THE PERIPHERAL EFFECTS OF ANESTHETICS[1,2]

By Milton H. Alper and Werner Flacke

Departments of Pharmacology and Anesthesia, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Massachusetts

Peripheral effects of general anesthetic agents are defined in this review as those effects exerted on organs and tissues outside the central nervous system. On one level, they can be considered to encompass a multitude of "side effects," both desirable and undesirable in clinical anesthesia. In this review, however, we are more concerned with assembling evidence, garnered from studies of the actions of anesthetics outside the central nervous system, which may provide us with some insight into the way these drugs work in any living structure. Indeed, most of the investigation of the basic actions of anesthetics has perforce been carried out in "peripheral" systems because of their easier accessibility and simpler organization.

Anesthetics are also of considerable general pharmacological interest since they probably act not by interacting with chemically specific tissue "receptors" (1) but rather by affecting the function of living tissue in a different, less specific way. General anesthetics include a wide variety of drugs of differing chemical structures; specific antagonists are not known, and there are remarkable correlations between certain physicochemical properties of these drugs and some of their pharmacological actions. We intend, therefore, to review some of the phenomena produced by anesthetics in a variety of peripheral systems in an attempt to draw certain inferences regarding the mechanism of action of this important class of drugs. We shall not discuss the intermolecular forces which may be involved, since that has been the subject of several recent reviews (2–6) and is covered in this volume by the contribution of Cherkin.

Claude Bernard (7) was probably the first to recognize that the ability to be "narcotized," i.e. to undergo a reversible inhibition of biological activity, is a general property of living matter, demonstrable in an amazing variety of biological systems. For example, he observed that narcotic agents reversibly inhibited the activity of *Paramecia*, phagocytosis, germination of spores, and growth of yeast. His reports stimulated a great deal of investigation over the ensuing 50 years. The results are summarized in Winter-

[1] The survey of the literature referred to in this review was concluded in July, 1968. The authors apologize for any omissions which may have resulted from either limitations of space or oversight.

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stein's monograph (8) which is highly recommended for anyone who wishes to inform himself of this extensive, and quite fascinating, albeit qualitative, body of work. For example, the biphasic nature of the action of anesthetics was pointed out by Johannsen (9) who demonstrated in 1902 that the inhibitory effect of anesthetics on germination and plant growth was preceded by a stimulatory effect.

Growth and Function of Relatively Unspecialized Cells

The study of "narcosis" in single cells and other relatively simple systems continues to intrigue investigators, who are often apparently unaware of their predecessors. Among the more recent studies is the demonstration that xenon inhibits the movement of *Paramecia* (10) and that the helium group gases (11, 12) and nitrous oxide (13) inhibit the growth of the mold, *Neurospora crassa*. In *Neurospora*, xenon and krypton, but not helium, reversibly inhibit protoplasmic streaming (14).

Of somewhat greater interest is the recent observation (15) that exposure to both halothane and ether reversibly abolished motion and protoplasmic streaming in the giant amoeba, *Chaos chaos*. The protoplasm was segmented into two distinct zones, a central granular area and a peripheral clear zone, with the latter developing a positive charge of 65 mV with respect to the central zone in the absence of an identifiable membrane. The authors speculate that the anesthetics interact with colloidal elements of the cell plasma to change its structure and create an electrochemical potential. This "stiffening" of the cytoplasm, also seen in leukocytes *in vivo* in the bat wing, was suggested as the mechanism whereby halothane abolishes the extravasation of leukocytes into the peritoneal cavity of mice in response to endotoxin (16). These actions may be of importance in the microcirculation and in the response to injury.

Another area of recent interest is the effect of anesthetics on cell growth and division. Gormsen in 1955 (17) described the development of thrombocytopenia and agranulocytosis in a patient with tetanus, treated by the prolonged administration of nitrous oxide. The inhibition of granulopoiesis by various anesthetics has subsequently been confirmed in both animals (18–21) and man (22). Indeed, nitrous oxide has been found to reduce the white cell count in patients with myelogenous leukemia (18, 23). In cell cultures of myocardial myoblasts of the mouse embryo, nitrous oxide was shown to be cytotoxic, inhibiting mitosis and inducing chromosomal abnormalities (24). The helium group gases and nitrous oxide also depress the growth of tissue cultures of HeLa cells in direct proportion to the lipid solubility of the gases (25). Fink & Kenny (26) have recently reported that chloroform, ether, fluroxene, halothane, methoxyflurane, trichloroethylene, and nitrous oxide all slow the growth of mouse heteroploid cells in tissue culture. In addition, various anesthetics have been shown to inhibit the growth of certain tumors (27, 28), to depress immune responses (29), and to be teratogenic in chick embryos (30, 31).

Another cell in which anesthetics have been studied is the erythrocyte. The biphasic nature of the effects of anesthetics on the red cell membrane was pointed out by Traube in 1908 (32). Low concentrations of anesthetics inhibit hemolysis by hypotonic solutions, whereas high concentrations by themselves produce hemolysis. This subject was recently reviewed by Seeman (33) under the heading "Membrane stabilization by drugs." Seeman pointed out the correlation over a wide range of absolute concentration between potency as a narcotic and potency in stabilizing against hypotonic hemolysis (34). The actions of anesthetics on transfer mechanisms in the red cell membrane were reviewed by Greene & Cervenko (35) who concluded that anesthetics may act as competitive penetrating inhibitors of glucose transport in a way as yet undefined but not related to stabilization of the membrane, to effects on metabolic processes, or to interaction with sulfhydryl or amino groups in the membrane.

The effects of anesthetics on sodium transport mechanisms in the short-circuited toad bladder are similarly complex. Andersen (36) found that halothane depressed sodium transport, cyclopropane and nitrous oxide stimulated it, while ether had a biphasic action. The stimulatory effect of cyclopropane was later found to be synergistic with that of epinephrine and antagonized by alpha adrenergic blocking drugs (37). On the basis of these findings, it was proposed that anesthetic stimulation of sodium transport represented an effect on alpha receptors, whereas the depression was the result of a direct action at another site. At high pressures, nitrous oxide (38), xenon, and krypton (39) inhibit sodium transport with a linear relationship between the effective pressure and the polarizability of the gases.

This brief review of the actions of anesthetics on various cellular functions merely introduces the enormous amount of investigation and the fascinating variety of effects that have been uncovered. Despite this, however, the nature and significance of these actions remain unknown.

Electrically Excitable Membranes

The fact that general anesthetic agents can block impulse conduction in peripheral nerves was recognized as long ago as 1881 when Szpilman & Luchsinger (40) showed that ether, chloroform, and ethanol blocked conduction in frog nerves. However, by raising stimulus strength, a response could still be elicited from the region of the nerve exposed to the anesthetics at a time when the response to a stimulus applied outside the narcotized region was already blocked, suggesting that the nerve was not depolarized by the anesthetics. Winterstein (8), in 1919, concluded that "undoubtedly" the reversible decrease in excitability of nervous tissues produced by narcotic agents was associated with a decrease in permeability, while the irreversible "toxic" effect of narcotics was associated with an increase in permeability. He stated that "further insight will only be possible when we understand better the processes of excitation." Studies from that point up to the advent of transmembrane electrical recording were reviewed in 1952 by Toman

(41). It had been established by Kato (42), that narcotic-induced block in single nerve fibers was all-or-none and not due to decremental conduction. Again, excitability in the narcotized area was depressed, but this could be overcome by an increase in stimulus strength. More recently, block of conduction by chemically inert gases has been reported (39, 43, 44). Skou (45) observed a close parallelism between the blocking dose of several alcohols and their ability to penetrate monolayers of nerve lipids.

The introduction of techniques for measuring transmembrane potentials or currents in single nerve fibers, the so-called voltage clamp technique, and the formulation of a complete hypothesis of the processes of excitation and conduction by Hodgkin and Huxley have set the stage for a new level of understanding of the effects of anesthetics.

However, evidence concerning the precise mechanism by which anesthetics block nerve conduction is still surprisingly scanty. Thesleff (46), in an early study of several nonvolatile anesthetics in frog skeletal muscle, reported no change in resting potential and no decrease in resting transmembrane resistance at a time when excitability was decreased, the rate of rise of the action potential was reduced, and conduction was blocked. He attributed these effects to a decrease in the rise of sodium conductance of the membrane which follows stimulation. His results were confirmed and extended to ether and to alcohol by Inoue & Frank (47, 48) who also showed that increasing the extracellular sodium concentration readily overcame the effects of the anesthetics. Modern electrophysiological methodology has been brought to bear on the question only recently and only for alcohols. Moore, Ulbricht & Takata (49), in a study of membrane currents in the squid axon with the sucrose gap technique and voltage clamp, observed that ethanol reduced both the maximum sodium and potassium conductances; Armstrong & Binstock (50), using coaxial electrodes, found sodium conductance much more affected than potassium conductance. Both groups reported that the kinetics of the conductance changes (time to peak of the early current, time course of sodium inactivation) were unaltered. Recently, barbiturates (sodium pentobarbital, sodium thiopental) were found to exert similar effects on lobster axons (51). Changes in sodium and potassium conductances during excitation, and the rate of conductance "turn-on" were reduced. The author concluded that the effect was probably not secondary to an action on cell metabolism because exposure of the membrane area to low pH antagonized the changes. Furthermore, he suggested that the barbiturate in its anionic form dissolved in the membrane lipids and thus affected binding of Ca^{++} and ionic permeabilities. No similar study of volatile anesthetic agents is known to the reviewers.

In view of the parallelism between lipid solubility and anesthetic potency of the commonly used anesthetics, it is of interest to summarize briefly the effects of drugs of differing solubilities on membrane conductances in crustacean axons. The compounds of one group have relatively specific effects on these axons. For example, tetrodotoxin selectively blocks the early

change in sodium conductance accompanying the action potential and only when applied to the outside of the membrane (52, 53). Both tetraethylammonium (54) and cesium ions (55) specifically block the late potassium conductance changes and only when applied to the inside of the membrane. All three of these agents are insoluble in lipid media.

By contrast, lipid soluble agents are less specific. Procaine, for example, blocks both early and late ionic currents (56) and works from either the inside or the outside of the membrane (53). Allethrin, a lipid-soluble pyrethrin derivative, resembles procaine in these respects (57).

Thus, while it has been clearly shown that the sodium and potassium conductance changes can be separated and specifically inhibited, lipid-soluble blocking drugs affect both processes in a seemingly nonspecific way. It would be surprising if inhalation anesthetics did not resemble the lipid-soluble agents in their effects on excitable membranes. That this is true for those narcotic agents that have been investigated has been reported above (49–51). The clinically useful inhalation anesthetics remain to be studied. There are some investigators who have already concluded that local anesthetics, general anesthetics, and perhaps other central nervous system depressants all share a common mechanism of action (58, 59). This hypothesis, though obviously stimulating, requires much more experimental scrutiny.

Electrically Inexcitable Membranes; Synaptic and Receptive Membranes

The observations of the preceding section are very important for our understanding of the actions of anesthetics and narcotics on membranes in general and on the processes of electrical impulse generation and conduction. However, it has long been known that electrically excitable membranes and conductive processes in the nervous system are not as vulnerable to inhibition by anesthetic agents as are synaptic events. This was pointed out, for example, by Larrabee & Posternak in 1952 (60) who showed that synaptic transmission was blocked by anesthetics in significantly lower concentrations than those needed to block axonal conduction.

Neurohumoral transmission is a complex series of events involving the presynaptic nerve endings (impulse conduction, transmitter synthesis, storage, transport, and release), the transmitter in the synaptic cleft (enzymatic destruction, reuptake), and events at the subsynaptic or receptive membrane (transmitter-receptor interaction, changes in the properties of the chemically excitable membrane, and changes in the adjacent electrically excitable membrane, which may affect the generation of conducted impulses). In addition, in interpreting the actions of drugs in these systems, one must be aware of the possibility of direct drug actions on the effectors themselves.

The differences between electrically excitable and electrically inexcitable membranes have been stressed especially by Grundfest (61–63). Some synaptic or receptive membranes do not respond to electrical depolarization with an all-or-none regenerative action potential but rather, in many cases,

these membranes are sensitive to chemical substances, often transmitters released by adjacent nerve terminals. For our purpose, it is enough to state that chemical agents (transmitters) produce graded responses in such membranes. Conducted spikes may result if the membrane is mixed with or adjacent to electrically excitable areas. Selective block of the sodium system by tetrodotoxin has been used recently with considerable success to separate more clearly the two types of membranes (64–67).

Another concept has to be kept in mind in interpreting experiments dealing with synaptic processes. Although the response to a transmitter may be graded, the conducted response of adjacent electrically excitable membrane is not. The amount of transmitter normally released may be many times in excess of the amount which would just produce a threshold depolarization, and an all-or-none response. This type of synaptic system is then relatively insensitive to minor alterations, up or down, of the amount of transmitter released or to changes in postsynaptic sensitivity. This "margin of safety" has recently been investigated for the neuromuscular system by Paton & Waud (68). On the other hand, suitable effector systems which respond to chemical agents with graded responses (e.g., some smooth muscles, slow striated muscles) may be used to advantage with proper precautions.

Cholinergic systems.—There is only one report indicating that the volatile anesthetic agents, chloroform and ether, depress the release of acetylcholine measured directly in electrically stimulated guinea pig intestine (69). In the same study, it was found that the responses to ACh and KCl were also depressed. When doses of hyoscine and anesthetics, which produced the same depression of the response to electrical stimulation, were tested for their antagonism to acetylcholine, hyoscine caused a greater depression of the dose ratio than did the anesthetics. In another study, in isolated guinea pig intestine (70), Rang found a similar depression of smooth muscle response by anesthetics. Both authors also found stimulatory effects which were antagonized by cholinergic blocking agents but not by hexamethonium and antihistaminics and were thus ascribed to actions on intrinsic postganglionic nerves.

The effects of halothane, ether, and thiopental on guinea pig tracheal rings were studied by Fletcher, Flacke & Alper (71). The anesthetics by themselves caused relaxation and antagonized the response to ACh and to histamine. The interaction of these agonists and the anesthetics were compatible with a physiological and not a pharmacological antagonism.

In these studies the effector organs were smooth muscle and the ACh effect was muscarinic. Torda in 1943 (72) studied the effects of ether and chloroform on the mechanical response to ACh of frog rectus abdominis muscle, a nicotinic preparation capable of graded response. She found a marked potentiation, as did Sachdev, Panjwani & Joseph (73), who also observed that the presence of an anticholinesterase agent did not alter the potentiating effects of the anesthetics. It was concluded that the mild anticholinesterase activity of the anesthetics was not the cause of the potentiation.

Adrenergic systems.—There is general agreement that cyclopropane

enhances the response of various organs and tissues to norepinephrine. Gravenstein, Sherman & Andersen (74) showed this in the nictitating membrane of the cat. A similar phenomenon was demonstrated by Price & Price (75) in rabbit aortic strips where cyclopropane increased the response, not only to norepinephrine, but also to 5-hydroxytryptamine, histamine, and angiotensin. Baez & Orkin (76) showed an enhanced reactivity of the microcirculation to epinephrine during cyclopropane anesthesia. In man, McArdle & Black (77) demonstrated a marked potentiation during cyclopropane anesthesia of the vasoconstrictor response to the intraarterial injection of norepinephrine. By contrast, the response of aortic strips (75) and forearm vasculature (78) to norepinephrine was decreased by halothane.

Emerson & Massion (79) found that both cyclopropane and halothane caused vasodilatation in innervated as well as in denervated forelimbs of dogs. Cristoforo & Brody (80) in a comparison of cyclopropane and halothane concluded that halothane decreased the responsiveness of vascular smooth muscle to norepinephrine whereas cyclopropane produced no change. In a second study by the same authors (81) in the isolated perfused dog gracilis muscle, both halothane and cyclopropane produced vasoconstriction unaffected by alpha adrenergic blockade. The direct effect of halothane was vasodilatation which was apparently masked by the liberation of a vasoconstrictor substance from the hypophysis. The direct effect of cyclopropane was that of a stimulant producing vasoconstriction, not mediated by the release of catecholamines.

Another system in which the interaction of anesthetics and norepinephrine has been studied is the heart. Flacke & Alper (82), in the heart-lung preparation of the dog, demonstrated that halothane exerted a physiological antagonism to the chronotropic effects of infused norepinephrine in the lower part of the dose-response curve, but a potentiation of the response to high rates of norepinephrine infusion. Similar results with both halothane and cyclopropane were obtained by Garfield et al. (83) in spinal dogs with stimulation of postganglionic sympathetic nerves. These observations were confirmed by Price et al. (84) in the intact open-chest dog. Naito & Gillis (85) in isolated atria found no change in the response to norepinephrine or sympathetic nerve stimulation in the presence of either cyclopropane or halothane, but these authors studied only low frequencies of stimulation.

In attempts to explain the altered response to norepinephrine, a few studies have been directed at elucidating what effects, if any, anesthetics might have on the disposition of catecholamines. Li, Laasberg & Etsten (86) found an increase in norepinephrine content of the heart after ether and cyclopropane anesthesia. The possibility that cyclopropane interferes with catecholamine biotransformation was studied by Gardier, Endahl & Hamelberg (87) who found a 29 per cent reduction in the *in vitro* transformation of epinephrine to metanephrine by catechol-O-methyltransferase from rat liver, but this was in the presence of an atmosphere of 100 per cent cyclopropane.

Another possibility, that of a cocaine-like action of the anesthetics pre-

venting reuptake of norepinephrine into the adrenergic nerve endings, was not supported by the work of Ngai, Ozernitsky & Diaz (88) and Price et al. (84), who found no increase in the norepinephrine content of coronary sinus blood during either cyclopropane or halothane anesthesia. Price and co-workers (84) also observed that cyclopropane anesthesia did not affect the ability of the heart to extract infused norepinephrine. More direct evidence against a cocaine-like action was obtained by Naito & Gillis (85) who found that anesthetics had no effect on the uptake of labeled norepinephrine by rat ventricle slices.

Thus, there is evidence for a direct depressant effect, especially of halothane on intestinal, tracheal, and vascular smooth muscle. As for the influence of anesthetic agents on the actions of agonists, both depression and potentiation have been observed in different systems and with different anesthetics, but there is no evidence for specific interaction at the same receptor. The work that has been done suggests that the anesthetics influence events beyond the receptor, as indicated by the fact that different agonists are often similarly affected. This conclusion is tenuous and studies to support or reject it are overdue.

Ganglionic transmission.—The effect of anesthetics on synaptic transmission has been studied extensively in peripheral ganglia, particularly in the cervical sympathetic ganglia of the cat, rat, rabbit, and dog. Many of the early studies of anesthetics in this system used, as an index of degree of blockade of ganglionic transmission, changes in the amplitude of the action potential in the postganglionic nerve.

In a series of papers in 1952 (60, 89, 90), Larrabee and co-workers demonstrated that anesthetics selectively inhibited synaptic transmission in sympathetic ganglia, both isolated and *in situ*. The concentration of anesthetic which blocked axonal conduction was never more than ten times that which blocked the synapse, a much less specific effect than that of nicotine, for example. They pointed out several important features of the action of anesthetics. The response to high-frequency stimulation was much more readily blocked than that to single stimuli. When the control response was small, anesthetic depression was more readily demonstrated. Transmission over the fastest conducting fibers was more sensitive to the anesthetics than that over the slower fibers. They conclusively established, then, the importance of rate and magnitude of nerve stimulation in determining the response to anesthetics.

A similar study in the cat cervical ganglion *in situ* (91) demonstrated a concentration-dependent depression of ganglionic transmission by ether. Again, the fewer the preganglionic fibers stimulated, the greater the sensitivity to block by ether. Thiopental had no effect, cyclopropane only a weak effect.

With the advent of halothane and its pronounced effects on the circulation, there developed a new interest in the actions of anesthetics on ganglionic transmission. Initial reports (92, 93) suggested, on the basis of indirect evidence, that halothane blocked ganglionic transmission.

Biscoe & Millar (94) compared the effects of halothane, ether, and cyclopropane in various ganglia of the cat and of the rabbit using a technique of single preganglionic stimuli of varying intensity, and measuring the height of the elicited postganglionic action potential. They found significant blockade of ganglionic transmission by all three anesthetics. By contrast, Li, Gamble & Etsten (95) found in the dog that halothane had no effect on postganglionic action potentials while stimulating preganglionically at constant frequency over a range of voltages.

Price & Price (96, 97) investigated in dogs the ganglionic actions of halothane, cyclopropane, and nitrous oxide using as an index of ganglionic block the difference between the heart-rate response to preganglionic and postganglionic sympathetic nerve stimulation. They found that 1 per cent halothane produced approximately a 40 per cent block of transmission and 2 per cent produced an 80 per cent block. Cyclopropane also produced a definite block, but it was not as potent as halothane. Nitrous oxide had an effect in only one animal. The interpretation of these data is difficult since all the dogs received atropine and no account was taken of the altered response to postganglionic stimulation (83).

It has recently been found that in the cardiac sympathetic ganglia of the dog and cat two cholinergic pathways exist in parallel: one which is labeled "nicotinic" since it is susceptible to blockade by agents such as hexamethonium and mecamylamine, and a second "muscarinic" pathway susceptible to block by atropine (98, 99). With this situation in mind, the effects of several anesthetic agents on transmission in the cardiac sympathetic ganglia were investigated in our laboratory (83). It was found that both ether and halothane in clinical concentrations blocked transmission as determined by the difference between heart rate responses to preganglionic and to postganglionic stimulation over a wide range of frequencies. The effect was markedly increased by small doses of atropine. It was concluded that the presence of the muscarinic system "masked" the effect of the anesthetics on transmission. Since the muscarinic system operates at higher frequencies of stimulation than does the nicotinic system, the influence of atropine is especially marked at these frequencies. Another important factor is the observation that halothane, but not ether, increased the response to high frequency postganglionic sympathetic stimulation. The response to preganglionic nerve stimulation in the presence of halothane was thus a balance between the increase in postganglionic response and the depression of the ganglion. Cyclopropane was found to behave like halothane in that it both potentiated the response to postganglionic nerve stimulation and blocked ganglionic transmission. We were unable to demonstrate any effect of nitrous oxide. From these experiments, we concluded that halothane, ether, and cyclopropane block transmission through the cervical sympathetic ganglion of the dog by acting primarily on the nicotinic system.

The peripheral sympathetic ganglion has proven to be a fruitful experimental model in which to study the effects of anesthetics on synaptic transmission. Unfortunately, it is often forgotten that both rate and intensity of

stimulation by themselves will alter the apparent response to anesthetics. Further, the attempt to derive quantitative data from either simple measurements of action-potential amplitude or response of heart rate to preganglionic stimulation alone is not adequate.

From the available data, we cannot specify the site of action of anesthetics within the ganglion. The two most likely possibilities are a decrease in transmitter release from the presynaptic nerve ending or an altered sensitivity of the post-junctional neuron to the transmitter. Studies of the effects of ether on monosynaptic pathways in the spinal cord (100–102) and of ether and halothane at the neuromuscular junction (103–105) support the latter alternative. Similarly, current investigations in our laboratory (106) have shown that halothane shifts the dose-response curve to intra-arterial injection of various ganglionic stimulating drugs, thus supporting a postsynaptic site of action.

The observations of the French group of investigators in ganglion cells of *Sepia, Aplysia,* and *Helix* have contributed much to our knowledge of the action of volatile anesthetics (107–112). A summary of this work has appeared in English (113). These investigators showed with transmembrane recording techniques that ether, chloroform, and halothane caused a biphasic change in the membrane potential, membrane resistance, excitability, and rate of spontaneous discharge. The initial event was a very brief depolarization followed by hyperpolarization. Excitability changed accordingly. Membrane resistance decreased continuously, as estimated from measurement of membrane potential change resulting from application of transmembrane current pulses. Excitatory postsynaptic potentials, evoked by stimulation of afferent nerve fibers, were abolished by the anesthetics before the somatic response to direct stimulation. The most resistant phenomenon was the excitation of the cell by antidromic stimulation. Narcotics also affected, to a different degree, the responses of a single cell to stimulation of two different afferent nerves.

Neuromuscular junction.—The effects of anesthetics at the neuromuscular junction have been relatively carefully studied and are therefore of great interest in terms of insight into actions of these drugs on a neuroeffecter system. Very early studies (114–118) suggested that ether had a curare-like action in that it antagonized the response of the muscle to nerve stimulation and intra-arterial acetylcholine, and partially blocked the potentiating action of physostigmine. These observations were confirmed by Gross & Cullen in 1943 (119) who also noted that cyclopropane had little effect. Poulsen & Secher (120–122) reported that ether affected the response to twitch stimulation to a greater degree than to tetanic stimulation and that neostigmine effectively antagonized the action of ether.

Naess (123–126) compared the effects of ether and curare in a more quantitative way by exploring complete frequency-response curves in the rabbit sciatic nerve-gastrocnemius muscle preparation. He found blockade of transmission by both drugs but neostigmine reversed only the curare

block and actually accentuated that produced by ether. Naess described post-tetanic potentiation during exposure to both ether and curare with a markedly greater depression by ether of the response to higher frequencies of nerve stimulation. From his observations, Naess concluded that ether blocked transmission at the neuromuscular junction in a manner qualitatively different from that of curare.

Watland et al. (127) in 1957 found marked differences among ether, cyclopropane, chloroform, and halothane in the sciatic nerve-gastrocnemius muscle preparation of the rabbit using both single stimuli and tetanic stimulation. Ether depressed the response to both twitch and tetanic stimulation; halothane had no effect at all. Cyclopropane and chloroform, interestingly, both increased the response to single stimuli but not to tetanic stimulation. All four anesthetics potentiated the actions of curare.

The response to cyclopropane was further studied by Sabawala & Dillon (128) in human intercostal muscle preparations. They found that 25 per cent cyclopropane resulted in a marked increase of the tension output of the muscle to both direct and indirect stimulation, whereas 50 per cent cyclopropane resulted in an increase in the tension output in response to direct stimulation, but a decrease in response to indirect stimulation after an initial increase. In the presence of curare, cyclopropane produced a marked increase in the response to direct stimulation, but further depressed the response to indirect stimulation. They also demonstrated a dual effect of ether and halothane. In low concentrations, there was an increased response to both indirect and direct stimulation; in higher concentrations, the response to indirect stimulation, and, in even higher concentrations, the response to direct stimulation was abolished (129). They concluded that all anesthetics affected both the neuromuscular junction and the skeletal muscle itself in a complex manner, certainly not like that of curare.

Ngai, Hanks & Farhie (130) obtained somewhat different results in the cat, where ether was the only agent which decreased the response to twitch stimulation of the nerve. Halothane, trichloroethylene, and methoxyflurane had no effect whereas chloroform, nitrous oxide, and especially cyclopropane accentuated the response to stimulation of the nerve. Cyclopropane also produced an increased twitch response in denervated muscle. Very recent studies (131, 132) in man failed to demonstrate a significant depression by ether or halothane of the response to single-twitch nerve stimulation at a time of good surgical relaxation. In the cat, high concentrations of ether depressed the response to intra-arterial acetylcholine, an effect not reversed by anticholinesterase agents. Both drugs significantly enhanced the blockade induced by curare.

Further light has been shed on the effects of ether, halothane, and cyclopropane in a recent series of papers (103–105) where the actions of these drugs were studied with modern single-fiber techniques in the sciatic nerve-sartorius muscle preparation of the frog. There was a direct relationship between the concentration of ether and the decrease of tension output

of the indirectly stimulated muscle (103) with a threshold effect at 1 per cent ether and abolition of contraction at 3 per cent ether. Edrophonium and succinylcholine, both of which reversed curare-induced blockade in the system, produced only a weak and transient reversal of the block produced by ether. Ether caused a slowing of the rate of rise of the end-plate potential and a marked prolongation of its duration with no change in the resting potential. In the muscle fiber itself, there was no change in the resting potential but a decrease in the overshoot of the action potential, an increase in the critical membrane potential at which the action potential was initiated and an increase in the negative after-potential. All these changes in the muscle occurred only at concentrations of ether which produced complete block of neuromuscular transmission. During the administration of ether there was a progressive decrease in amplitude and eventual abolition of the spontaneously occurring miniature end-plate potentials. Those which did occur were markedly prolonged as compared with the controls. Ether decreased the response of the end-plate potential to carbamylcholine.

In the same preparation (104) halothane also blocked neuromuscular transmission in a manner quite analogous to that previously observed with ether. Complete block of the muscle response to nerve stimulation was observed at 1.5 per cent halothane, a concentration without effect on the nerve or on the muscle. Microelectrode studies at the end-plate revealed a slowing of the rate of rise of the end-plate potential, a decrease in response to carbamylcholine and acetylcholine, and a decrease in amplitude and eventual abolition of the miniature end-plate potentials. Simultaneous observation of the prejunctional action potential showed it to be unchanged at a time when the response of the post-junctional membrane was completely blocked.

Cyclopropane (105) in concentrations up to 20 per cent resulted in an increase in the tension output of the frog sartorius muscle. At higher concentrations of cyclopropane, neuromuscular transmission was blocked. A much smaller initial potentiation was also seen with halothane.

From these elegant experiments, several important points have emerged. Both ether and halothane affect neuromuscular transmission in this *in vitro* system in concentrations effective *in vivo* and by an apparently identical mechanism, i.e., an altered response of the post-junctional membrane to acetylcholine. That the action is not presynaptic is inferred from the finding of normal presynaptic action potentials at a time of transmission block. Other indirect evidence against the hypothesis of impaired release of ACh is the prolonged duration of the end-plate potential and the inability of anticholinesterase agents to reverse the block. In addition, there is direct evidence of an impaired response of the post-junctional membrane to the transmitter substance. One postulated mechanism of action is an interference by these drugs with the mechanisms controlling ionic permeability in the end-plate such that the rise in sodium conductance in response to ACh is of lesser

magnitude but of longer duration. Direct evidence dealing with these ionic phenomena is lacking.

A different mode of action has been postulated in a series of preliminary observations of van Poznak (133–135). He found that both cyclopropane and ether abolished post-tetanic repetitive activity in motor nerve terminals of the cat. In addition, cyclopropane enhanced such activity in skeletal muscle. The possibility that anesthetics affect motor nerve endings cannot then be entirely ruled out.

From the gross studies in various species, several points of general agreement emerge. Ether, in almost every study, definitely impaired the response to nerve stimulation. The differences among the results may be attributable to lack of attention to the importance of frequency of nerve stimulation and nonuniformity of experimental techniques. The clinical importance of this action as compared to effects in the central nervous system is open to question. It is clear, however, that there is a marked synergism between ether and curare. The recent electrophysiological studies suggest that both ether and halothane alter the response of the motor end-plate to acetylcholine by a mechanism as yet undetermined, different in type, but additive with that of curare. A similar mode of action has recently been described for local anesthetics (135a).

A second interesting feature of the actions of anesthetics, especially cyclopropane, is their effect on the strength of contraction of skeletal muscle in response to both indirect and direct stimulation. From the repeated observations cited above, all anesthetics seem to share this action in some experiments. This is obviously another area requiring further study.

Sensory receptors.—Sensory receptors represent another case of a membrane selectively sensitive to specific energies. The response to such specific energies is graded and translated into conducted all-or-none action potentials whose frequency is related to the input energy and the induced generator potential. It has been shown that tetrodotoxin abolishes the conducted responses without affecting the generator potential (136). A recent review of receptor physiology can be found in reference 136a.

A review of the effects of anesthetics on receptors (137) reveals their effects to be biphasic in most cases with initial stimulation followed by depression. There are differences among the anesthetics in their action on a given receptor and also for the same anesthetic on different receptors (137–141). Analysis of the site and nature of the change induced by anesthetics has not been carried very far in view of the existing possibilities.

HEART

A number of recent reviews and monographs (142–146) have dealt with the effects of anesthetics on cardiovascular function and regulatory mechanisms. Of special interest is the recent paper by Katz & Epstein (147) who discuss in great detail, with a thorough review of the literature, the multi-

plicity of factors, both cardiac and extracardiac, involved in the genesis of cardiac arrhythmias during anesthesia. We shall not discuss this topic any further here, but rather focus upon the direct actions of anesthetics upon the various functions of the heart.

Some anesthetics directly affect the rate of the denervated heart. Price & Helrich (148) noted in the dog heart-lung preparation that ether usually caused a tachycardia, whereas cyclopropane and nitrous oxide had no consistent effect. Flacke & Alper (82) described a concentration-dependent, negative chronotropic effect of halothane unaffected by prior depletion by reserpine of intrinsic stores of norepinephrine. A similar action of halothane was noted in dogs with totally denervated hearts (149), in the isolated perfused cat heart (150), and in the areflexic, spinal dog (151).

The only attempt to elucidate the mechanism of these effects of halothane on cardiac pacemaker activity is that of Hauswirth & Schaer (152) who studied the electrical activity of single pacemaker fibers from the sino-atrial node of the rabbit. They found that the slope of the slow diastolic depolarization (pacemaker potential) was decreased to two thirds of control value by 1 percent halothane and to less than 50 per cent of control value by 2 per cent halothane. This action tends to decrease the rate of firing of the pacemaker. Simultaneously, 2 per cent halothane also decreased the magnitude of the maximal diastolic potential (from 65 to 56 mV) bringing it closer to the threshold potential, which changed relatively little. This change in diastolic potential tends to increase the rate of pacemaker firing. The interpretation of these findings in the light of changes in ionic fluxes in the membrane must await further investigation.

Studies on other electrical properties of the heart are equally rare. Smith et al. in 1962 (153) found that both cyclopropane and halothane decreased excitability in the dog heart and increased the absolute refractory period, whereas ether had no effect. All three agents increased both atrial and atrio-ventricular conduction times, with halothane having the most profound effect. Galindo & Sprouse (154) in a similar group of experiments in dogs, found that halothane prolonged the absolute refractory period, whereas ether and cyclopropane did not. All three agents decreased cardiac excitability, as evidenced by an increase in ventricular diastolic threshold. In a careful study in vagotomized spinal dogs, Kohli et al. (151) found that both halothane and ether prolonged the functional refractory period of atrio-ventricular conduction to a greater extent than did cyclopropane. Thus, in terms of gross effects on excitability and conduction, all anesthetics appear to decrease excitability and slow conduction, although there are inconsistencies perhaps attributable to differences in experimental techniques.

Cyclopropane has been more carefully studied in isolated rabbit atria (155). Measurements of atrial transmembrane potentials showed that cyclopropane accelerated the repolarization rate of the first and second phases of the atrial action potential with little effect on membrane resting potential or

action potential amplitude. These changes were associated with a decreased excitability of right atrial tissue, an increased excitability of left atrial tissue, and a negative inotropic action.

Davis et al. (156), in a study of transmembrane potentials in single cells from the dog heart, observed that cyclopropane caused an increased rate of repolarization of phase 2 and a decreased rate of repolarization of phase 3 of the action potential in Purkinje fibers. The net result was a shortening of the functional refractory period. No definite effect of cyclopropane could be demonstrated in ventricular or atrial muscle fibers. The authors point out the similarity of this response to that produced by an increase in the extracellular concentration of calcium.

In a subsequent paper, Temte, Helmer & Davis (157) demonstrated the dependence of this effect of cyclopropane on the extracellular concentration of calcium. Lowering the concentration of calcium prevented, and increasing the concentration of calcium enhanced, the effect of cyclopropane in shortening the functional refractory period by accelerating repolarization. In addition, the combination of cyclopropane and elevated calcium increased the rate and magnitude of diastolic depolarization (pacemaker potential), although no spontaneous firing was observed. These are important observations in that they indicate a link between a gaseous anesthetic and calcium in affecting membrane properties of an excitable cell.

Other electrophysiological studies of anesthetics in the heart are those of Awalt & Frederickson (158) who found no effects of halothane on transmembrane potentials of rabbit atria at a time when there was significant negative inotropic action.

Despite some differences in methods and results, it seems clear that cyclopropane significantly affects repolarization in Purkinje fibers in such a way as to shorten the refractory period and that this action is linked with the concentration of calcium. Cyclopropane itself does not alter the rate of the isolated heart, nor does it affect diastolic depolarization. These observations have certain implications for the genesis of cardiac arrhythmias (see reference 147). Halothane, which slows the rate of the isolated heart, also slows the rate of diastolic depolarization in isolated pacemaker cells; but at the same time, it decreases the magnitude of the maximum diastolic potential. The mechanisms are still a matter of speculation; there is a need for further investigation of the interaction of anesthetics with catecholamines and calcium in the heart.

The effects of anesthetics on myocardial contractility are of equal interest. In this review we shall deal only with studies in the heart isolated from extrinsic influences (hormonal, neurogenic, and hemodynamic) which may affect cardiac output, blood pressure, ventricular function curves, strain gauge arch measurements, etc. General reviews of overall cardiac performance during anesthesia can be found in references 142 to 146.

There have been numerous studies of the effects of anesthetics on contractility in isolated atrial tissue. An early example is that of Acierno &

DePalma (159) who demonstrated in isolated atria of the cat that ether, cyclopropane, and chloroform produced a concentration-dependent decrease in contractility. These observations have been confirmed and extended by Levy, Ichiyanaga & Frederickson (155) and by Naito & Gillis (85). In the dog heart-lung preparation, Price & Helrich (148), in a comparative study of cyclopropane, ether, nitrous oxide, and thiopental, found that all four agents depressed contractility as indicated by the depression of the curve relating cardiac output to right atrial pressure. They measured arterial blood concentrations of the anesthetics and found approximately equal degrees of depression by all four agents at equianesthetic concentrations. Halothane had a similar effect in the same preparation (82).

It is important to realize that there are both advantages and limitations in studies such as these in the isolated heart. One can observe the effects of anesthetics directly on the heart free of extraneous influences and under conditions probably closer to physiological than those attainable in isolated tissues bathed in salt solutions. However, it is also important to realize that the choice of conditions such as output or arterial resistance setting greatly affect the results of such studies. For example, in 1949, Moe et al. (160) pointed out that cyclopropane in the heart-lung preparation depressed to a much greater extent the ability of the heart to perform external work induced by increasing arterial resistance than that induced by increasing venous return. Alper & Flacke (161) reported similar results with halothane thus confirming the importance of the nature of the load imposed upon the heart in assessing the effect of drugs on contractility. Much of the investigation of the effects of anesthetics on myocardial contractility suffers from a lack of definition or control of these crucial factors. Comparisons can be made only if experimental conditions are comparable, but this is not to imply that the drug effects are fundamentally different depending upon experimental conditions. The consequences of the same impairment of contractility for the functioning of the intact heart as a pump depend upon the geometry of the heart as a sphere and the restrictions imposed by the experimental conditions. For a thorough review of these matters, see Blinks & Koch-Weser (162) and Braunwald, Ross & Sonnenblick (163).

More recent studies have applied the techniques developed in skeletal muscle to the analysis of the action of anesthetics on heart muscle. The first such report was that of Goldberg & Ullrick (164) who studied the effects of halothane on isometric contractions of isolated rat ventricular muscle under carefully controlled conditions. They found reductions in the peak developed tension and the maximum rate of tension development proportional to the concentration of halothane. By contrast, there was no change in time to peak tension or in total twitch duration. These changes were fully reversible. In a second paper, Goldberg & Phear (165) described concentration-dependent symmetrical depression by halothane of both length-tension and force-velocity curves. In similar studies of halothane in cat papillary muscle, Sugai, Shimosato & Etsten (166) reported depression of the force-

velocity curve, power, and work of cardiac muscle, without change in the stiffness of the series-elastic element. Similar results have also been reported with methoxyflurane (167, 168).

There are, then, good descriptions of the negative inotropic action of all anesthetics on cardiac muscle. Cyclopropane and ether remain to be studied in the same way as have halothane and methoxyflurane. From these carefully done studies, it appears that halothane and methoxyflurane cause a reduction in the magnitude of the active state of cardiac muscle.

In attempting to define the cause of this phenomenon, Brodkin, Goldberg & Kayne (169) showed that halothane depressed the ability of cardiac myofibrils to hydrolyze ATP. In this connection, both ether and halothane have been shown (170) to interfere with the utilization of ATP in the production of luminescence by firefly extracts. It is possible, then, that anesthetics interfere in some way with energy conversion in cardiac muscle, although further studies are required to prove this.

Another intriguing possibility deals with the role of calcium in the heart. It is beyond the scope of this paper to review in any detail the role of calcium in excitation, contraction, and excitation-contraction coupling in the heart. The reader is referred to several recent reviews (171–173). It is, however, becoming increasingly obvious that the role of calcium is a key one both in normal function and in the mode of action of cardioactive drugs, including, possibly, the anesthetics. For example, Daniel, Johnston & Foulks (174) in 1962, related the depression of myocardial contractility of pentobarbital in rabbit ventricles to an effect on binding and mobilization of calcium. Broadbent (175), in isolated perfused frog hearts, noted similarities between low calcium concentrations, ether, and thiopental in their ability to depress myocardial function. Those drugs which strongly antagonized the depression by low calcium concentrations (e.g., ouabain) also stimulated strongly those hearts depressed by ether and thiopental. Broadbent suggested that the narcotic drugs somehow interfered with the functions of calcium.

Flacke & Alper (82), in the heart-lung preparation of the dog, noted the inability of norepinephrine even in maximal doses to overcome the depression of myocardial contractility induced by halothane. On the other hand, elevation of serum calcium, which has a powerful positive inotropic effect on the normal heart (176), always reversed the depression (177). The limited ability of either norepinephrine infusion or sympathetic nerve stimulation to overcome the negative inotropic effect of halothane has also been noted by other investigators (95, 96).

There are several observations of the ability of cardiac glycosides to overcome the negative inotropic actions of halothane (178–179). On the other hand, Morrow & Townley (180) demonstrated that the dose of ouabain required to produce ventricular arrhythmias in the dog was markedly increased by halothane, decreased by cyclopropane, and unaltered by pentobarbital. These reports suggest that the relationship between digitalis glyco-

sides and anesthetics is complex, and is not adequately understood. There seems to be little doubt, however, that calcium is somehow associated with the action of both types of drugs.

The only direct study of the interaction of an anesthetic with calcium is that of Schaer (181) who described in guinea pig atria a complex relationship between the effects of sodium pentobarbital on heart rate and varying concentrations of calcium in the bathing medium.

From these fragments of evidence, it is apparent that the interaction of calcium and anesthetics merits careful investigation. This will be complicated because calcium has effects on the membrane, on excitation-contraction coupling, and on the contractile elements themselves. However, in view of the many tantalizing clues cited above, this investigation deserves high priority. It is also noteworthy that of all the peripheral structures studied, it is in the heart that the depressant effects of the anesthetics most closely parallel their anesthetic potency.

Metabolism

This review has emphasized the actions of anesthetic agents which may be characterized as "membrane" effects. There is, of course, a voluminous literature dealing with the effects of anesthetics on biochemical processes. Space limitations prevent us from including this body of literature, which has at any rate been reviewed frequently and recently (3, 4, 182–184). It seems to us that the crucial question which remains to be decided is that of *"post hoc"* or *"propter hoc,"* i.e., whether the biochemical effects are primary and causative of the functional effects of anesthetics or secondary to the impairment of function by the agents. There is good evidence that functional effects can occur which are not the consequences of biochemical lesions (e.g., the effects in isolated axons of squid and lobsters). The observations of Larrabee, Ramos & Bülbring (90) that ganglionic transmission was impaired by anesthetics without decrease in oxygen consumption should be mentioned here, although the authors point out that total oxygen consumption may not reflect inhibition at a specific site which accounts for only an insignificant fraction of the total. If the metabolic theory of anesthesia were to gain wider acceptance, such crucial functions would have to be identified. Metabolic inhibition and alteration of function by anesthetics should be parallel or the former should clearly precede the latter. An interesting example of this type may be found in the work of Negishi & Svaetichin (185, 186) who observed that the so-called "controller" cells in the retina of fish respond within seconds to anoxia and hypercarbia (185). The same cells respond rapidly and sensitively to alcohols and several volatile anesthetics (186). The authors advance the concept that similar "controller cells" may be present in the CNS proper and may exert a modifying influence upon activity of other cells. The metabolism of such cells would be only a small fraction of total brain metabolism and their inhibition would hardly be detectable by measurement of total metabolism. It must be real-

ized that the concept that anesthetics act by virtue of affecting the metabolism of controller cells in the CNS is only speculative.

Conclusions

In this review we have emphasized the effects of anesthetic agents upon biological functions outside the central nervous system. The considerable volume of literature dealing with correlations between the physiocochemical properties of anesthetics and anesthetic activity has been little considered. We believe that the reason for these efforts in the past has been the desire to gain some insight, albeit indirect, into the mode of action of anesthetics because the more direct approaches appeared too difficult or were unavailable. This is no longer so. On the one hand, the interaction between anesthetics and cell components of model systems is being studied directly by modern techniques (5, 6, 187–189). Furthermore, our increasing knowledge of structure and function of nervous tissue is bringing us closer to an understanding at the level of molecular structures and forces.

It seems therefore no longer useful to stop with the classification of drug action as "chemical" or "physical" when we already know that there is overlap and when we can be more specific in some instances. Anesthetic agents may prove to be useful tools in investigating the ways in which chemical agents can influence biological functions, not by combination with a well defined chemical entity, the "receptor," but by subtly changing molecular conformation and intermolecular forces. Understanding of the clinical state of anesthesia will probably follow from a better understanding of the intimate physiology of the peripheral and central nervous systems and of the subtle modifications produced by anesthetic agents (190, 191). The details of these modifications may well vary from drug to drug; it is even conceivable that the same functional effect may be the result of different basic mechanisms. With this approach we may eventually answer the question of how so many different drugs of such differing molecular structures can produce so many different effects in such a wide variety of biological systems and yet all cause the same clinical syndrome which we label "general anesthesia."

LITERATURE CITED

1. Waud, D. R., *Pharmacol. Rev.*, **20,** 49–88 (1968)
2. Featherstone, R. G., Muehlbacher, C. A., *Pharmacol. Rev.*, **15,** 97–122 (1963)
3. Bunker, J. P., Vandam, L. D., *Pharmacol. Rev.*, **17,** 183–263 (1965)
4. Latner, A. L., *Proc. Roy. Soc. Med.*, **58,** 895–900 (1965)
5. Schoenborn, B. P., Featherstone, R. G., *Advan. Pharmacol.*, **5,** 1–17 (1967)
6. Symposium on "Molecular Pharmacology of Anesthesia". Various authors. *Federation Proc.*, **27,** 870–913 (1968)
7. Bernard, Claude, *Anesthesiques et l'asphyxie* (Baillière, Paris, 1875)
8. Winterstein, H., *Die Narkose,* (Springer, Berlin, 1919)
9. Johannsen, W., *Naturwissenschaftl. Wschr.*, **18,** 97–109 (1902)
10. Sears, D. F., Gittleson, G. M., *Federation Proc.*, **20,** 142 (1961)
11. Schreiner, H. R., Gregoric, R. C., Laurie, G. A., *Science,* **136,** 653–54 (1962)
12. Buchheit, R. G., Schreiner, H. R., Doebbler, G. F., *J. Bacteriol.*, **91,** 622–627 (1966)
13. Schreiner, H. R., Gregoric, R. C., *Federation Proc.*, **22,** 424 (1963)
14. Schreiner, H. R., Buchheit, R. G., Corio, A. J., Doebbler, G. F., *Pharmacologist,* **6,** 183 (1964)
15. Bruce, D., Christiansen, R., *Exptl. Cell Res.*, **40,** 544–53 (1965)
16. Bruce, D. L., *J. Cellular Comp. Physiol.*, **68,** 81–88 (1966)
17. Gormsen, J., *Danish Med. Bull.*, **2,** 87–89 (1955)
18. Eastwood, D. W., Green, D. C., Lambdin, M. A., Gardner, R., *New Engl. J. Med.*, **268,** 297–99 (1963)
19. Bruce, D. L., Koepke, J. A., *Anesthesiology,* **27,** 811–16 (1966)
20. Parbrook, G. D., *Brit. J. Anaesthesia,* **39,** 119–27 (1967)
21. Aldrete, J. A., Virtue, R. W., *Anesthesiology,* **28,** 238–39 (1967)
22. Lassen, H. C. A., Henriksen, E., Neukirch, F., Kristensen, H. S., *Lancet,* **1,** 527–30 (1956)
23. Lassen, H. C. A., Kristensen, H. S., *Danish Med. Bull.*, **6,** 252–55 (1959)
24. Kieler, J., *Acta Pharmacol. Toxicol.*, **13,** 301–08 (1957)
25. Bruemmer, J. H., Brunetti, B. B., Schreiner, H. R., *J. Cellular Comp. Physiol.*, **69,** 385–92 (1967)
26. Fink, B. R., Kenny, G. E., *Anesthesiology,* **29,** 505–16 (1968)
27. Fink, B. R., *Anesthesiology,* **27,** 214 (1966)
28. Parbrook, G. D., *Brit. J. Anaesthesia,* **39,** 549–53 (1967)
29. Wingard, D. W., Lang, R., Humphrey, L. J., *J. Surg. Res.*, **7,** 430–32 (1967)
30. Rector, G. H. M., Eastwood, D. W., *Anesthesiology,* **25,** 109 (1964)
31. Andersen, N. B., *Anesthesiology,* **29,** 113–22 (1968)
32. Traube, G., *Biochem. Z.*, **10,** 371 (1908)
33. Seeman, P. M., *Intern. Rev. Neurobiol.*, **9,** 145–221 (1966)
34. Seeman, P. M., *Biochem. Pharmacol.*, **15,** 1632–37 (1966)
35. Greene, N. M., Cervenko, F. W., *Acta Anaesthesiol. Scand., Suppl.* 28 (1967)
36. Andersen, N. B., *Anesthesiology,* **27,** 304–10 (1966)
37. Andersen, N. B., *Anesthesiology,* **28,** 438–45 (1967)
38. Gottlieb, S. F., Savran, S. V., *Anesthesiology,* **28,** 324–26 (1967)
39. Gottlieb, S. F., Cymerman, A., Metz, A. V., *Aerospace Med.*, **39,** 449–52 (1968)
40. Szpilman, G., Luchsinger, B., *Pflügers Arch. Ges. Physiol.*, **24,** 347 (1881)
41. Toman, J. E. P., *Pharmacol. Rev.*, **4,** 168–218 (1952)
42. Kato, G., *Cold Spring Harbor Symp. Quant. Biol.*, **4,** 202–13 (1936)
43. Carpenter, F., *Am. J. Physiol.*, **178,** 505–09 (1954)
44. Gottlieb, S. F., Weatherly, J. M., *Am. J. Physiol.*, **208,** 407–11 (1965)
45. Skou, J. C., *Biochim. Biophys. Acta,* **30,** 625–29 (1958)
46. Thesleff, S., *Acta Physiol. Scand.*, **37,** 335–49 (1956)
47. Inoue, F., Frank, G. B., *Can. J. Physiol. Pharmacol.*, **43,** 751–61 (1965)
48. Inoue, F., Frank, G. B., *Brit. J. Pharmacol.*, **30,** 186–93 (1967)
49. Moore, J. W., Ulbricht, W., Takata, M., *J. Gen. Physiol.*, **48,** 279–95 (1964)

50. Armstrong, C. M., Binstock, L., *J. Gen. Physiol.*, **48,** 265–78 (1964)
51. Blaustein, M. P., *J. Gen. Physiol.*, **51,** 293–307 (1968)
52. Narahashi, T., Moore, J. W., Scott, W. R., *J. Gen. Physiol.*, **47,** 965–74 (1964)
53. Narahashi, T., Anderson, N. C., Moore, J. W., *J. Gen. Physiol.*, **50,** 1413–28 (1967)
54. Tasaki, I., Hagiwara, S., *J. Gen. Physiol.*, **40,** 859–85 (1957)
55. Chandler, W. K., Meves, H., *J. Physiol. (London)*, **180,** 788–820 (1965)
56. Taylor, R. E., *Am. J. Physiol.*, **196,** 1071–78 (1959)
57. Narahashi, T., Anderson, N. C., *Toxicol. Appl. Pharmacol.*, **10,** 529–47 (1967)
58. Shanes, A. M., *Pharmacol. Rev.*, **10,** 59–164, 165–273 (1958)
59. Frank, G. B., Sanders, H. D., *Brit. J. Pharmacol.*, **21,** 1–9 (1963)
60. Larrabee, M. G., Posternak, J. M., *J. Neurophysiol.*, **15,** 91–114 (1952)
61. Grundfest, H., General physiology and pharmacology of junctional transmission, in *Biophysics of Physiological and Pharmacological Actions*, 329–89 (Shanes, A. M., Ed. AAAS, Washington, 1961)
62. Grundfest, H., Comparative electrobiology of excitable membranes, in *Advan. Comp. Physiol. Biochem.*, Vol. II, 1–116 (Lowenstein, O., Ed. Academic Press, New York, 1966)
63. Grundfest, H., *Ann. N.Y. Acad. Sci.*, **137,** 901–49 (1966)
64. Hagiwara, S., Nakajima, S., *Science*, **149,** 1254–55 (1965)
65. Ozeki, M., Freeman, A. R., Grundfest, H., *J. Gen. Physiol.*, **49,** 1319–34 (1966)
66. Nonomura, Y., Hotta, Y., Ohashi, H., *Science*, **152,** 97–99 (1966)
67. Kurijama, H., Osa, T., Toida, N., *J. Physiol. (London)*, **191,** 225–38 (1967)
68. Paton, W. D. M., Waud, D. R., *J. Physiol. (London)*, **191,** 59–90 (1967)
69. Speden, R. N., *Brit. J. Pharmacol.*, **25,** 104–18 (1965)
70. Rang, H. P., *Brit. J. Pharmacol.*, **22,** 356–65 (1964)
71. Fletcher, S. W., Flacke, W., Alper, M. H., *Anesthesiology*, **29,** 517–22 (1968)
72. Torda, C., *J. Pharmacol. Exptl. Therap.*, **77,** 350–56 (1943)
73. Sachdev, K. S., Panjwani, M. H., Joseph, A. D., *Arch. Intern. Pharmacodyn.*, **145,** 36–43 (1963)
74. Gravenstein, J. S., Sherman, E. T., Andersen, T. W., *J. Pharmacol. Exptl. Therap.*, **129,** 428–32 (1960)
75. Price, M. L., Price, H. L., *Anesthesiology*, **23,** 16–20 (1962)
76. Baez, S., Orkin, L. R.: *Anesthesiology*, **24,** 568–79 (1963)
77. McArdle, L., Black, G. W., *Brit. J. Anaesthesia*, **35,** 352–57 (1963)
78. Black, G. W., McArdle, L., *Brit. J. Anaesthesia*, **34,** 2–10 (1962)
79. Emerson, T. E., Massion, W. H., *J. Appl. Physiol.*, **22,** 217–22 (1967)
80. Cristoforo, M. F., Brody, M. J., *Anesthesiology*, **29,** 36–43 (1968)
81. Cristoforo, M. F., Brody, M. J., *Anesthesiology*, **29,** 44–56 (1968)
82. Flacke, W., Alper, M. H., *Anesthesiology*, **23,** 793–801 (1962)
83. Garfield, J. M., Alper, M. H., Gillis, R. A., Flacke, W., *Anesthesiology*, **29,** 79–92 (1968)
84. Price, H. L., Warden, J. C., Cooperman, L. H., Price, M. L., *Anesthesiology*, **29,** 478–83 (1968)
85. Naito, H., Gillis, C. N., *Anesthesiology*, **29,** 259–66 (1968)
86. Li, T. H., Laasberg, L. H., Etsten, B., *Anesthesiology*, **25,** 641–49 (1964)
87. Gardier, R. W., Endahl, G. L., Hamelberg, W., *Anesthesiology*, **28,** 677–79 (1967)
88. Ngai, S. H., Ozernitsky, S., Diaz, P. M., *Anesthesiology*, **29,** 207 (1968)
89. Larrabee, M. G., Holaday, D. A., *J. Pharmacol. Exptl. Therap.*, **105,** 400–08 (1952)
90. Larrabee, M. G., Ramos, J. G., Bülbring, E., *J. Cellular Comp. Physiol.*, **40,** 461–94 (1952)
91. Normann, N., Löfström, B., *J. Pharmacol. Exptl. Therap.*, **141,** 231–39 (1955)
92. Raventos, J., *Brit. J. Pharmacol.*, **11,** 394–410 (1956)
93. Purchase, I. F. H., *Brit. J. Anaesthesia*, **37,** 915–28 (1965)
94. Biscoe, T. J., Millar, R. A., *Brit. J. Anaesthesia*, **38,** 3–12 (1966)
95. Li, T. H., Gamble, C., Etsten, B., *Anesthesiology*, **29,** 444–55 (1968)
96. Price, H. L., Price, M. L., *Anesthesiology*, **27,** 764–69 (1966)

97. Price, H. L., Price, M. L., *Anesthesiology*, **28**, 349–53 (1967)
98. Brown, A. M., *J. Physiol. (London)*, **191**, 271–88 (1967)
99. Flacke, W., Gillis, R. A., *J. Pharmacol. Exptl. Therap.* (In press)
100. Austin, G. M., Pask, E. A., *J. Physiol. (London)*, **118**, 405–11 (1952)
101. Somjen, G. G., Gill, M., *J. Pharmacol. Exptl. Therap.*, **140**, 19–30 (1963)
102. Somjen, G. G., *J. Pharmacol. Exptl. Therap.*, **140**, 396–402 (1963)
103. Karis, J. H., Gissen, A. J., Nastuk, W. L., *Anesthesiology*, **27**, 42–51 (1966)
104. Gissen, A. J., Karis, J. H., Nastuk, W. L., *J. Am. Med. Assoc.* **197**, 770–74 (1966)
105. Karis, J. H., Gissen, A. J., Nastuk, W. L., *Anesthesiology*, **28**, 128–34 (1967)
106. Alper, M. H., Fleisch, J. H., Flacke, W., Unpublished observations
107. Arvanataki, A., Chalazonitis, N., *J. Physiol., Paris*, **48**, 374 (1956)
108. Chalazonitis, N., Arvanataki, A., *Compt. Rend. Acad. Sci.*, **246**, 161 (1958)
109. Arvanataki, A., Chalazonitis, N., *Compt. Rend. Acad. Sci.*, **252**, 193 (1961)
110. Chalazonitis, N., Fink, B. R., *Compt. Rend. Soc. Biol.*, **158**, 1898 (1964)
111. Chalazonitis, N., Takeuchi, H., *Compt. Rend. Soc. Biol.*, **160**, 1918 (1966)
112. Takeuchi, H., Chalazonitis, N., *Compt. Rend. Soc. Biol.*, **160**, 1915 (1966)
113. Chalazonitis, N., *Anesthesiology*, **28**, 111–22 (1967)
114. Auer, J., Meltzer, S. J., *J. Pharmacol. Exptl. Therap.*, **5**, 521 (1914)
115. Githens, B., Meltzer, S. J., *J. Pharmacol. Exptl. Therap.*, **5**, 523 (1914)
116. Forbes, A., McIntosh, R., Sefton, W., *Am. J. Physiol.*, **40**, 503 (1916)
117. Simonart, A., Simonart, E. F., *Arch. Intern. Pharmacodyn.*, **49**, 302–28 (1934)
118. Brown, G. L., Dale, H. H., Feldberg, W., *J. Physiol. (London)*, **87**, 394–424 (1936)
119. Gross, E. G., Cullen, S. C., *J. Pharmacol. Exptl. Therap.*, **78**, 358–65 (1943)
120. Poulsen, T., Secher, O., *Acta Pharmacol. Toxicol.*, **5**, 196–212 (1949)
121. Poulsen, T., Secher, O., *Acta Pharmacol. Toxicol.*, **5**, 213–21 (1949)
122. Secher, O., *Acta Pharmacol. Toxicol.*, **6**, 371–85 (1950)
123. Naess, K., *Acta Physiol. Scand.*, **19**, 187–206 (1949–50)
124. Naess, K., *Acta Physiol. Scand.*, **19**, 350–69 (1949–50)
125. Naess, K., *Acta Physiol. Scand.*, **20**, 117–24 (1950)
126. Naess, K., *Acta Physiol. Scand.*, **20**, 241–52 (1950)
127. Watland, D. S., Long, J. P., Pittinger, C. B., Cullen, S. C., *Anesthesiology*, **18**, 883–90 (1957)
128. Sabawala, P. B., Dillon, J. B., *Anesthesiology*, **19**, 473–77 (1958)
129. Sabawala, P. B., Dillon, J. B., *Anesthesiology*, **19**, 587–94 (1958)
130. Ngai, S. H., Hanks, E. C., Farhie, S. E., *Anesthesiology*, **26**, 162–67 (1965)
131. Katz, R. L., *Anesthesiology*, **27**, 52–63 (1966)
132. Katz, R. L., Gissen, A. J., *Anesthesiology*, **28**, 564–67 (1967)
133. Van Poznak, A., *Federation Proc.*, **22**, 390 (1963)
134. Van Poznak, A., *Anesthesiology*, **26**, 263–64 (1965)
135. Van Poznak, A., *Anesthesiology*, **28**, 124–27 (1967)
135a. Steinbach, A. B., *J. Gen. Physiol.*, **52**, 144–61 (1968)
136. Lowenstein, W. R., Terzuolo, C. A., Washizu, Y., *Science*, **142**, 1180–81 (1963)
136a. *Cold Spring Harbor Symp. Quant. Biol.*, Vol. 30 (1965)
137. Paintal, A. S., *Pharmacol. Rev.*, **16**, 341–80 (1964)
137a. Price, H. L., Widdicombe, J., *J. Pharmacol. Exptl. Therap.*, **135**, 233–39 (1962)
138. Morse, H. T., Price, M. L., Price, H. L., *Federation Proc.*, **22**, 187 (1963)
139. Biscoe, T. J., Millar, R. A., *J. Physiol. (London)*, **173**, 24–37 (1964)
140. DeJong, R. H., Nace, R. A., *Anesthesiology*, **28**, 851–55 (1967)
141. Biscoe, T. J., Millar, R. A., *Brit. J. Anaesthesia*, **40**, 2–12 (1968)
142. Price, H. L., *Physiol. Rev.*, **40**, 187–218 (1960)

143. Etsten, B., Li, T. H., *Am. J. Cardiol.*, **6,** 706–15 (1960)
144. Fabian, L. W., Ed., Anesthesia and the Circulation, *Clinical Anesthesia,* Vol. 3 (1964)
145. Price, H. L., Cohen, P. J., Eds., *Effects of anesthetics on the circulation* (Chas. C. Thomas, Springfield, Ill., 1964)
146. Price, H. L., *Circulation during anesthesia and operation* (Thomas, C C, Springfield, Ill., 1967)
147. Katz, R. L., Epstein, R. A., *Anesthesiology,* **29,** 763–84 (1968)
148. Price, H. L., Helrich, M., *J. Pharmacol. Exptl. Therap.,* **115,** 206–16 (1955)
149. Morrow, D. H., Gaffney, T. E., Holman, J. E., *Anesthesiology,* **22,** 915–17 (1961)
150. Purchase, I. F. H., *Brit. J. Anaesthesia,* **38,** 80–91 (1966)
151. Kohli, J. D., Tuttle, R. R., Dresel, P. E., Innes, I. R., *J. Pharmacol. Exptl. Therap.,* **153,** 505–10 (1966)
152. Hauswirth, O., Schaer, H., *J. Pharmacol. Exptl. Therap.,* **158,** 36–39 (1967)
153. Smith, S. L., Webb, W. R., Fabian, L. W., Hagaman, V. D., *Anesthesiology,* **23,** 766–75 (1962)
154. Galindo, A. H., Sprouse, J. H., *Anesthesia Analgesia,* **41,** 659–69 (1962)
155. Levy, J. V., Ichiyanagi, K., Frederickson, E. L., *Anesthesiology,* **24,** 185–93 (1963)
156. Davis, L. D., Temte, J. V., Helmer, P. R., Murphy, Q. R., *Circulation Res.,* **18,** 692–704 (1966)
157. Temte, J. V., Helmer, P. R., Davis, L. D., *Anesthesiology,* **28,** 354–62 (1967)
158. Awalt, C. H., Frederickson, E. L., *Anesthesiology,* **25,** 90 (1964)
159. Acierno, L. J., DiPalma, J. R., *Anesthesiology,* **12,** 567–73 (1951)
160. Moe, G. K., Rennick, B. R., Freyburger, W. A., Malton, S. D., *Anesthesiology,* **10,** 706–13 (1949)
161. Alper, M. H., Flacke, W., *Anesthesiology,* **24,** 121–22 (1963)
162. Blinks, J. R., Koch-Weser, J., *Pharmacol. Rev.,* **15,** 531–99 (1963)
163. Braunwald, E., Ross, J. Jr., Sonnenblick, E. H., *New Engl. J. Med.,* **277,** 794 (1967)
164. Goldberg, A. H., Ullrick, W. C., *Anesthesiology,* **28,** 838–45 (1967)
165. Goldberg, A. H., Phear, W. P. C., *J. Pharmacol. Exptl. Therap.,* **162,** 101–08 (1968)
166. Sugai, N., Shimosato, S., Etsten, B., *Anesthesiology,* **29,** 267–74 (1968)
167. Sugai, N., Shimosato, S., Etsten, B., *Anesthesiology,* **29,** 215 (1968)
168. Shimosato, S., Shanks, C., Etsten, B., *Anesthesiology,* **29,** 538–49 (1968)
169. Brodkin, W. E., Goldberg, A. H., Kayne, H. L., *Acta Anaesthesiol. Scand.,* **11,** 97–101 (1967)
170. Ueda, I., *Anesthesiology,* **26,** 603–06 (1965)
171. Brady, A. J., *Ann. Rev. Physiol.,* **26,** 341–56 (1964)
172. Nayler, W. G., Am. Heart J., **73,** 379–94 (1967)
173. West, T. C., *Federation Proc.,* **27,** 120–25 (1968)
174. Daniel, E. E., Johnston, P. D., Foulks, J. G., *Arch. Intern. Pharmacodyn.,* **138,** 276–301 (1962)
175. Broadbent, J. L., *Brit. J. Pharmacol.,* **21,** 78–83 (1963)
176. Seifen, E., Flacke, W., Alper, M. H., *Am. J. Physiol.,* **207,** 716–20 (1964)
177. Alper, M. H., Flacke, W., Seifen, E., Rutledge, C. O., *Federation Proc.,* **22,** 247 (1963)
178. Goldberg, A. H., Maling, H. M., Gaffney, T. E., *Anesthesiology,* **22,** 974–76 (1961)
178a. Goldberg, A. H., Maling, H. M., Gaffney, T. E., *Anesthesiology,* **23,** 207–12 (1962)
179. Shimosato, S., Etsten, B., *Anesthesiology,* **24,** 41–50 (1963)
180. Morrow, D. H., Townley, N. T., *Anesthesia Analgesia,* **43,** 510–19 (1964)
181. Schaer, H., *J. Pharmacol. Exptl. Therap.,* **146,** 111–16 (1964)
182. Hunter, F. E., Jr., Lowry, O. H., *Pharmacol. Rev.,* **8,** 89–135 (1956)
183. Greene, N. M., *Inhalation anesthetics and carbohydrate metabolism* (Williams and Wilkins, Baltimore, 1963)
184. Quastel, J. H., *Brit. Med. Bull.,* **21,** 49–56 (1965)
185. Negishi, K., Svaetichin, G., *Pflügers Arch. Ges. Physiol.,* **292,** 177–205 (1966)
186. Negishi, K., Svaetichin, G., *Pflügers*

Arch. Ges. Physiol., **292**, 218–28 (1966)
187. Schoenborn, B. P., *Federation Proc.*, **27**, 888–94 (1968)
188. Metcalfe, J. C., Seeman, P., Burgen, A. S. V., *Mol. Pharmacol.*, **4**, 87–95 (1968)
189. Feinstein, M. B., *J. Gen. Physiol.*, **48**, 357–74 (1964)
190. Paton, W. D. M., Speden, R. N., *Brit. Med. Bull.*, **21**, 44–48 (1965)
191. Miller, K. W., Paton, W. D. M., Smith, E. B., *Nature*, **206**, 574–77 (1965)