91) and one derivative of hydantoin (140). Some differences were found between the isomers in each case, but these differences are not great and the presentation of the data unfortunately does not permit a judgment of the significance of the

[2] Richet's rather casual proposal of this rule (132), which he only intended to apply to closely related compounds, was based on the study of the toxicity of seven substances for fish.

[3] The convenience of high water solubility is perhaps one reason why these drugs have continued even into recent years to be popular in the study of "narcotics" on isolated tissues and lower organisms. Since chloral hydrate is probably reduced by all cells, it is particularly inappropriate for this use. The result is a mixture of chloral hydrate and trichloroethanol of unknown proportions, the proportions changing continuously with time.

differences. No difference was found between the optically isomeric *sec*-butyl alcohols, and it was shown that the doses producing equivalent anesthetic effects could not differ by as much as 10 per cent (27). The greatest difference between enantiomorphic anesthetics that has been reported is for the α-arabinochloraloses (22). The derivative of L-arabinose is about twice as active an anesthetic as the antipodal derivative of D-arabinose. This demonstrates that anesthetic activity is not always determined entirely by physical properties, and that no rule relating activity to any physical property or properties whatever can be adequate to predict activity quantitatively. It is conceivable that anesthetic activity depends solely on physical properties in some classes of drugs, such as the alcohols, and not in other classes, such as the chloraloses. Yet the theories are deficient in that they cannot predict to what classes of drugs they are applicable.

In most attempts to correlate anesthetic activity with physical properties, attention has been unduly focused on compounds known to have some degree of anesthetic activity. This approach is unsound if it is desired to find what properties distinguish anesthetics from non-anesthetic drugs. It has not been sufficiently emphasized that compounds having water solubilities and oil/water distribution coefficients in the same range as those of active anesthetics may have no anesthetic properties, may in fact be convulsants. Camphor, for instance, is a substance having low solubility in water and high solubility in fats. γ-Hexachlorocyclohexane, which is convulsant, does not differ greatly in its solubilities from the δ-stereoisomer, which is depressant (109, 139). No way is known of predicting from physical properties that a barbituric acid will be convulsant rather than anesthetic. Some derivatives of barbituric acid have both convulsant and anesthetic properties, the former being the more prominent in low doses or in certain species of animals. These facts may be indicative that convulsant and anesthetic effects are produced through fundamentally similar mechanisms, similar properties in a drug being essential for either effect. Nevertheless, it is a matter of practical importance whether a drug is convulsant or anesthetic, and it must be admitted that we have no theory adequate to explain why a drug should have one of these effects rather than the other.

It cannot be claimed that the anesthetics show any perfect regularity in the relationship of their potency to any physical property. We are not only unable from physical measurements to predict quantitatively anesthetic doses, but we are even unable to predict with any reliability the qualitative nature of the pharmacological action. Such rules as have been proposed are more nearly adequate to describe the properties necessary for anesthetic activity than those sufficient. Yet the extent to which anesthetic activity is associated with physical properties is enough to provide some suggestions regarding the mechanism of anesthetic action.

(ii) *Theoretical implications of the relations between physical properties and activity*

The inadequacies of the empirical rules discussed above are not surprising. Intermolecular forces even in the simplest of systems are tremendously complex

and difficult of analysis. Even if anesthesia should depend upon a relatively simple physico-chemical process, it could not be expected that the anesthetic dose would be related to any single physical constant by a simple mathematical function. It is still not obvious how much importance should be attached to conspicuous deviations from the rules as indicative of different mechanisms of action. The concept of one all-inclusive theory of narcosis is not to be accepted without question. It is conceivable that in the cell, the normal function of which depends on many chains of complex physical and chemical events, the interference with these processes at any one of many points might lead to reversible depression of irritability. The nature of depressant drugs furnishes some suggestions that similar final effects may indeed result from different mechanisms of action. It is difficult to conceive of the magnesium ion or the bromide ion producing thhe depressant effects in the same way as the organic compounds, and most theorists have not wished to postulate a common mechanism. Even among the organic anesthetics there are some indications that there may not be a single fundamental mechanism. For instance, the evidence of an asymmetric process in the action of the arabinochloraloses and the lack of such evidence in the action of the *sec*-butyl alcohols suggest, without of course conclusively proving, that anesthesia may be brought about in more than one way.

The lack of structural specificity in anesthetics, the reversible nature of the anesthetic process, and such correlations as there are with physical properties have been taken as indications that anesthesia is produced by a "physical" rather than a "chemical" mechanism. To attempt distinctions between "chemical" and "physical" forces is no longer profitable. The specific, short-range, oriented forces that have been termed "chemical" are ultimately explicable in "physical" terms, and nothing is elucidated by caviling over nomenclature. All evidence would indicate that anesthesia does not depend upon participation of the drug itself in chemical reactions, at least in the generally accepted sense of the word. A number of anesthetics can be recovered from the body almost quantitatively in an unchanged form. Of course the possibilities have not been excluded that the drug has entered into a completely reversible reaction or that the small amount unaccounted for has been responsible for the pharmacological effect. When an anesthetic does undergo chemical reactions in the body, the only apparent result is usually the inactivation of the drug. The lack of "chemical" reactivity in a drug capable of producing a derangement of function of the central nervous system—whether or not it should be termed anesthesia—is decisively exemplified in xenon, an atom incapable of forming "chemical" bonds. The forces operative between the xenon atom and a molecule in the cell are those due to the interaction of a dipole in the molecule with the dipole induced in the xenon atom and the inductive forces due to the rapid variation of charge distribution around the apparently symmetrical atom. Thus forces usually considered "physical" in nature must be responsible for the pharmacological action of xenon.

Neither is there any reason to believe, however, that the high degree of structural specificity characterizing some other classes of drugs indicates that these agents enter into chemical reactions with cellular constituents. The molecular

interactions that determine pharmacological response are dependent in these cases upon a highly specific spatial pattern of forces. No such specific pattern is essential in anesthetic action. This distinction between anesthetics and structurally specific drugs does not, however, necessarily indicate that the forces involved are ultimately of a different physical nature.

It was pointed out above in the discussion of homologous series that the various physical properties of substances are closely interrelated. Consequently, as has been emphasized by Ferguson (46), the different theories that seek to correlate physical properties with anesthetic activity are not to be regarded as really independent. Although they may not be equally valuable for the prediction of potency, they actually have a common theoretical basis. The essential identity of the theory of equal thermodynamic activities with "Richet's rule" has already been mentioned. Again, if anesthetics are effective at equal thermodynamic activities and if the solution of the anesthetic in oil does not depart greatly from ideality, the correlation of Meyer and Overton will result. In a homologous series of surface active compounds, surface activity is approximately proportional to the activity coefficient in dilute aqueous solution (20). Thus it seems doubtful whether any experiment involving only measurements of concentrations of drugs in a circumambient medium and the response of a living system can point decisively to the physical nature of the mechanism. The same data could be equally consistent with theories postulating different physical processes. Various experimental procedures have been devised in an effort to decide whether adsorption[4] or solution in a lipide phase is the basis of narcotic effects, but none of the results can be considered conclusive in this regard.

The theoretical implications of anesthesia being produced by equal thermodynamic activities are by no means simple or obvious. This amounts essentially to a substitution of the distribution coefficient between water and the pure liquid or solid phase of the anesthetic substance for the distribution coefficient between water and fat of the Meyer-Overton theory. This is difficult of theoretical interpretation, since it involves the properties of the pure substance, which itself constitutes a complex system. It has been shown (21) that the decreasing solubility in ascending the homologous series of alcohols is in very large part attributable to the interaction of the alcohol molecules with each other in the pure liquid. It would seem likely then that the only physical meaning implied in the correlation between anesthetic doses and activity coefficients is that anesthesia somehow involves intermolecular forces of the same nature as those prevailing between the molecules of the anesthetic agent itself. The rough correlations that have been found cannot accordingly be considered to be of any simple, direct theoretical significance.

From the foregoing discussion it can be concluded that the study of the physical properties of anesthetics is a method possessing distinctly limited possibilities for disclosing the actual mechanism of anesthesia. An elaborately reasoned inference that a "physical" process is involved represents really little progress

[4] The sense in which the term "adsorption" has frequently been employed by biologists is so loose and vague as to be almost meaningless.

beyond the obvious. The nature of the physical effect is not clarified, and the consequences of the primary action that lead to depression of cellular function remain to be described. The suggestions furnished by the study of the properties of anesthetics have, however, been the starting point for theories that have attempted to describe the mechanisms by which depression is actually brought about.

Lipide solubility is the property of anesthetics that has received most attention from theorists. Although the original versions of the lipide theory did not attempt any very definite description of the interaction of drugs with the cellular lipides or the mechanism by which this would alter cellular function, there have subsequently been numerous suggestions as to the specific manner in which the property of lipide solubility might be involved in the process leading to anesthesia. Most of these theories have been frankly speculative and of such a nature that direct experimental evidence would be difficult to adduce. Only a few of the more recent suggestions will be mentioned.

X-ray diffraction studies have revealed something of the manner in which lipide molecules are associated with each other and how organic solvents affect this association. In the dry state the nerve lipides appear to be arranged in bimolecular leaflets with the polar groups in apposition and the hydrocarbon chains closely packed in parallel arrangement (5). In aqueous emulsions the water comes between the polar interfaces of the bimolecular leaflets (124). A similar arrangement has been found in sodium oleate emulsions (90). In this emulsion benzene could enter between the apolar interfaces, causing a separation of as much as 36 A. Benzene did not increase the distance between the hydrocarbon chains in the plane of the layer. A similar phenomenon was observed in the case of lipides in ether (126). Schmitt and Palmer (136) remarked that this phenomenon was of interest in connection with the mechanism of narcosis, but they made no more specific suggestion. In Danielli's model of the cellular membrane (37) a double layer of lipide with the apolar interfaces in apposition is postulated. If the anesthetic molecules entered between these interfaces, it is not difficult to conceive that this might so alter the properties of the membrane as profoundly to affect cellular function. Theories involving the membrane will be considered in some detail in a later section.

The property of lipide solubility in anesthetic agents is not necessarily indicative that the lipides themselves are the cellular constituents involved in the critical interaction. There have been suggestions that the same properties might equally well indicate an action upon proteins. Östergren (122) suggested that attractive forces between the anesthetic molecule and the lipophilic side chains of the protein would cause a folding of the polypeptide chain with a change of the fibrous protein molecule to a corpuscular shape. A somewhat similar theory has been proposed by Gavaudan, Dodé, and Poussel (61, 41, 126). Lipides are pictured as being "dissolved" in the lipophilic chains of protein and it is suggested that the anesthetic displaces the lipides from this solution. It is emphasized by the authors that this process would take place in accord with the rule of equal thermodynamic activities. McElroy (108) has suggested that the action of a narcotic on a lipo-protein complex might involve penetration of the lipide phase

with increase in its volume and subsequent unfolding or denaturation of the associated protein. Ball and Cooper (3) presented the idea that phospholipides may act as cementing substances to hold in close association groups of enzymes that perform together in metabolic cycles. They raised the question whether the inhibitory effect of 2-hydroxy-3-alkylnaphthoquinones on succinate oxidase might be attributable to the affinity of the drugs for the lipides of the enzyme complex. It is conceivable that through a similar mechanism the lipide-soluble anesthetics might derange the coordinated action of a structurally associated group of enzymes.

Implicit in all the foregoing reasoning is the assumption that the physical properties of the drugs are an important factor in the actual process that results in anesthesia. Yet this is not the only possible interpretation of a correlation between physical properties and anesthetic properties. The physical properties might merely be a factor regulating the access of the drug to its site of action. Lipides constitute an important structural component of the cell membrane, and lipide solubility has been shown to be a factor in determining the penetration of substances into many varieties of cells (37). The property of lipide solubility in anesthetics might accordingly be regarded equally logically as indicating either (a) that the action of the drug is exerted, as postulated by Meyer, *within* the lipide phase, or (b) that the site of action lies *beyond* the lipide phase. The cell membrane is not the only structure where lipide solubility is an important factor in determining the rate of penetration. In this respect the permeability of the capillaries of the central nervous system resembles that of cellular membranes and differs fundamentally from that of capillaries in all other organs (95). Water-soluble organic compounds such as sugars and urea penetrate the capillaries of the central nervous system very slowly, while lipide soluble substances penetrate rapidly. The great rapidity with which most anesthetics enter the brain from the blood is demonstrated by the fact that the maximal effects from an intravenous injection are attained at a time scarcely greater than that required for the anesthetic-bearing blood to circulate to the brain. The only classes of anesthetics that show any measurable delay in the full development of their action following an intravenous injection are the chloraloses and the 5,5-disubstituted derivatives of barbituric acid and hydantoin (23). Among the 5,5-disubstituted barbituric acids there is a statistically significant association between anesthetic dose and lag, the more active drugs tending to have the more rapid onset of action. Since anesthetic activity and the fat/water distribution coefficient are also associated properties, the lag in onset might be explained as the time required for the penetration of the drug into or through a non-aqueous phase of lipoidal character. This suggests as the site of the delay either the capillary wall or the surface membrane of the brain cell. However, when the barbituric acids are compared with other chemical classes having immediate onset of action, it becomes evident that the delay is not determined simply by anesthetic activity or fat/water distribution or molecular dimensions. The reason is not yet apparent why a lag in onset of action should be found in such a limited number of chemical classes of anesthetics.

The rate at which a drug attains equilibrium between blood and brain is evi-

dently a factor of the greatest importance in determining the characteristics of the anesthesia and the possibilities of practical application. If a hypothetical anesthetic should enter the brain as slowly as does, say, urea, not only would the drug be valueless for practical anesthesia, but even the detection of its anesthetic properties would be difficult. For anesthesia to be a feasible procedure at all, the anesthetic agent must penetrate to its site of action in a rather short time. For anesthesia to be controllable, either in an intravenous or an inhalation procedure, it is essential that equilibrium between blood and brain be attained with very great rapidity. A drug with even as slow an onset of action as barbital would not give sufficiently controllable effects to make it safe for intravenous anesthesia. Drugs that are suitable for intravenous anesthesia, the 1-methyl barbituric acids and the 2-thio barbituric acids, enter the brain with great rapidity, as evidenced by the almost immediate onset of action. Furthermore, greater rapidity of penetration into the brain than into other bulkier tissues and the ensuing redistribution may play an important part in bringing about the quick termination of the anesthetic activity of these drugs.

Thus the physical properties that are associated with anesthetic activity may conceivably have no direct relation to the actual mechanism of anesthesia but may rather be the properties essential for the arrival of the drug at its site of action in a time compatible with its recognition as an anesthetic. On the other hand, this argument should not be used to depreciate the theories based on inferences from the physical properties of anesthetics. The fact that a property is essential for access to the site of action is in no way indicative that the same property is not essential in the final mechanism. If it were to be assumed that all physical properties common to the anesthetics are concerned only with their transport to the site of action, then the remarkable conclusion would have to be accepted that the actual mechanism of action requires no common property either structural or physical and that any substance whatever reaching the critical site will be effective.

THEORIES INVOLVING THE CELLULAR MEMBRANE

The cellular membrane being generally regarded as playing an essential role in the propagation of excitation, it would be logical to expect that a drug interfering with this process might have its site of action in the membrane. Furthermore, the lipide theory has attracted attention to the membrane. Meyer did not specify what lipides would be affected by narcotics, but evidence has accumulated for a long time indicating the importance of lipides in the structure of the membrane and it might naturally be supposed that these would be the lipides the alteration of which would most likely disturb cellular function. Although theories focusing attention on the membrane are naturally allied to the lipide theory, this is not to imply that changes in the membrane brought about by other processes than solution of the drug in the membrane lipides are not altogether conceivable. In fact the first theories suggesting the membrane as the site of narcotic action did not concern themselves with lipides.

Lillie (103) clearly formulated a theory explaining how modification of the properties of the membrane by drugs might abolish excitability. The drug would decrease the permeability of the membrane or would at least stabilize the membrane in such a way that it would be incapable of undergoing an increase in permeability under conditions that would normally bring this about. Thus the increase in permeability that presumably gives rise to the depolarization accompanying a wave of excitation could not occur. This theory was based on numerous observations on various types of cells in which narcotics produced decreases in permeability or protected against increases in permeability. Subsequently there have been a number of investigations that have failed to support the concept of a universal permeability-decreasing effect of narcotics (37, 17, 102). This has been regarded by some theorists as a fatal blow to Lillie's theory. Although not in accord with Lillie's original ideas, these experiments really have no bearing on the essential feature of his theory as it concerns the abolition of excitability. "Permeability" is not the simple affair that it once was thought to be, and it need not be expected that an alteration of "permeability" will manifest itself in the same way in all cells and with respect to all penetrating substances. Lillie's theory, so far as it applies to excitability, is concerned only with the very special increase in permeability that accompanies excitation. Experiments relating to any other type of "permeability" are irrelevant. The only question is whether drugs stabilize the membrane in such a way as to prevent the depolarization of excitation. In regard to the theory of general anesthesia, the question is whether such a stabilization occurs in the membranes of the neurones of the central nervous system in such a way as to interfere with synaptic transmission.

There is some experimental evidence that can be considered to bear upon this question. The prolonged negative potentials of the spinal cord evoked by reflex stimuli and transmitted electrotonically along the ventral and dorsal roots have been studied in connection with drug action by Bremer, Bonnet and Moldaver (15), Bonnet and Bremer (13), Bremer and Bonnet (14), Eccles (42), Eccles and Malcolm (43), and Brooks and Eccles (18). These "synaptic potentials" presumably arise in the motoneurones when they are subjected to synaptic excitation and have been regarded by these workers as the causal factor in setting up the discharge of the motoneurone. The only anesthetic studied by Eccles and his colleagues was pentobarbital. This drug was found to have little effect on the synaptic potential until anesthesia was very deep, and it was concluded that its blocking action on synaptic transmission is due to a stabilization of the soma membrane so that the discharge of an impulse is not initiated by a synaptic potential that normally would be effective. The blocking action of anoxia, on the other hand, appeared to depend upon an entirely different process, the depolarization of presynaptic fibers. Eccles' view of the action of pentobarbital, it will be noted, is essentially in accord with Lillie's theory. The results of the Belgian group's work with barbituric acids (15, 14) are not in disagreement with those from Eccles' laboratory, but different types of anesthetics were found to produce different effects. Urethane and chloralose, like anoxia, depressed the synap-

tic potentials without changing the voltage at which discharge appears (13). It was concluded that these anesthetics block central transmission by lowering the voltage of the synaptic potential below the critical level. Van Harreveld (145) found no effect of ether and pentobarbital on the potential between the gray matter of the cord and a dorsal root. This was interpreted as indicating uniform depolarization of the entire neurone. This interpretation was based on indirect evidence derived from the effects of the drugs on asphyxial depolarization and is not convincing.

The electrical potentials arising from junctional regions within the central nervous system are still very imperfectly understood and the few studies of anesthetics are not altogether in agreement. Further study of these potentials should, however, provide a profitable approach to a comprehension of the fundamental physiological alteration of the nerve cell in anesthesia.

The peripheral never fiber furnishes a more accessible system in which to study the effects of drugs on polarized membranes, although it is questionable how much of the information acquired from this preparation is applicable to the central synapse. In peripheral nerve it can be demonstrated that impulse transmission can be blocked through a stabilization of polarization. This effect is produced by cocaine (12, 9, 106), procaine (9, 143), a number of other basic local anesthetics (9), amyl alcohol (12), urethane (106, 35), physostigmine (143), and DFP (143). On the other hand, anoxia (62, 106, 153), ethyl alcohol (153, 60), and ether (106, 153) cause a depolarization and produce block in this way. It would appear that excitability of nervous tissue can actually be abolished by an alteration of the properties of the membrane in a manner similar to that suggested by Lillie.

THEORIES INVOLVING INHIBITION OF OXIDATIVE REACTIONS

A very active oxidative metabolism having long been recognized as essential for the function of the central nervous system, it is understandable that the question should have arisen whether anesthesia is caused by a derangement of this metabolism. In recent years a great deal of effort has been devoted to the investigation of this question. It will accordingly be discussed in considerable detail here. The modern theories of metabolic inhibition in the course of their development have diverged so far from Verworn's original formulation of his asphyxial theory that the older experimental evidence offered in support or refutation of that theory can no longer be considered relevant. The present discussion will therefore be limited to recent work. Since the postulated metabolic inhibitions have usually been attributed to direct effects upon enzymes, the question of the action of anesthetics on enzymes must be considered in a discussion of the modern theories. Anesthetics, or at least some anesthetics, in high concentrations have been shown to inhibit a variety of enzymes. The older work in this field has been reviewed by Winterstein (151). The present discussion will be concerned chiefly with those enzymatic inhibitions, or apparent enzymatic inhibitions, produced in brain tissue by relatively low concentrations of anesthetics.

(a) Oxidative inhibitions produced by anesthetics in brain tissue in vitro

The recent revival in interest in a theory of this general nature is due in large part to the work of Quastel and his associates (129, 130, 81, 82, 80, 127), in which it was shown that a number of anesthetics inhibit the consumption of oxygen by brain tissue *in vitro*. The number of drugs that have been studied for this effect is by now rather large. Some of the anesthetics that have been shown to inhibit oxygen consumption of brain tissue *in vitro* will be listed here, no consideration being given for the moment to the concentrations required to produce the effect. This listing is not intended to be complete. The group of drugs most extensively studied are the derivatives of barbituric acid. Among the anesthetic members of this series[5] that have been found to inhibit oxygen consumption of brain are allylbarbituric acid (55), amobarbital (55), aprobarbital (129, 55), barbital (129, 55, 19), butethal (55, 19), cyclopal (55), hexethal (55), hexobarbital (80, 76), pentobarbital (72, 55, 65, 149, 137, 150, 44), phenobarbital (129, 130, 152, 81, 80, 76, 55, 148), probarbital (55), seconal (55), and thiopental (55). Other types of anesthetics that have been shown to have the effect include acetylene (129), chloral hydrate (129, 80, 76, 19), chlorobutanol (129, 130, 81, 80, 76, 65), chloroform (129, 65), 5,5-dipropyl-2,4-oxazolidinedione (53), ethanol (80, 72, 56, 19), ether (129, 82), the magnesium ion (80), nitrous oxide (129), paraldehyde (129), tribromoethanol (80), and urethane (129, 80, 76).

Brain tissue of several different species of laboratory animals has been studied. Slices, chopped tissue, and homogenates have been used. Slices of rat brain have been investigated more extensively than any other preparation.

The inhibition produced by most drugs develops rapidly and, under certain conditions at least, changes little with time. The effects of chlorobutanol and of phenobarbital have been shown to be reversible (130). The effects of ether, on the contrary, proved to be irreversible (82). The inhibitory effect of ether continues to increase with time, and by extrapolation it may be inferred that immediately after addition of the drug the effect is nil. Ethanol resembles ether in this respect (80, 56), and the effect of this drug is also presumably irreversible. Even between two 5, 5-disubstituted-2,4-oxazolidinediones there is a difference in reversibility of effects. The inhibition produced by the dipropyl derivative is partially reversible, while that produced by the diphenyl derivative is not (54).

In all the studies of anesthetics in which this point has been investigated, it has been found that oxidation of succinate is not inhibited as is that of glucose, pyruvate or lactate (129, 81, 82, 65, 44). This applies to homogenates as well as slices. Curiously enough, this same difference in effect on succinate and glucose oxidations is found alike with numerous drugs quite heterogeneous in their chemical and pharmacological nature: not only the anesthetic barbituric acids with their reversible action and ether with its irreversible action but also a convulsant

[5] The nonproprietary names of barbituric acid derivatives used here are those of the U. S. Pharmacopoeia or the 1949 edition of New and Nonofficial Remedies. Some of these names are not yet in general use.

barbituric acid (57), a convulsant oxazolidinedione (54), and several non-anesthetic amines (128).

(b) *Inquiries into the general nature of the oxidative inhibitions produced by anesthetics*

A number of different anesthetics have thus been shown capable of inhibiting certain oxidative reactions in the brain. The question of the causal relation of this phenomenon to anesthesia will be deferred in order first to consider work designed to elucidate the nature of such inhibitions as have been demonstrated to occur.

(i) *Proposed mechanisms of enzymatic inhibition*

It has usually been assumed that the observed inhibitions of oxygen consumption are caused by a direct action of the drug upon some enzyme. As will appear later in the discussion, this is not an inescapable conclusion, but we shall first consider some of the speculations concerning the mechanism by which an anesthetic drug might inhibit an enzyme. There are a number of different ways in which enzyme inhibitors have been demonstrated to act or may be imagined to act (47, 108), but whether any type of process hitherto proposed is involved in the action of anesthetics on the oxidative system of the brain is not known. The lack of structural specificity among the anesthetics can be regarded as evidence against any process involving competition between the drug molecule and a specific cellular component or any process involving chemical inactivation of a specific functional group of the enzyme.

Studies of the inhibition of luminescence of bacteria by drugs and the influence of temperature and pressure on the process (78, 79, 108) have led to the theory that some chemical inhibitors act by influencing a reversible equilibrium assumed to be normally present between native and denatured forms of a protein-enzyme. The drugs are thought to shift the equilibrium toward the denatured form, which is enzymatically inactive and has a larger volume than the active form. Among the drugs that appeared to act through this mechanism were ethanol, chloroform, urethane and ether. The inhibitory effects of barbital and chloral hydrate were not reversible by increase of pressure, and there was accordingly no evidence that these drugs acted by influencing the denaturation equilibrium. The denaturation theory is based on studies of luminous bacteria, and there is no direct evidence that it is applicable to the brain. There are of course many other ways in which an anesthetic could conceivably affect an enzyme system. Suggestions that have appeared over a period of many years include adsorption of the drug on the enzyme, displacement of the enzyme from its normal locus by the drug, and the various postulated alterations of protein and lipo-protein complexes that have been mentioned in an earlier section of this review.

(ii) *Attempts to localize the site of enzymatic block*

The simplest, although not the only possible, explanation of the inhibition of oxygen consumption is that the drug is inactivating some enzyme in the main respiratory pathway. The assumption that such is the case has prompted ef-

forts to identify the affected enzyme. The process by which hydrogen is removed from a substrate and transferred to molecular oxygen involves a complex chain of reactions, and it is not a simple task to locate the point in this chain at which a drug is interfering with the process. Since many enzymes can be inhibited by sufficient concentrations of some anesthetics, there are numerous experiments performed with high concentrations that are not pertinent to the problem of locating the most sensitive component of the oxidative system of the brain. The work bearing most directly on this specific question is that of Michaelis and Quastel (119) and of Greig (65, 66). Although an exact site of anesthetic action has evaded direct identification, progress has been made in the recognition of some separate steps in the oxidative chain that are not sufficiently sensitive to the drugs to account for the inhibition of the complete process.

The relative insensitivity of dehydrogenases of brain, muscle and yeast to chlorobutanol was demonstrated by the unimpaired oxidation of substrates by ferricyanide and pyocyanine (119). By this procedure the steps involving transfer of hydrogen from the substrate to the dehydrogenase and from the dehydrogenase to its coenzyme are excluded as possible sites of the block. By the use of methylene blue, which oxidizes substrates through an additional step involving a flavoprotein (40), it has been possible to exclude one more step in the oxidative chain, that involving transfer of hydrogen from the reduced coenzyme to the flavoprotein. In brain homogenates the inhibition of oxidation of pyruvate produced by pentobarbital and chloroform and the inhibition of oxidation of glucose produced by chlorobutanol and chloroform could be reversed by the addition of methylene blue (65). In a simple system containing only methylene blue, heart flavoprotein and dihydro-coenzyme I under anaerobic conditions it was shown that pentobarbital did not inhibit the reduction of methylene blue by dihydrocoenzyme I (65). Essentially the same information was furnished by a more complex system consisting of lactate, muscle extract, coenzyme I, heart flavoprotein and methylene blue. Oxygen uptake by this system was not inhibited by chlorobutanol (119). Further evidence excluding the step between coenzyme and flavoprotein was furnished by the demonstration that dihydro-coenzyme I does not accumulate in brain homogenate in which the oxidation of lactate is being inhibited by pentobarbital (65). A number of reports concerned with several different anesthetics have been in agreement that the oxidation of succinate is not inhibited by concentrations of the drugs that do inhibit the oxidation of glucose, lactate and pyruvate. This is indicative that cytochrome oxidase and the cytochrome system, or that part of it involved in the oxidation of succinate, are not sensitive to anesthetics. If the scheme of progressive oxidative steps that has been assumed is actually an adequate representation of brain metabolism, the only step not excluded is that between flavoprotein and the cytochrome system. This portion of the oxidative chain is still very incompletely understood, but it has been suggested that the block may be between flavoprotein and cytochrome b (65). The effect of ascorbic acid in reducing the inhibitory effect of pentobarbital on brain oxidations has also been explained on the basis of the block being at this step (66).

(c) *Evidence relating to causal connections*

The theory of metabolic inhibition, as it appears to be conceived by some of the workers now active in the field, has assumed a form that involves a sequence of two causal connections: inhibition of an oxidative enzyme causes slowing of oxygen-consuming reactions; slowing of these reactions in turn causes anesthesia. We shall now examine the evidence bearing upon both of these relationships.

Attention will first be directed to the relation between anesthesia and such oxidative inhibitions as have been demonstrated *in vitro*. The logical difficulties of either confirming or refuting the hypothesis that a causal relationship between these two phenomena exists will be considered below. The type of evidence necessary and sufficient is not readily definable. There are, however, four general lines of endeavor, not altogether independent, that have been followed in an effort to support the hypothesis: (i) to demonstrate that concentrations of drugs known to be effective *in vivo* are sufficient to produce a demonstrable effect *in vitro*; (ii) to establish a correlation, qualitative and quantitative, between activity in the effect *in vitro* and in the gross effect *in vivo*; (iii) to demonstrate in the brain *in situ* the same effect that is observed *in vitro*; and (iv) to demonstrate *in vivo* a pharmacological antagonism to anesthetics by substances that prevent the oxidative inhibition *in vitro*. These will be discussed in order below.

(i) *Concentrations effective in vivo and in vitro*

As will be seen below, this is not necessarily a vital theoretical point, but some theorists have gone to great pains to establish that concentrations occurring *in vivo* produce demonstrable effects *in vitro*. Jowett and Quastel (81) and Jowett (80) were the first to demonstrate inhibitory effects with concentrations of drugs that might be expected to be compatible with life and to emphasize the importance of this as supporting evidence for their theory. On the assumption that anesthetic doses of the drugs are uniformly distributed throughout the total body weight, calculation showed that concentrations that would occur during anesthesia were sufficient to cause measurable inhibition *in vitro* in the cases of urethane, chloral hydrate, chlorobutanol, tribromoethanol, phenobarbital, hexobarbital and the magnesium ion (80). Some of the anesthetic doses used in the calculations are excessive, but the conclusion is probably acceptable that sublethal, if not minimal anesthetic, concentrations of most of these drugs are sufficient to produce some detectable effect *in vitro*.

Attempts to correlate the concentrations effective *in vivo* and *in vitro* are handicapped by lack of adequate information concerning the concentrations occurring *in vivo*. For only a few anesthetics are there satisfactory quantitative data on blood and brain concentrations. The assumption of uniform distribution quite likely gives values of the proper order of magnitude for most of the familiar anesthetics, but this assumption cannot be adopted *a priori* with any real assurance.

Ether, a drug for which there is adequate knowledge of concentrations *in vivo*, is conceded by Jowett and Quastel (82) not to produce significant effects *in vitro* at anesthetic concentrations. The most indisputable example of failure

to inhibit *in vitro* at anesthetic concentrations is furnished by ethanol. The data from several different investigations (80, 72, 56, 19) are in agreement in showing inhibitory concentrations *in vitro* far in excess of any concentration that could ever occur in a living mammal.

Of particular interest are the barbituric acids because of the extensive investigation of this series and because the inhibitory concentrations reported have frequently been of an order of magnitude that might be expected to occur *in vivo*. Unfortunately the knowledge of concentrations of barbituric acids in blood and brain is far from satisfactory. The analytical methods that have been used are subject to question in regard to their specificity and their quantitative reproducibility. The reliability of these methods has been examined in some detail in the review of Maynert and van Dyke (111) and will not be discussed further here. Several different series of measurements employing both the cobalt color method (94, 39) and the ultraviolet spectrophotometric methods (51, 63, 133) have given results indicative that the concentrations of barbituric acids in blood are of the order of those predicted on the assumption of uniform distribution. Most of the measurements of brain concentrations by both of these methods have shown no very great differences between the concentrations in brain and in blood (94, 39, 146, 63, 133). The outstanding divergence from this pattern is found in a report on the distribution of amobarbital in rabbits (142). By a gravimetric method depending on isolation of the drug by sublimation, it was found that the concentrations of amobarbital in the brain were as much as five times as high as those in blood. The blood concentrations were near the value predicted from uniform distribution. This report formed the basis of an argument by Fuhrman and Field (55, 54) that the concentrations studied *in vitro* should be compared not with the concentration calculated for uniform distribution *in vivo* but with a value four times this high. Most of the barbituric acids studied by these authors would produce significant degrees of inhibition *in vitro* at a concentration four times that resulting from uniform distribution of an anesthetic dose. Even at this concentration, barbital and probarbital failed to have much effect. Aside from the question of whether the drugs are actually concentrated to this extent in the brain, it is doubtful whether the logic of the argument is valid. If the drug were present in a much higher concentration in brain than in blood *in vivo*, as was reported for amobarbital, concentration might be expected to take place likewise *in vitro* from the suspending medium into the brain slices. Then the average concentration in the contents of the Warburg vessel would be more nearly comparable with the blood concentration than with the brain concentration *in vivo*. Even in the report on amobarbital (142) the blood concentration did not differ greatly from that expected from uniform distribution. The only concentration effect that would be expected to occur *in vivo* and not *in vitro* would be that due to passage of the drug from the blood into the brain at a more rapid rate than into other tissues. As a result of this effect, the initial period after an intravenous injection might be characterized by higher concentrations in both brain and blood than would be attained later when the drug is more uniformly distributed. This may well be an important effect with barbituric acids of rapid onset of ac-

tion, but would not be expected to be so with those drugs having slow onset and long duration of action.

An experimental technic that avoids the troublesome question of analytical methods and makes a direct attack on the problem of the relation between concentrations effective *in vivo* and *in vitro* is the use of blood from an anesthetized animal as the suspending medium for brain tissue *in vitro*. In this way it was shown (137) that the concentration of pentobarbital in the blood of dogs during anesthesia is sufficient to produce inhibitory effects on rat brain *in vitro*.

In the efforts to show that inhibition is produced *in vitro* by concentrations of drugs effective *in vivo*, attention has been focused almost entirely on the concentrations associated with full surgical anesthesia. This is the level of neurological derangement most readily studied in laboratory animals, but it should not be forgotten that definite effects, though more difficult of objective demonstration, are produced by far lower doses of drugs than are required for full anesthesia. For instance, in many human subjects unmistakable hypnotic effects appear after a dose of pentobarbital as low as 0.5 mgm. per kgm. Yet the dose of this drug required to anesthetize a man is of the order of fifteen times that amount (1). If it is considered of crucial importance to demonstrate an effect *in vitro* at concentrations effective *in vivo*, the concentrations associated with the first subjective effects would be more appropriate than those associated with the complete abolition of consciousness. These much lower concentrations have not been claimed to produce any demonstrable effects *in vitro*.

(ii) *Correlations between activities in vivo and in vitro*

The data available scarcely allow a judgment of the degree to which the potency of different types of anesthetics is correlated with their inhibitory activity *in vitro*. In any one study the number of drugs included has usually been small, and it is not possible to make comparisons with experiments performed by other workers with different technics. From what fragmentary data are available for comparison, it is evident that there is no very close correspondence between activity *in vitro* and *in vivo*. The largest series of closely related compounds subjected to the same experimental technic is the group of barbituric acids studied by Fuhrman and Field (55). Among the eleven 5,5-disubstituted barbituric acids in their series, the concentrations inhibiting oxygen consumption *in vitro* are correlated with the anesthetic doses to a degree that can be considered statistically significant. Irregularity in the relation between the two values is nevertheless conspicuous. In the discussion of physical properties in an earlier section of this review, mention was made of the hazards of basing theoretical inferences on correlations between two biological effects produced by a group of closely related compounds.

More convincing than a correlation between quantitative activities of anesthetics *in vitro* and *in vivo* would be the demonstration that the enzymatic inhibition supposed to be the cause of anesthesia is an effect produced only by anesthetics. This demonstration is wanting. Attention has been unduly directed toward the known anesthetics, but a sufficient number of drugs having other

types of pharmacological action have been studied to make it evident that inhibition of oxygen consumption of brain tissue is not an effect uniquely characteristic of anesthetics. Among the non-anesthetic substances that have been shown to inhibit brain oxidation *in vitro* are phenethylamine (128), α-(aminomethyl)-benzyl alcohol (128), tyramine (128), indole (128), isoamylamine (128), mescaline (128), atropine (129), scopolamine (129), cocaine (129), 5-(1,3-dimethylbutyl)-5-ethyl barbituric acid (57), 5,5-diphenyl-2,4-oxazolidinedione (54), metrazol (138) and picrotoxin (92). It is to be noted that the barbituric acid and the oxazolidinedione[6] as well as metrazol and picrotoxin are convulsants. There are some qualitative differences that have been reported in the effects produced by different drugs, but if there is any characteristic difference between the metabolic effects of convulsants and those of typical anesthetics, this has yet to be elucidated. The puzzling relationship between anesthetics and convulsants has been remarked upon earlier in this review. Even though a theory should contain some intimations of the true nature of anesthesia, it is a serious deficiency to be unable to explain the difference between anesthetics and convulsants. This is a deficiency that the metabolic theory shares with all other theories that now demand any serious consideration.

(iii) *Effect of anesthetics on the oxygen consumption of the brain in situ*

There have been several reports of a reduction of the cerebral arteriovenous oxygen difference produced by anesthesia (2, 154, 36, 99, 107, 45). However, since the rate of blood flow cannot be assumed to remain constant, these measurements are not necessarily indicative of a reduced rate of oxygen consumption in the brain. Studies including determinations of cerebral blood flow as well as oxygen content of blood are more pertinent to the present question. By perfusion of the vertebral arteries of the dog with a pump (134), it was possible to show that ether and morphine decreased oxygen consumption. Perfusion of the head of the dog (67) revealed a similar effect of pentobarbital. The cerebral blood flow of the monkey was measured directly with a bubble flow meter placed in the stream of blood passing through the carotid arteries, the basilar artery having been tied (135). In these animals, which were already anesthetized with pentobarbital and thiopental, an additional dose of thiopental caused a decrease in cerebral oxygen consumption. In more recent studies the indirect nitrous oxide method (88) has been found useful. This method applied to dogs under thiopental anesthesia (74) showed that the brain takes up oxygen at a slower rate in deep anesthesia than in light anesthesia. In man the nitrous oxide method has revealed a decrease in cerebral oxygen consumption during thiopental anesthesia (71). In another study, subanesthetic doses of thiopental and amobarbital failed to produce significant effects on the cerebral metabolism of human subjects (89).

[6] Despite the fact that the grossly observable effects of 5,5-diphenyl-2,4-oxazolidinedione are of an excitatory nature, the drug has antiepileptic activity. Association of excitatory and antiepileptic properties is also seen in diphenylhydantoin.

(iv) *Antidotal action of substances preventing inhibition of oxygen consumption*

If anesthesia were due to inhibition of oxygen consumption by brain cells, it might be expected that substances preventing the inhibition *in vitro* would have an antidotal effect *in vivo*. An opportunity might be offered not only of theoretical corroboration of the metabolic theory but also of development of a therapeutic procedure of practical value.

The oxidation of succinate not being inhibited by anesthetics as is that of glucose, lactate and pyruvate, it was suggested by Soskin and Taubenhaus (141) that anesthetics might be antagonized pharmacologically by succinate. Sodium succinate was accordingly tested as an antidote against pentobarbital and amobarbital in rats and was reported to be effective. Subsequent attempts to confirm the antidotal action of succinate against barbituric acids in laboratory animals have not yielded consistent results (11, 97, 125, 34, 38, 133). Some of the authors found smaller effects than those reported by Soskin and Taubenhaus while others failed to demonstrate any significant effect. In dogs anesthetized with chloralose, sodium succinate has been reported to have a stimulant effect on respiration independent of the chemoreceptors of the sino-aortic system (70). Succinate has also been tried in cases of human poisoning with barbituric acids. On account of the notorious difficulties in the appraisal of antidotes in any type of human poisoning, the reports of the few observations on man will not be cited here.

It might be thought that antidotal action of succinate or lack of antidotal action should constitute evidence for or against the theory of metabolic inhibition. However, even if it were not for the contradictory experimental results, no decisive theoretical inferences would be possible. If succinate does antagonize anesthetics, this need not be indicative of action through restoration of oxidative metabolism. De Boer, who found sodium succinate to shorten the effects of pentobarbital, thought that this action was due to diuresis (38). On the other hand, if succinate fails to show an antidotal action, this is not evidence that anesthesia is not caused by inhibition of oxidative reactions. It might be that even if succinate were oxidized by the brain in the presence of the anesthetic, the energy provided would not be utilizable for the maintenance of the critical metabolic process (97, 58). Furthermore, it is doubtful whether any significant amount of an intravenously injected dose of succinate is ever metabolized in the brain. In common with many other highly ionized substances, succinic acid appears to pass very slowly if at all from the blood to the brain (93). This would be sufficient to explain the failure of succinate to counteract the electroencephalographic effects of hypoglycemia in hepatectomized animals (110). If succinate is pharmacologically ineffective as an antidote against anesthetics, this likewise might be only the consequence of its failure to reach the brain. Lack of action would accordingly have no bearing on the theory of anesthesia.

Deficiency of ascorbic acid in guinea pigs has been reported to affect the reactions of the animals to barbituric acids. The sleeping time of the deficient animals was found to be prolonged when pentobarbital was given but not affected

in barbital and thiopental anesthesia (131). In another laboratory it was found that deeper depression was caused by pentobarbital and phenobarbital when guinea pigs were on a low intake of ascorbic acid (64). Although the authors of these reports did not have in mind any direct antagonism between ascorbic acid and the barbituric acids, it was later suggested (66) that there might be a connection between their results and the effect of ascorbic acid in counteracting the depression of oxygen consumption produced by pentobarbital in brain *in vitro*. However, the physiological derangements in ascorbic acid deficiency are so extensive and so complex that it would scarcely be safe to interpret the experiments as indicating a direct effect of the vitamin in antagonizing barbituric acids *in vivo*.

(d) General discussion of the theory of inhibition of oxidative metabolism

The measurements of oxygen consumption of brain tissue *in vitro* do not furnish very satisfactory support of the theory of metabolic inhibition. Even at concentrations that would cause deep anesthesia in the intact animal, the inhibitions of oxygen uptake are for the most part either small or not demonstrable at all. On the other hand, failure to demonstrate large inhibitions cannot be taken as convincing evidence that anesthesia is not due to inhibition of an oxidative reaction. There are two general lines of argument that have been used to discount the negative evidence.

Quastel and Wheatley (129) pointed out that the oxygen consumption measured is the average for a relatively large mass of tissue. If the inhibition were particularly intense at certain specific loci, this would not be manifest in the average value as measured. Quastel and Wheatley regarded the unequal influence of anesthetics on different neurological functions as evidence that the drugs are indeed present in unequal concentrations at different centers of the nervous system and accordingly exert unequal metabolic effects at different centers. There is no adequate reason for belief in any pronounced inequalities in the distribution of anesthetics in the brain. The reports of Keeser and Keeser (84, 85, 86, 87, 83), which were at one time quoted extensively as showing concentration of barbituric acids in certain parts of the brain, were based on methods that could yield nothing of quantitative significance. Other workers failed to confirm their conclusions (94, 146, 39). On account of the uncertain value of some of the analytical methods used for the determination of barbituric acids, the most convincing refutation of the idea of concentration has been furnished in the recent demonstration that barbital labeled with N^{15} is distributed essentially uniformly throughout the brain (112).

The unequal influence of drugs on different functions of the central nervous system does not demand acceptance of any assumptions of localization of drugs or even of chemical differences among the neurones. Bárány (4) has pointed out how specific functional effects of drugs might arise from anatomical differences in the synaptic organization of the different functions. Some explanation of the general nature of that of Bárány would appear more plausible than any involving specific physical localization of drugs. The different neurological effects of dif-

ferent anesthetics could be a reflection of differences in the fundamental nature of the physiological alterations produced in neurones by the various drugs and need not be attributed to differences in distribution of the drugs in the central nervous system. Even without unequal distribution of the drug, it is still conceivable that the effects on enzyme systems may not be equal in all cells of the brain. However, there is no direct evidence to support this view. Samples from different parts of the brain have not shown any great differences in respiratory inhibition produced by drugs (76, 72, 150).

There is yet another argument that has been used to discount the significance of any failure to observe large effects of drugs on oxygen consumption. Fisher and Stern (50) in a study of the relation of urethane concentration to depression of oxygen consumption by yeast found that this was not in agreement with that to be expected if urethane were reacting reversibly with a single enzyme in accordance with the mass action law. Although there are other possible interpretations (79, 108, 17), the authors showed that the relationship found by them was compatible with the assumption that urethane is acting upon two independent parallel respiratory systems, one of these being inhibited at a lower concentration of urethane than the other. Inhibition of the more sensitive of these two hypothetical systems would be complete at a concentration of urethane that is just sufficient to stop cell division. Fisher and his colleagues (48, 123, 49) found quite similar relationships for protozoa and fertilized sea urchin eggs and pointed out that published observations on brain slices and on luminous bacteria were also compatible with the hypothesis. This work led to the theory that the oxidative metabolism of cells comprises two distinct parallel systems, an "activity" system and a "basal" system. The former being the more sensitive to narcotics, the specific function that it supports might be abolished without great effect on other activities of the cell and with only a partial inhibition of the total consumption of oxygen. This theory has been elaborated upon by McElroy (108), who suggests that the inhibition of assimilative reactions will, by decreasing the amount of phosphate incorporated into esters, increase the concentration of inorganic phosphate and thereby accelerate carbohydrate metabolism.

Thus in its modern refinements the theory of inhibition of oxidative metabolism has become more and more elusive of experimental test. No amount of experimentation restricted to measurements of oxygen consumption can ever definitely refute it. Yet it must be conceded that the theory is left with a very weak structure of positive evidence to support it.

It is still not permissible to disregard the fact that inhibitions have been produced *in vitro* and that the oxygen consumption of the brain in the living animal is reduced in anesthesia. If these inhibitions are not the cause of anesthesia, what is their relation to anesthesia? Are anesthesia and inhibition of oxidation two unrelated phenomena both produced through independent mechanisms by the same drug, do they both have a common immediate cause, or is anesthesia the cause of the decreased oxygen consumption? One possibility that suggests itself particularly with respect to the brain *in situ* is that the anesthetic reduces the number of neuronal discharges occurring in a given period of time. This is an

effect that almost certainly does occur, and it is immaterial to the present argument by what mechanism the drug brings this about. Elimination of a discharge, by whatever means the elimination is accomplished, will abolish the extra oxygen consumption normally evoked by the discharge. It is noteworthy that Schmidt *et al.* (135) found that the cerebral oxygen uptake of the monkey changed in the same direction as cerebral functional activity, regardless of the cause of the change of activity. It is quite possible that the same interpretation is applicable to brain slices. Although there is no direct knowledge of the matter, it has been suggested by Larrabee (98) that there might possibly be considerable spontaneous activity on the part of the neurones in brain slices. The abolition of this activity by any mechanism would reduce oxygen consumption. Even in the absence of a discharge, it is conceivable that a drug might so alter the membrane that the energy required to maintain polarization would be reduced. Thus a theory postulating a physical alteration of the cellular membrane, such as has been discussed in an earlier section, might be able to account for the inhibition of oxygen consumption in the intact brain or in slices without the necessity of any assumption of a direct effect of the drug on the main respiratory pathway. The drug would be affecting respiration indirectly through the mechanism by which cellular activity regulates respiration.

A more accessible synaptic system, where activity is subject to experimental control, is available in the autonomic ganglia. In the excised superior cervical ganglion of the rabbit it has been shown by Larrabee and García Ramos (98) that synaptic transmission can be blocked with pentobarbital, chloroform, chlorobutanol, ether and alcohols in concentrations lower than those required to cause a measurable decrease in oxygen consumption. Although there is need for caution in assuming common physiological properties for synapses in autonomic ganglia and in the central nervous system, these experiments do furnish additional reason to doubt that decreased oxygen consumption of the brain is the cause of synaptic block.

It might appear that inhibitions produced in brain homogenates, where there are few intact cells, would be contradictory to the idea that the oxidative inhibition is secondary to decreased activity. However, it is not inconceivable that some related mechanism might come into play even in disrupted cells. The rate of oxygen consumption of a cell is obviously determined by the rate at which it expends energy, but the details of the mechanism by which this regulation is accomplished remain to be elucidated. Since adenosinetriphosphate (ATP) is regarded as the universal source of directly utilizable energy, the controlling mechanism might be supposed to involve phosphate, either inorganic or in high-energy compounds. Suggestions have been made of ways in which phosphate could determine the rate of oxidation or glycolysis (77, 96, 108). In any case, through the intervention of some mechanism presumably not involving direct alteration of the activity of oxidative enzymes, the rate of oxidative reactions can be caused to vary over a wide range. A drug affecting this regulatory mechanism could thereby affect oxygen consumption. Even in the absence of intact cells, it is conceivable that a part of the energy derived from oxidation could be stored

in some potential form through the mediation of energy-rich phosphate bonds and that a drug might, by preventing in some way the degradation of this potential energy, depress oxygen consumption.

Direct evidence that such a process can occur is lacking, but there are some fragmentary pieces of information that may prove to be relevant. In brain homogenates ATP is very rapidly hydrolyzed. This hydrolysis has been attributed to an adenosinetriphosphatase ("ATP-ase"), the greater part of which is bound to the particulate elements of the homogenate (117). Under certain conditions, the activity of the ATP-ase on the particles can be inhibited with octyl and decyl alcohols (118). ATP-ase activity in killed yeast is strongly inhibited by these alcohols as well as by phenylurethane, phenylurea, tolylurea and toluene (116). Whether or not the effect is really directly on the hydrolytic enzyme, the significant fact is that compounds ordinarily thought of as "narcotic" are capable of inhibiting the breakdown of ATP even in disrupted or dead cells. It is not difficult to conceive that this inhibition of hydrolysis of ATP could lead to a decreased rate of oxygen consumption. The fluoride ion, which inhibits brain ATP-ase (121), also inhibits oxygen consumption (120). It has not been shown, however, whether the former phenomenon is the cause of the latter. There is still little knowledge of the effects of anesthetics on phosphate metabolism. One study (44) of the effect of pentobarbital in brain homogenate in the presence of fluoride showed that phosphate uptake was inhibited by the anesthetic to the same extent as oxygen uptake when pyruvate was the substrate. With succinate as substrate, neither oxygen nor phosphate uptake was affected. The simplest view to take of these results is that the primary action of the drug is on the oxidative reactions and that the coupling of these with phosphorylation is unaffected. The possibility is not exluded, however, that the primary action could be on the phosphorylative mechanism rather than the oxidative mechanism. The absence of effect on succinate metabolism is unexplained whichever the primary action may be on pyruvate metabolism.

Scrutiny of the causal connection between decreased oxygen consumption and anesthesia has already led into the question of the other relationship under consideration, that between enzymatic inhibition and decreased oxygen consumption. The possibility has been suggested that a decrease in the rate of oxidative reactions might result from some mechanism other than direct action of the drug upon any enzyme lying in the main oxidative pathway. The question now arises whether this view is compatible with those previously cited experiments that were designed to locate the site of an oxidative block. It will be noted that all the inferences concerning the location of a block have as yet depended upon a process of exclusion. This fact invites a close search for any possibility that a block may not exist even at the non-excluded step. Moreover, for numerous drugs of diverse structure to have the same specific action on the same enzyme is a situation without parallel among other known types of inhibition and calls for careful consideration of any alternative hypothesis. The suggestion has been offered above that there may be such an alternative. Whether any of the experiments showing separate segments of the oxidative chain to be insensitive to

anesthetics are contradictory to this view is not clear. When the flow of electrons proceeds through abnormal channels as in these experiments, the normal yield of high-energy phosphate bonds may not be obtained. This is a circumstance that in turn can influence the rate of oxygen consumption and render the interpretation of the experiment difficult. It has been reported, for instance, that methylene blue, although accelerating respiration, disrupts the link to phosphorylation and replaces inorganic phosphate (105). If an anesthetic were depressing oxygen consumption by inhibiting the utilization of energy stored in phosphate bonds, methylene blue might abolish the depression of oxygen consumption or even bring the rate above normal.

Brilliant as have been the advances in knowledge of biological oxidations during the past few years, understanding of these processes is still in a primitive state. The complexity of the interrelationships prevailing among the enzymatic processes is enormous, and attempts to study any single part usually entail profound derangements of the coordination of the system in its entirety. It is not surprising then that it should prove difficult to discover the means by which a drug is affecting the function of the integrated whole.

The view that has been favored in the foregoing discussion is that cellular function is deranged not by an interference with the processes by which energy is acquired from the environment and is stored but with the process by which the stored energy is utilized. This is nothing more than a very general conception of the nature of anesthesia and is in no sense a complete or adequate theory of anesthesia. Present knowledge scarcely justifies an attempt to describe the process by which utilization of energy is blocked. This might conceivably be either by direct action on an enzyme or by some process, such as structural alteration of the membrane, not directly involving any enzyme. This hypothesis rejects the concept of oxidative inhibition as the cause of anesthesia. Anesthesia, on the contrary, can be viewed as the cause of oxidative inhibition or the two phenomena can be considered to have a common immediate cause. The present suggestions concerning the causal sequences are in accord with the experimental evidence indicating that conduction and excitation can be blocked without depolarization of the membrane. Energy derived from oxidative reactions is utilized in maintaining polarization. If the drug were interfering directly with oxidative reactions, it might be expected that this process, like simple anoxia, would result in depolarization. It must be conceded that the simplest and most obvious explanation of an inhibition of oxygen consumption is a direct action on one of the oxidizing enzymes. However, for the reasons that have been outlined above, any alternative hypothesis, even though it may be devious and strained and speculative, has attractions lacking in the simpler interpretation.

The present status of the theory of inhibition of oxidative enzymes may then be summarized as follows. The concentrations of anesthetics that are known or can reasonably be supposed to be associated with the lightest recognizable degrees of depression of the central nervous system have not been shown to produce metabolic changes in brain tissue *in vitro*. Concentrations associated with deep surgical anesthesia may in some cases produce demonstrable inhibition

of oxygen consumption *in vitro*. The same type of inhibition is also produced by some non-anesthetic drugs. It has not been conclusively demonstrated that inhibition of oxygen consumption produced by anesthetics is due to a direct action of the drug upon any enzyme in the main oxidative chain or, for that matter, upon any enzyme whatever. Even in cases where inhibition of oxygen consumption is found, a causal connection between this phenomenon and that of general anesthesia has not been established. Further experimentation confined to measurements of oxygen consumption would appear to be futile, since it cannot conclusively establish or refute the theory that anesthesia is caused by inhibition of an oxidative reaction.

Careless statements suggesting that nearly all effects of drugs are, or will doubtless prove to be, produced through specific actions on enzymes have appeared all too frequently and have enjoyed all too uncritical a reception. There is today a widespread predisposition to favor any pharmacological theory involving enzymes as being in accord with modern scientific concepts. If any distinction is made between actual accomplishments and fantasies of future developments, it must be realized that, as of the date of the present writing (the close of 1949), the number of pharmacological effects that can with any assurance be attributed to a direct action of a drug on a known enzyme is still very small indeed. General anesthesia is not among this number.

CONCLUDING REMARKS

Despite the numerous attempts to explain the phenomenon of general anesthesia, nothing has yet emerged that can be called an adequate theory. In the present inconclusive state of knowledge, preference for one theory or another is likely to be determined by the field of training or interest of the person attempting judgment. This is not to say that all proposed theories have been entirely devoid of any sort of validity or entirely unfruitful of practical results. There is reason to doubt whether the search for a single theory to explain the effects of all anesthetics may not have been a misguided effort. The metaphysical desire for unification on the part of Claude Bernard and his intellectual heirs among the general physiologists may well have delayed arrival at a more satisfactory solution. Even in this review, where an effort has been made to avoid the idea of an all-inclusive phenomenon of "narcosis," failure of a theory to achieve universal validity may have been used unjustifiably as an argument against it.

The long period that has elapsed since the first attempt at a theoretical explanation of general anesthesia has seen the development of large parts of the present-day structures of the experimental sciences of physiology, biochemistry and pharmacology. The numerous theories of anesthesia that have appeared during that time consequently reflect changing philosophical attitudes, the development of new experimental technics, and the appearance of new concepts in the physical and biological sciences. General anesthesia is probably the first pharmacological phenomenon to have evoked scientific theorizing in any modern sense, and theories of other types of drug action have been obviously influenced by the theories of anesthesia.

It may be permissible to make some general observations about the various theories of anesthesia, some of these observations being equally applicable to other pharmacological theories. Some theories have had as their sole basis experiments on some simple physical model that may or may not have any counterpart in the living cell. Some theories have been little more than loose speculation couched in a jargon borrowed from the physical sciences. This can be mistaken for scientific insight. New technics and new discoveries in related fields determine temporary vogues in experimental method and theoretical thought. This concentration of effort along a single line can be mistaken for an indication of the approach to the final solution.

Theorists concerned with anesthesia have had the advantage of having at their disposal a large and ever increasing number of drugs—a circumstance that should favor discovery of the essential property of the agents. On the other hand, they have been under a disadvantage in their concern with the most complex and inaccessible of biological systems, the central nervous system. So long as even the general nature of the process of synaptic transmission is a matter for controversy, it is not reasonable to expect a satisfactory description of the effect of a drug upon that process. If the attempt to explain the mechanism of anesthesia has hitherto been unsuccessful, it must be kept in mind that very few other pharmacological effects have been explained any more successfully. Vague references to enzymes and primitive diagrammatic attempts to represent cellular "receptors" are no adequate disguises for almost total ignorance.

If contemporary theories are not satisfactory, what would constitute a satisfactory theory? It will probably be conceded that what is desired by most biologists is an interpretation expressed in terms of the known behavior of non-living matter. Even aside from abstract philosophical questions, it is not obvious how complete a description of this sort can be achieved. The early theories of anesthesia were developed in a period when there was a general feeling that physical laws had received essentially their final formulation. The demonstration that the principle of conservation of energy is applicable (within the limits of experimental error) to living organisms had inspired confidence in many biologists that all phenomena of life would prove to be comprehensible in terms of the physics and chemistry of the day. The most extreme expression of this view was voiced early in this century by an influential biologist, who declared that "nothing . . . indicates that the artificial production of living matter is beyond the possibilities of science" (104). Opinions of this sort reflect not only the mechanistic philosophy but a propensity to discount the complexity of living systems. It is not surprising then that there was a time when serious consideration was given to pharmacological theories involving nothing more than some simple physico-chemical change in colloids. An era in which the recognized complexities of vital phenomena have been seen to multiply endlessly and in which it is realized that the constitution of non-living matter is yet to be fully described is more conducive to a cautious attitude toward the adequacy of current physical concepts to describe biological functions. Yet for the present purpose it is almost irrelevant to ask what range of biological phenomena are explicable in terms of

the physical concepts of 1949 and how completely they are explicable in those terms. There can be no doubt that the present knowledge of inanimate matter and even the present experimental technics are capable of yielding a much more complete and satisfying picture than has yet appeared. Each individual is privileged to adopt his own criteria of what constitutes a satisfactory explanation. Obviously no one can expect anything approaching a complete mathematical description of the behavior of a living cell. If there were no other obstacle, complexity is a factor sufficient to preclude any such achievement. On the other hand, theories no more complete or convincing than those hitherto formulated cannot be considered satisfactory from the point of view of ability to afford either intellectual gratification or predictions of practical value.

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