MECHANISMS OF GENERAL ANESTHESIA BY NON-HYDROGEN-BONDING MOLECULES[1,2,3]

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Neural activity can be disrupted in many ways. General anesthesia, a special case of reversible disruption, has been reviewed recently from various standpoints (23, 47, 48, 65, 74, 82). In this review, anesthesia is considered as one aspect of the molecular pharmacology of certain non-hydrogen-bonding compounds—the volatile "physical anesthetics"—that interact with biological systems by van der Waals attractions only. These compounds, all gases or volatile liquids of low chemical reactivity coupled with high biological reactivity, include the noble gases, chemically "unreactive" fixed gases, and aliphatic, alicyclic, aromatic, and halogenated hydrocarbons (Table I). Oxygen (66) and carbon dioxide (26) also show anesthetic effects, confounded by other biological interactions.

Theories of anesthesia are characterized by the critical phase with which the anesthetic molecules are considered to interact, e.g., lipoid (24, 54, 55), water (57, 58, 68, 69), protein (5, 73, 74), or membrane (8, 15, 39, 63). Unitary theories ascribe anesthesia to a primary action in a single phase, but simultaneous actions in more than one phase presumably occur. Wall (85) listed seven components of synaptic transmission, each vulnerable to interference by anesthetic molecules that could act by decreasing the excitatory effect or increasing the inhibitory effect of individual impulses, or by disorganizing the spatial and temporal pattern of bombardment, or by combinations of these actions. Reversible unconsciousness follows such diverse events as inhalation of chloroform, injection of thiopental, hypothermia, electrical stimulation of the brain, or cranial trauma. Furthermore, chloroform stimulates odor and taste receptors, inhibits enzyme activity, stimulates or inhibits reactivity in isolated neurons or axons, and stimulates,

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[2] The author wishes to acknowledge the excellent bibliographic aid supplied by the UCLA Brain Information Service, under Contract #DHEW-PH-43-66-59 to the NINDB.

[3] For supplementary bibliographic material (176 references) order NAPS Document 00090 from ASIS National Auxiliary Publications Service, c/o CCM Information Sciences, Inc., 22 West 34th Street, New York, N.Y. 10001. Remit with your order $1.00 for microfiche or $3.00 for photocopies.

TABLE I

ANESTHETIC POTENCIES

Compound	P_a[a] dog	P_a[b] mouse	Compound	P_a[b] mouse
	torr	torr		torr
N_2O	1,430	1,140	C_2H_6	988
Xe	904	836	C_2H_4	836
c-C_3H_6	133	83.6	C_3H_8	684
$CF_3CH_2OCHCH_2$	45.5	—	C_2H_2	646
$(C_2H_5)_2O$	23.1	24.3	CH_3CHF_2	342
$CF_3CClBrH$	6.6	12.9	C_3H_6	304
$CHCl_2CF_2OCH_3$	1.8	—	CF_2Cl_2	304
He	—	144,000	$CFCl_3$	114
Ne	—	>83,600	CH_3F	106
H_2	—	64,600	CH_3Cl	106
N_2	—	26,600	CH_3I	53.2
Ar	—	18,200	C_2H_5Cl	30.4
CF_4	—	14,400	C_2H_5Br	30.4
SF_6	—	5,240	CH_2Cl_2	22.8
CH_4	—	4,480	CH_3CHCl_2	19.8
Kr	—	2,960	$CHCl_3$	6.1

[a] Alveolar partial pressure to prevent response to painful stimulus, in dogs (24).

[b] Best estimate of inspired partial pressure to block righting response, in mice (54, 55).

anesthetizes or kills protozoa, paramecia, arthropods, fish, amphibia, birds, and mammals. Are common mechanisms, relevant to surgical anesthesia, to be found here?

Clearly, anesthetics cause such diverse effects at the molecular, cellular, tissue, and organism level, that the question of mechanisms may amount to: "Under a given set of conditions, what are the relative contributions of the various interactions, at the various sites of action, to the overall biological effect of interest?" Consciousness depends upon the integrity of many interdependent reactions and may be abolished by a sufficient perturbation of any one, or a cooperation of lesser perturbations. This review calls attention to many candidate perturbations, emphasizing those that occur within the range of 0.5 to 2.0 times the anesthetizing partial pressures and that take effect quickly. In the absence of a crucial experiment, theory must be based upon a convergence of experimental correlations. Exceptions can then be considered as secondary effects, to be resolved individually; the alternative is to risk discarding a useful theory because of a trivial exception.

PHARMACOLOGIC CONSIDERATIONS

Mechanisms of anesthesia may be considered at three levels: (*a*) the primary interaction between the anesthetic molecule and some molecular constituent or constituents of nervous tissue; (*b*) one or more intermediate

neural steps; and (*c*) reversible unconsciousness. Unconsciousness may be reached via progressive depression ("true anesthesia") or via disorganizing excitation (87); clinical "anesthesia" may thus reflect either depression or hyperexcitation. Either of these, in turn, can result from the identical primary interaction, since both excitation and inhibition are characteristic of anesthetic molecules (16, 67, 81, 87). A unitary primary mechanism therefore requires expansion to mechanisms of actions, to describe general anesthesia. There are so many levels between the primary interaction and unconsciousness that physicochemical correlations valid at the primary level may become distorted at the level under observation. Fortunately, electrophysiological techniques are clarifying the selective sensitivity of neural structures to anesthetics and pointing to the specific sites relevant to anesthesia (*vide infra*).

Pharmacokinetics.—Some effects of an anesthetic are manifested within a minute, others only after hours or days. Rapid effects, such as excitation or anesthesia, are certainly related to the molecules administered. Slow effects may result from an initial interaction relevant to anesthesia mechanisms, or from a slow irrelevant reaction, or from metabolic products of the anesthetic. Slow effects include arrest of mitosis by many anesthetics, including ether, nitrous oxide, propane, and argon (3); impaired embryogenesis by cyclopropane (4); and impaired hemopoiesis by xenon (1). A better understanding of the pharmacokinetics of inhalational anesthetics is needed, for proper correlation of laboratory results under steady-state conditions with clinical experience under dynamic conditions. Paton & Speden (67) derived the expression: $\log (C_1 - C_e/C_1) = -kT_1$, where C_1 is the concentration of anesthetic agent that produces anesthesia in time T_1, C_e is the equilibrium concentration, and k is a proportionality constant. The exponential relationship emphasizes the slow final approach to true equilibrium and the impracticability of reaching it in a real experiment, if the noxious effects of excessively prolonged anesthesia are to be avoided. Cerebral circulation is an added source of variation; cerebral blood flow measured with Kr^{85} was increased by ether, chloroform, and possibly halothane, but not by trichloroethylene (49).

Potency.—The ratio of the partial pressure of an anesthetic in the gray matter of the brain to the inspired partial pressure varies widely with the agent, its inspired partial pressure, and the duration of administration (50). After 60 min, the partial pressure of ether in the gray matter is computed to be 25 per cent of the inspired partial pressure; the corresponding figure for halothane is 61 per cent and for nitrous oxide or cyclopropane it is 93 per cent (50). In most experiments, involving less than 30 min inhalation, the differences are even more marked. Thus, potency values based on inspired partial pressures are probably skewed in the direction of assigning relatively lower potency to compounds with slower uptake, i.e., with higher solubility in water. Use of the steady-state MAC (minimum alveolar concentration to prevent response to tail clamp or 40V shock, in volume per cent at 1 atm ambient pressure) eliminates confounding variables of uptake

and distribution by allowing 15 min after reaching a constant alveolar partial pressure, or by saturating the animal with the more soluble gases, before testing for anesthetic effect (24). In dogs, the equipotent MAC's are: methoxyflurane (0.23), halothane (0.87), ether (3.04), fluroxene (6.0), cyclopropane (17.5), xenon (119), and nitrous oxide (188). These values correlate more closely with the oil/gas partition coefficient than with vapor pressure, or hydrate dissociation pressure at 0°C, or other physical constants.

Dose-response.—A characteristic property of anesthetics is the steep quantitative dose-response relationship, conveniently expressed as the slope function, i.e., the ratio between two doses that cause responses differing by one probit. This corresponds, for example, to increasing the proportion of animals anesthetized from 31 per cent to 69 per cent. Reported slope functions are 1.20 to 1.28 for N_2O, SF_6, Ar, and N_2 in mice (12), 1.06 to 1.23 for N_2O, SF_6, N_2, CF_4 and CF_2Cl_2, in mice (55), and 1.10 to 1.35 for ether, chloroform, halothane, and methoxyflurane in goldfish at 20° to 30°C (17). The small slope functions reflect a small individual variability to the anesthetic effect.

Dose-effect.—A small change in the dose of an anesthetic causes a large change in the qualitative effect. For example, the anesthetic and the lethal partial pressures are often separated by a factor ("therapeutic index") of less than 2.0 (16). This suggests caution in appraising the relevance to mechanisms of anesthesia of interactions in the lethal range, i.e., at partial pressures much more than 2.0 times the anesthetizing partial pressure.

At low doses, stimulatory effects are characteristic of anesthetic molecules and are usually ascribed to selective depression of inhibitory neurons or synapses, but there is abundant evidence for direct stimulation (16). Sensitization or stimulation by anesthetics occurs in pulmonary stretch receptors, baroreceptors, chemoreceptors, muscle spindles, frog sciatic nerve, ganglion cells of *Limulus,* the mammalian sympatho-adrenal system, guinea pig intestinal nerve, smooth muscle (67). All volatile anesthetics have an odor, i.e., they directly stimulate the olfactory receptors. Parallelisms between olfaction and anesthesia have attracted attention (62). Discussions of olfaction theory are cited in reviews by Wenzel & Sieck (86) and Moulton & Beidler (61). Eyring's theoretical analysis of olfaction (28), based on the Davson-Danielli membrane model, appears applicable to the stimulatory effects of anesthetic molecules.

The non-hydrogen-bonding compounds with anesthetizing partial pressures below 1 atm stimulate olfaction; Mullins (62) suggested that a molecular volume of about 100 cm^3/mole appeared optimal. The C_3 and C_4 fluorocarbons, with molecular volumes (117 to 147 cm^3/mole) near the optimal range, are exceptions since they are odorless or nearly so. They also have unusually low anesthetic potency ($P_a > 600$ torr). In contrast, perfluorobenzene has a strong odor and is a potent anesthetic ($P_a = 6$ torr). A quantitative comparison of the olfactory thresholds and anesthetizing partial pressures of pure perfluorocompounds would be of interest. Computer

analysis of molecular model silhouettes, used to correlate molecular shape with odor (2), offers promise in correlating molecular shape with anesthesia.

There are, of course, differences between anesthesia and olfaction. Olfaction is stimulated at much lower partial pressures than anesthesia (16) and has a much flatter dose-response relationship; for example, the slope functions for pentane, hexane, and heptane in rats (35) may be calculated as approximately 100 to 10,000.

Stimulation of other senses; excitation; convulsions.—The relationships between olfactory, excitatory, and convulsant action have been pointed out (62). The evidence for interaction of anesthetics with sensory systems other than olfaction is more indirect. The receptor site concept of Hodgson (38) provides a theoretical accommodation for nonspecific chemosensitivity of all sensory receptors. Comparison of taste with anesthesia appears not to have been explored, possibly because the compounds of interest (76, 88) are so different. The sweet taste of chloroform and other compounds has been ascribed to interactions based on London dispersion forces (76); aqueous solutions of halothane and methoxyflurane also taste sweet. A systematic study of the taste of non-hydrogen-bonding anesthetics might produce a useful correlation.

Electrophysiology provides a tool for tracing the sequence of events during anesthesia induction, maintenance, and recovery (80, 87). Of great interest are the widespread findings of neural excitation as the characteristic immediate effect of most anesthetics. Since excitatory effects usually precede depressant effects, occur at lower partial pressures, and are sometimes prolonged, the state of excitation may be as relevant as the state of unconsciousness to the mechanisms of interaction of anesthetic molecules with neural systems. In the case of the inhalation anesthetic agents, a pure central nervous system depressant activity is unknown; transient or prolonged excitatory effects are so widespread (13, 15, 16, 32, 39, 43, 53, 60, 67, 81, 87) that they may characterize such agents fully as well. At the level of gastropod ganglion cells, "it appears that excitation and inhibition are two facets of the same mechanism" (81). Cyclopropane, halothane, and ether increase preganglionic sympathetic activity in rabbits (53). Ether causes a characteristic prolonged behavioral excitation in the "Shaker" mutant of *Drosophila* (43); this observation opens the exciting prospect of a genetic approach to mechanisms of anesthesia.

Winters et al. (87) reported a progression of changes in cats under γ-hydroxybutyrate that suggested "a continuum of electrical patterns similar to the stages induced by increasingly more potent CNS excitant agents," as follows: desynchronization, intermittent hypersynchrony, continuous hypersynchrony, spikes, spikes with electrical silence, and generalized seizures. Nitrous oxide (80 per cent), ether (17.5 to 35 per cent), and trichloroethylene (2.5 to 5 per cent) caused hypersynchrony, with the latter compound causing all states up to generalized seizures. At slow rates of administration, halothane (2 to 6 per cent) also showed hypersynchronous activ-

ity. Winters et al. (87) criticized the classification of clinical anesthetics as CNS depressants and proposed three classes, each inducing levels of unresponsiveness considered appropriate for surgical procedures, but by three different mechanisms: hallucinatory, epileptoid, and true general anesthesia. Hallucinatory effects, shown by ether, nitrous oxide and trichloroethylene, include inappropriate movements and hypersynchrony. Epileptoid effects, shown by trichloroethylene, include myoclonic jerks, generalized seizures, spikes or generalized seizure waves, or both. True general anesthesia, shown by ether and halothane, includes muscle relaxation, progressive EEG flattening, and low voltage burst suppression. At very slow rates of administration, even the "true general anesthetics" halothane and pentobarbital showed evidence of excitation. Electrophysiological comparisons under steady state, exactly isonarcotic partial pressures in the brain would be of great interest.

The clinical convulsant agent flurothyl ($CF_3CH_2OCH_2CF_3$) may be regarded as diethyl ether modified by fluorination in such a way as to become "a potent, but clinically unusable, CNS depressant which produces a highly exaggerated version of stage II anesthesia" (40). This is a reasonable interpretation of several experiments. Flurothyl induced reversible unconsciousness in goldfish (16) at a supraconvulsive concentration (3m*M*, at 10°C) and blocked excitability of the frog sartorius muscle at 23.2 m*M* (40). Sub-anesthetic doses of ether, fluroxene, methoxyflurane, and halothane completely suppressed the convulsant effect of flurothyl in mice (13). Mixtures of fluorothyl in certain ratios with ether, methoxyfluorane, fluroxene, and ISO [$(CF_3)_2CHOCH_3$] were "inert," i.e., they caused neither behavioral excitation nor depression; other ratios accentuated the depressant effects but not the excitatory effects of the two agents in the mixture (13).

Toxicity.—In a number of studies, anesthetics were used for prolonged periods ($>$ 1 hr) or at concentrations exceeding three times the anesthetic level; they merit mention because of their relevance to the mechanisms of toxic effects. Cyclopropane (3 to 12 hr) increased abnormalities and mortality in 4-day chick embryos (4). Multiplication of mouse heteroploid or sarcoma I cells in tissue culture was inhibited by 96-hr exposure to nitrous oxide, chloroform, ether, fluroxene, halothane, methoxyflurane or trichloroethylene (31). Xenon, krypton, and nitrous oxide reversibly inhibited sodium active transport across frog skin and conduction in frog sciatic nerve; the conduction blockade pressure was correlated with the molecular polarizability (36). Cerebral motor cortex excitability and EEG were considered most suitable for analysis of the mechanism of toxic action of non-hydrogen-bonding compounds used as solvents (52).

Mechanisms of Action

Danielli (20) cautioned against "theories which postulate similar mechanisms of action for all agents producing similar biological responses" and "superficial interpretations based simply on correlations between various

physico-chemical and physiological properties." In the absence of a crucial experiment, such caution is especially necessary, in appraising the convergence of evidence that provides the support for a given theory.

Recent reviews of mechanisms of anesthesia have emphasized: physicochemical correlations (47); van der Waals forces (74); comparisons of current theories (82, 85); transport and neurophysiology (23); and biochemical mechanisms, including stabilization or block of presynaptic or subsynaptic membranes, interference with the sodium pump mechanism, interference with adenosine triphosphate production, and increased production of inhibitory transmitters (48); and effects on physiological systems (65).

Anatomic sites of action; selective sensitivity.—A complete theory of anesthesia must account for the fact that certain neural structures are little affected by concentrations of anesthetics that largely suppress the activity of other structures (15, 21, 33, 45, 60, 72, 78). Simultaneous recordings in different single neurons in the visceral ganglion of *Aplysia* (sea hare) showed selective sensitivity to halothane and chloroform (15). Individual motoneurons of mammalian spinal cord varied widely in sensitivity to ether (78). Ether (10 to 15 per cent), halothane (0.25 to 2.0 per cent) or cyclopropane (40 per cent) depress response to preganglionic but not postganglionic electrical stimulation in the spinal dog (33). Atropine or nicotinic blocking agents increase the depressant effect; the indicated action is decreased release of transmitter from preganglionic nerve terminals or depressed sensitivity of the postganglionic sites to the transmitter (33). Halothane (2 per cent) blocked transmission of cutaneous impulses in the monkey without blocking impulses from joint movements; the increased effect upon nonlemniscal afferent pathways was proposed as a fundamental property of anesthetics (21).

The recovery cycle of the cat primary auditory cortex was depressed by halothane and methoxyflurane when the amplitude of cortical-evoked responses was not depressed (45). In the *encéphale isolé* cat, ether (3 or 5 per cent) and nitrous oxide (50 or 75 per cent) depressed evoked click potentials in both the auditory cortex and inferior colliculus; halothane (1 or 3 per cent), however, depressed cortical activity more than subcortical (72). Ether and halothane caused marked depression of reticular activity in the cat, while auditory input was minimally affected (60). The visually evoked response recorded in the cortex of the monkey persisted during anesthesia with halothane or methoxyflurane, except at levels sufficient to cause hypotension (22).

Folkman et al. (32) diffused volatile anesthetics directly into a localized region of the cat midbrain through an implanted silicone rubber membrane; nitrous oxide had no effect, teflurane or methoxyflurane caused sleep and hypalgesia, and ether or halothane caused severe seizures. The technique may permit identifying the site of action with isotopically labeled anesthetics.

Lipid phase.—The Meyer-Overton theory was based on the correlation

of anesthetic potency with oil/water partition ratios. A better correlation exists, however, with oil/gas ratios (24, 54, 55). The correlation of anesthetic potencies (Table I) with solubility in olive oil was better than with ideal solubility, solubility in water, heat of solution in water, or hydrate dissociation pressure at 0°C (54, 55). The nonideal solubility behavior of fluorinated compounds was considered to afford an opportunity for assessing divergent theories of anesthesia (54, 55). CF_4 and SF_6 showed the greatest deviations from predicted potencies; the deviations from predictions based on hydrate dissociation pressure were so large as to cause K. W. Miller, Paton & Smith (54, 55) to question the validity of regarding the aqueous phase as relevant to anesthesia. The value used for the dissociation pressure of CF_4 hydrate (1 atm) was, however, too low by a factor of 41.5 (59). The fit of the corrected values to the regression line relating potency to hydrate dissociation pressure is now not unreasonable.

The "unusual set of physical properties" of the saturated fluorocarbons —low boiling points, surface tensions, refractive indices, dielectric constants, and solubilities—are well known (6). Sulfur hexafluoride shows anomalous behavior in various biological systems, for example, in depressing the growth of *Neurospora crassa* and the activity of acetylcholinesterase (75).

Aqueous phase.—Pauling (68, 69) and S. L. Miller (57, 58) proposed water as the primary reactant with the non-hydrogen-bonding anesthetic molecules. Structural effects in water are receiving growing attention, as key phenomena affecting biological systems (27, 34, 51, 64). Eisenberg & Kauzmann (27) have written a critical summary of the most important data and theories of the structure of water. The structuring effect of xenon on liquid water at 21°C, shown by nuclear magnetic resonance, indicates that "in essence, the xenon lowers the effective temperature of the water" (34). Mautner (51) has reviewed the role of water structure in drug action and has suggested how water structure could influence enzyme reaction rates. Némethy (64) favors the concept that structures similar to those in the gas hydrates, but incomplete, must exist around hydrocarbons and the hydrocarbon moieties of organic compounds. He cautions that the ordering of water around dissolved macromolecules and at interfaces, facilitated by nonpolar compounds, must be limited in extent and fluctuating with time, but still sufficient to slow the penetration of reacting groups; however, anesthesia need not require more.

Cyclopropane forms both Structure I and Structure II hydrates (58). Crystalline hydrates of halothane (mp 7°C), and of $CFCl_3$ (mp 14.5°C) have been prepared with the aid of H_2S as a helper gas (83). Fernández-Morán (30) published an electron micrograph (X1,330,000) of lecithin micelles exposed to Ar, with electron-dense structures about 10 to 20Å in diameter, that he interpreted as representing hydrate microcrystals. Jeffrey & McMullan (41) reviewed the structures of clathrate hydrates, including stable structures that involve ionic or hydrogen-bond forces in addition to van der Waals forces, and have melting points as high as 62°C; they anticipate that future research will uncover clathrate crystal structures not

yet known. Kamb (42) described a clathrate crystal of silica, analogous to the clathrate hydrates of composition $6X \cdot 46H_2O$ or $8X \cdot 46H_2O$, with the interesting ability to hold hydrocarbons that are too large to fit within a single polyhedron. Kamb speculated that straight-chain hydrocarbons might fit into two adjacent tetrakaide cahedral cavities, with the "waist" of the carbon-carbon bond accommodated within the hexagonal opening between adjacent cavities. This concept suggests a plausible structure for hydrates containing long-chain anesthetic molecules, under the Pauling theory.

Pauling (68) suggested that mixtures of different hydrate-forming classes "would act to some extent synergistically (and also to some extent competitively . . .)." S. L. Miller (57) suggested that gases forming Structure I hydrates would act additively in mixtures; for Structure II hydrates, nonadditive effects would be observed if the occupied sites were not independent. Specific efforts to test these suggestions have been interpreted as showing additivity, only. For example, a mixture of Xe (Structure I; ~ 0.33 MAC) and halothane (Structure II; ~ 0.67 MAC) acted like 1 MAC of either Xe or halothane alone (19). The simple additivity was considered to be inconsistent with the suggestion of potentiation but consistent with lipid theory. Caution needs to be exercised in interpreting such results. First, the expected extent of potentiation has not yet been calculated. Second, it is not clear from the data how much potentiation would be required to permit detection by this method and synergism determination is a highly complicated problem. For example, ether, chloroform, nitrous oxide, cyclopropane, and halothane show additive and synergistic effects with a wide variety of hydrogen-bonding anesthetics and other drugs (79). Indeed, ether and curare combine to produce complete neuromuscular block, at doses of each that are ineffective alone (44). Action at more than one synapse in a chain can play an important role in the pattern of drug action (10). The fact that compounds of different types, and action at different sites, can show additivity or potentiation makes difficulties for any unique interpretation of potentiation effects of non-hydrogen-bonding compounds.

When S. L. Miller (57) suggested study of the temperature-dependence of anesthesia as a test of the hydrate theory, he pointed out that temperature-dependence would be complicated by temperature effects on nerve activity and metabolism. This objection was raised (29), and rebutted (18), to the conclusion that anesthetic potency in goldfish decreases with rising temperature (17). Eger, Saidman & Brandstater (25) considered that their findings of reduced anesthetizing concentration of cyclopropane or halothane at reduced body temperature in dogs could not be explained on the basis of a depressant effect of cold alone. Regan & Eger (70) suggested that a direct anesthetizing effect of cold in the dog augumented the increased potency of cyclopropane, ether, halothane, methoxyflurane, and fluroxene caused by reduced temperature (27°C). They also provided systematic data on partition coefficients over a range of temperatures (approximately 25° to 37°C). Water/gas and oil/gas coefficients increased logarithmically with fall in temperature. Oil/water coefficients calculated from their data show irregular temperature dependence; cyclopropane decreases with

rising temperature, ether increases, halothane and methoxyflurane show a minimum at about 30°C, and fluroxene shows a maximum. The poor correlation between the oil/water partition ratio at 37°C and the anesthetic potency in normothermic dogs throws into question the oil/water correlations used to support the Meyer-Overton theory, as well as efforts to relate temperature-dependence to potency through parallel effects upon oil/water partition coefficients.

Regan & Eger (70) discuss the confounding variables affecting uptake and distribution, in determining anesthetic potencies and anesthetic indices, and the complex role of temperature changes in homeotherms. Acclimated poikolotherms appear better suited for temperature-dependence studies. Carpenter (11) demonstrated in *Aplysia* neurons a membrane potential hyperpolarization of about 1.5 mV per °C rise and suggested that the increased excitability of mammalian neurons under hypothermia is also because less depolarization is necessary to reach the critical firing threshold.

Schreiner (75) examined the biological effects of the helium-group gases and hydrogen, nitrogen, and nitrous oxide at the molecular, subcellular, and cellular level; in general, increasing potency was correlated with increasing molecular polarizability.

Protein interactions.—Structural changes of solvent water underlie the hydrophobic interaction between hydrocarbon-like groups, e.g. alanyl and leucyl residues of proteins. A novel model system (14) for studying hydrophobic interactions utilizes glass beads (0.2 to 0.3 mm) coated with methyl groups by treatment with dichlorodimethyl silane vapor. Aggregation of the beads when immersed in aqueous solutions was judged analogous to hydrophobic bonding; addition of compounds affecting water structure caused dispersal. Anesthetic potency has been correlated with hydrophobic interaction strength between anesthetics and proteins, but at partial pressures approximately 20 times the anesthetizing partial pressures (5). Furthermore, hydrophobic bonds become stronger with increasing temperature, up to about 65°C (64) whereas anesthetic potency decreases with increasing temperature (17, 25, 70). It appears that hydrophobic interactions play only a minor role in mechanisms of general anesthesia with non-hydrogen-bonding molecules.

Schoenborn & Featherstone (74) have stressed the small size of molecules of anesthetics compared with the large size of proteins or lipids that make up membranes; typical proteins are 300 times larger than a large anesthetic molecule like halothane (M=197). Thus, the direct interactions can involve only a very small fraction of the entire macromolecule. Using X-ray diffraction analysis, Schoenborn (73) demonstrated specific binding of xenon to hemoglobin, methemoglobin, metmyoglobin, and deoxymyoglobin, and of cyclopropane to myoglobin. Such direct binding represents an additional mechanism that may be involved in anesthesia, and correlation of the binding forces with anesthetic potencies would be of interest.

Membranes.—Membrane effects are certainly involved in anesthesia; any lipid, aqueous, or protein interactions can obviously occur in or at membranes. Alternative models of membrane structure (71) may force

reconsideration of theories of anesthesia based on the Davson-Danielli model. Mullins (63) emphasized that oil/water partition coefficients give excellent correlation with anesthetic potency for small molecules but poor correlation with larger molecules. The correlation of anesthetic potency of large molecules (e.g., $CHCl_3$, $C_2H_5OC_2H_5$, and C_6H_6) with uptake by hemoglobin crystals was much better. He suggested that the anesthetic molecules plug channels in the nerve fiber membrane, obstructing passage of Na^+ and preventing repolarization after excitation. It should be noted that his Na^+-efflux data were based on the effect of ether at 95 m*M* on the squid axon at 20°C; the anesthetizing concentration for goldfish at 20°C is 29 m*M* (17); for nonsynaptic pathways in the cat ganglion it is 96 m*M*; and for synaptic pathways it is 34 m*M* (46). The argument for relevant membrane involvement would be strengthened by showing efflux and conduction depression at ether concentrations nearer 30 m*M*.

At anesthetic levels, nitrous oxide, halothane, methoxyflurane, cyclopropane, and ether had variable effects on transport rates of various monosaccharides into erythrocytes, used as a membrane model; glucose transport was not affected (37). In the frog sartorious model (39), low concentrations of ether in Ringer's solution, corresponding to 1.1 to 2.8 per cent ether vapor, caused excitation; depression occurred at higher concentrations, with complete block at concentrations corresponding to 11 per cent ether vapor. The mechanism of ether block was considered to be antagonism of the sodium conductance through the membrane and this antagonism was proposed to represent "the basic mechanism of action of all general-anesthetic agents" (39). Ringer's solution equilibrated with vapor of ether (3 per cent), cyclopropane (>20 per cent), or halothane (1.5 per cent) produced complete neuromuscular block in the frog sartorius preparation at room temperature, attributed to reduction of the sensitivity of the postjunctional membrane to acetylcholine (44). Axonal conduction in the isolated frog sciatic nerve trunk was not affected. The partial pressures were within 1.0 to 1.7 MAC (if normalized to 37°C, they correspond to about 3 to 5 MAC). Chalazonitis (15) studied the effects of halothane, ether, and chloroform at the single neuron level. He observed in neurons of the sea hare or snail a transitory hyperexcitability, ascribed to brief depolarization of the neural membrane, followed by depression of membrane excitability, ascribed to increase of the membrane conductance by the anesthetic molecules.

Bennett & Hayward (8) measured changes in K^+, Na^+ and Cl^- in the cerebrospinal fluid of cats exposed to nitrogen (8 atm) or argon (8 atm), each with oxygen (2 atm). Sodium and chloride concentrations decreased, as compared to controls using helium. They concluded that the electrolyte changes reflected adsorption of nitrogen and argon on cell membranes, altering permeability to ions, and that this is the basis of inert gas anesthesia. It should be noted that the mean decrease of Na^+ and Cl^- were small (4.1 to 8.0 per cent).

Van Dyke & Chenoweth (84), impressed by the metabolic transformations undergone by ether, chloroform, halothane, and methoxyflurane, suggested that a particular chemical reactivity might be essential to the anes-

thetic effect of certain volatile compounds, but it seems difficult to reconcile slow biotransformation with swift anesthetic effect.

Paton (66) suggested that oxygen be considered an anesthetic gas, except that it enters into oxidative processes, and that its convulsive and depressant properties fall into place with those of the nonspecific gases, with a P_a *of* >11 atm. Carbon dioxide may be similarly considered although Eisele, Eger & Muallem (*26*) concluded that CO_2 anesthesia (MAC = 32 per cent) results solely from its hydrogen ion effect and not from any inert gas property. CO_2 and N_2O have similar molecules but CO_2 is six times as potent as N_2O; presumably, the other physiologic effects of CO_2 do augment its inert gas effect.

Helium at 125 atm was nonanesthetic in mice and at 135 to 145 atm it was lethal; neon showed similar effects (56). Hydrostatic experiments in newts showed equivalent loss of the righting reflex at 165 to 245 atm, independently of whether the pressure was applied by He, by Ne, or hydrostatically (56). Pure pressure effects during experiments above 100 atm may add to the gas effect, with the result that the latter appears augmented.

A molecular sieve model was used (9) to argue for electrostatic interactions between anesthetic molecules and charged sites of lipid cell walls, as being more reasonable than the hydrate theories. The model was criticized on the basis that molecular sieves sorb guest molecules into cavities rather than on surfaces (*77*) and that the sorption of nonpolar gases by biological systems is due mainly to dispersion forces (*7*) rather than to the postulated electrostatic forces.

Conclusions

Mechanisms of action.—There are as yet no facts that permit confident exclusion of any current theory of anesthetic action. Anesthetic molecules that are "unreactive" chemically are so highly reactive biologically, at anesthetizing partial pressures, that multiple primary interactions probably occur with water, lipids, and proteins, in membranes and elsewhere. The subsequent steps may lead to clinical anesthesia via hyperexcitation or depression. The direct excitatory effects characteristic of sub-anesthetic partial pressures merit increased attention. Reliable potency values, for precise correlation with accurate physicochemical parameters, require careful attention to the details of pharmacokinetics, dose-response, dose-effect, pressure effects, and temperature-dependence. Temperature-dependence (*17*) and synergism (*19*) are not likely to yield a crucial experiment because the results are open to divergent interpretations.

The impetus given by modern theories has already resulted in increased attention to the important roles of water structure and of "inert gases" in biological systems, improved physicochemical data on anesthetic compounds, and more reliable potency values. Promising avenues of future research include study of "exceptional" compounds, such as the fluorocarbons (55), focusing upon the sites that are selectively sensitive to the effects of anesthetics (60), direct administration into localized brain regions (32), further analysis of hyperexcitation vs depression (87), configuration analysis by

computer (2), and other approaches borrowed from research on odor, taste, and toxicity (16), and genetic approaches using favorable mutants (43).

Clinical implications.—A few rules-of-thumb from research into mechanisms of general anesthesia confirm clinical experience. Anesthetic agents show marked changes in effect, with small changes in dose. They can be expected to show about a 15 per cent fall in potency for each 2°C rise in body temperature, and a 15 per cent rise in potency for each 2°C fall. Combinations of anesthetics can be expected to display an approximately additive depressant effect. Prolonged anesthesia, especially with compounds subject to metabolic transformation, e.g., the halogenated anesthetics, can be expected to result in toxic effects not seen in brief anesthesia.

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