DRUGS AND NERVE CONDUCTION[1,2]

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Propagation of the action potential in nerve fibers is dependent on two related processes—the electrical excitability of the membrane ahead of the actively depolarized region, and the magnitude and temporal characteristics of the transitory depolarization that constitutes the action potential. The now classical work of Hodgkin (45, 46) on vertebrate nerve trunks demonstrated the dependence of conduction on the electronic spread of depolarization induced by "local current" from the region of the action potential to that ahead of it to cause enhanced excitability and excitation. The subsequent studies of Tasaki and associates, and others on large, single myelinated fibers, summarized in Tasaki's monograph (97), were of special importance in demonstrating saltatory conduction (i.e., conduction by activation of successive nodes of Ranvier) and a safety factor of 5 to 7 (i.e., that the action potential is 5 to 7 times larger in maximum amplitude than that just needed to excite the adjacent node of Ranvier); moreover, Tasaki & Takeuchi (103) showed that the threshold of excitability and the height of the action potential vary inversely, as might be expected from recent evidence that both are normally dependent on the ability of the excitable membrane to undergo permeability changes to Na^+ and K^+ (84, 87, 104).

The process of conduction is dependent not only on the kinetics of the transitory permeability changes underlying excitability and the action potential, but also on the physical conditions governing the spread of local current ahead of the action potential. Among the physical conditions are (*a*) the critical length of the fiber, determined by the resistances of the membrane, axoplasm, and extracellular spaces, which governs the spatial features of the electrotonic spread of depolarization ahead of the action potential, and (*b*) the capacitance of the excitable membrane and other structures (myelin) that, with the associated resistance, determines the temporal characteristics of the rise of the electrotonic potential. These have been reviewed recently by Fatt (30). Of course, the presence of heavily myelinated internodes that give rise to saltatory conduction in "myelinated" nerve, as distinguished from continuous propagation in "unmyelinated" (actually weakly myelinated) fibers, is also an important consideration in any general analysis of conduction among different fibers. Since the resistances, capacitances, and general histology vary with fiber size, everything else being equal

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the potencies of drugs can be expected to vary correspondingly. For example, since the axoplasmic resistance will be greater in smaller fibers, the current intensity across the membrane at a given distance ahead of an action potential will be smaller, which would contribute to a lower velocity of propagation and to a smaller safety factor. Attempts to explain differences in the sensitivity of different size and especially different type fibers to a given drug should first consider this and structural details before assigning different specificities to the interactions. This point has been clearly made by Nathan & Sears (65), and by Didisheim & Posternak (24) in their recent reexamination of differential nerve block by stabilizers. The physical factors in various types of fibers remain to be delineated as clearly as they have been in large, single myelinated and in large invertebrate fibers.

It must be emphasized that drug studies with multifiber preparations should be tempered by a hypercritical evaluation of the errors that may arise. Thus, the importance of evaluating critical threshold levels of action rather than speed of action by supracritical concentrations has been pointed out (24, 65) because of the misleading results obtained with the latter by virtue of diffusion limitations in whole tissue. Recently Rud (74) has carefully evaluated the complex factors involved in drug diffusion in sheathed and desheathed peripheral nerve trunks *in vitro*, and enumerated the uncertainties arising from the use of a heterogeneous tissue. Moreover, the change in maximum amplitude after propagation through a treated region (e.g., 12) may reflect not a reduction in the number of conducting fibers but a widening of the range of conduction velocities which thereby reduces superposition of action potentials from the individual fibers—an effect which would be minimized by measurement of action potential area rather than amplitude. In any case, maximum action potential amplitude after passage through a treated region remains among the least useful of measurements if an understanding of the detailed mechanisms and differences in drug action are to be understood; it provides at best very indirect information regarding effects on the parameters underlying propagation. At least threshold and demarcation potential data—obtainable with the same equipment—should be supplied as well.

This review is a selection largely of investigations which have provided the more detailed measurements necessary for an understanding of drug effects or for which such data are available from other sources. Less complete studies that seemed of special importance have also been considered. These measurements, as pointed out earlier (83, 84), consist of the resting membrane potential and resistance, temporal configuration of the spike and of the underlying changes of resistance (or the inverse of this, the membrane conductance or the related ion permeability), and, where possible—so far in giant axons of the squid and lobster and in the node of Ranvier of frog myelinated fibers—the inward (sodium) and outward (potassium) membrane currents during "voltage clamp." The strict limitation of space has precluded inclusion of pertinent data from muscle studies and much of the recent work

with ions. The papers referred to date chiefly from 1958 because earlier work, examined from the standpoint presented here, was described in a recent review [Shanes (83, 84)]. This review should be consulted for more detailed literature on methodology and principles.

Components of The Action Potential

The passage of the action potential causes a given region of the membrane to undergo first a rapid decrease and usually a reversal of the transmembrane electrical potential difference, so that the interior becomes positive briefly with respect to the outside, followed by a return toward, but seldom to, the original level of potential; this is the familiar "spike." If, as in tetraethylammonium treated fibers, the return is greatly delayed and depolarization remains large—of the order of the spike maximum and for many, even tens of milliseconds—this is regarded as part of the spike and referred to as a "plateau;" an appreciable delay in repolarization, but with a residual depolarization of only a fraction of the spike amplitude, constitutes the "negative after-potential." In fibers with low resting potentials, the negative after-potential may be separated from the spike by a brief hyperpolarization known as the "positive overshoot" or "positive potential." This often is not apparent until after the plateau in fibers that exhibit this larger residual depolarization. The brief and early positive overshoot must be distinguished from a much later, usually smaller and much longer phase of hyperpolarization that frequently follows the negative after-potential or positive overshoot—the "positive after-potential." The positive potential is caused by the delay in the decline of the elevated potassium permeability that frequently terminates the spike, while the positive after-potential is linked to active ion transport concerned with recovery from activity (83, 84).

Most of these components of the action potential were distinguished in early studies that demonstrated differences in their amplitudes and durations among A, B, and C fibers (43). Their functional importance lies in associated changes in membrane excitability, thereby influencing the ability of the fibers to conduct a succession of action potentials. Currently these various "potentials," and the underlying processes of passive and active ion transfer provide important clues concerning the mechanism of drug action.

Spike

General.—Additional voltage clamp studies on the amphibian node of Ranvier (26, 27, 32, 33, 34, 35, 67), and on the squid giant axon (11, 105) as well as other studies (e.g., 56) have demonstrated again the essential similarity of the two types of fibers in giving rise during excitation to (*a*) an initial increase in sodium permeability, P_{Na}, followed by (*b*) a decrease in P_{Na} (due to the process designated "inactivation"), and by (*c*) an increase in potassium permeability, P_K, which in amphibian fibers also undergoes inactivation (36). These investigations have also confirmed the earlier conclusion (84) that the oscillatory behavior of membrane currents at small clamping potentials,

assumed to render incorrect the interpretation of voltage clamp data (99, 100, 102), is a technical artifact resulting from inadequate control of membrane potential.

Further support of the voltage-clamp approach for an understanding of the bioelectrical processes underlying conduction, as well as of conduction itself, is seen in the broadened application of the equations developed by Hodgkin & Huxley (48) to describe the time-voltage dependence of membrane permeability in clamped giant axons. This approach has been applied without *ad hoc* assumptions to describe many new as well as older experimental results on conduction—results not only with the giant axon of the squid but with other nerve (and muscle) fibers as well. Thus, the effects of lowering extracellular calcium on oscillatory potentials and repetitive firing are accounted for by Huxley (50) in terms of previously measured changes in the voltage dependence of the rate coefficients controlling the permeability changes of sodium and potassium. Furthermore, the prolongation of the spike by tetraethylammonium, predicted earlier on qualitative grounds to be due to a delay in the increase in P_K (84), has now been shown quantitatively to conform in many of its properties to those predicted merely from a lengthening of the rate constants for P_K increase (31, 39)—a result confirmed experimentally by Hagiwara & Saito (43a) in a voltage-clamp study of giant nerve cells of Onchidium. Similarly, the prolongation of the spike at nodes of Ranvier by Ni^{++} (94) is explainable from alterations in the rate constants (25).

Most data are in agreement with the view that in excited nerve fibers the entry of sodium constitutes the normal source of diffusing charge which, upon entry during the phase of enhanced permeability to sodium, causes the initial depolarization and in particular the reversal of potential. The dependence of the rate of spike development and of spike amplitude on extracellular sodium concentration, $(Na)_o$, and more recently (6) on intracellular concentration, $(Na)_i$ (since the maximum reversal of potential during the peak of the spike is given by $59 \log[(Na)_i/(Na)_o]$ in millivolts at 25°C when P_{Na} greatly exceeds other permeabilities), to be expected from this view, has been verified in additional fibers, such as cockroach, lobster, and crayfish giant axons (16, 17, 119) and B fibers of the frog vagus (9). In such studies a suitable substitute for Na^+ must be used that will not function like Na^+. Choline has usually been the cation of choice. Depending on the fibers employed, however, various cations, organic and inorganic, including choline itself, appear to replace Na^+ as the carrier of charge, e.g., guanidinium in myelinated fibers of the frog (18, 55) and in B fibers of the frog vagus (9), Ba^{++} and Sr^{++} in B and C fibers of guinea pigs (42), and Li^+ in practically all fibers (18, 81). Ba^{++} cannot substitute for Na^+ in frog A fibers (42). The conclusion that such and other cations serve as diffusing charge has been most strongly supported by their ability, at increasing concentrations, to contribute increasingly to the rate of depolarization or to the amplitude of the spike during excitation. This conclusion remains to be validated by analytical data such

as were obtained earlier for sodium, for other interpretations of the results are possible (9, 55, 101). In any case, whether these interpretations are valid or not they serve to focus attention on the change in the excitable membrane which causes cations to be transferred more readily during excitation rather than on the specific ion involved. They also emphasize differences among membranes in their ability to transport sodium substitutes, differences which will have to be taken into account in molecular theories of excitation.

The possibility of another source of potential change, perhaps intrinsic to the membrane itself in the absence of external sodium, appears to have been demonstrated for frog spinal ganglion cells by Koketsu *et al.* (51 to 53). In these studies, microinjection of tetraethylammonium or hydrazinium restores a large bioelectrical response (but much prolonged) to electrical stimulation even though the sodium chloride of the bathing medium has been completely replaced by sucrose.

Drugs.—Many of the sodium substitutes not only restore the spike in certain nerve fibers and cells but, more usually, at low concentrations, and even when restoration does not occur in the absence of external sodium, they prolong the duration of the spike. In the case of tetraethylammonium, this appears to involve action intracellularly, perhaps on the inner lipid layer of the membrane, since its effects were shown by Tasaki & Hagiwara (101) to be induced only upon microinjection into giant axons of the squid. As in many other fibers, e.g., (43a), it acts from the outside as well as by microinjection into frog spinal ganglion cells (52). Hydrazinium when injected acts similarly (53). The effects, also seen with Ba^{++} (62, 83, 84), may include a decrease in resting potential, and an increase in resting resistance as well as the much more striking prolongation of the spike leading to a plateau. As pointed out recently on the basis of earlier literature (83, 84), these effects indicate (*a*) a decrease in the resting P_K, when present, and (*b*) a delay in the turning on of the increase in P_K during excitation. The former is in keeping with a decrease by tetraethylammonium of the depolarizing action of K^+ (98). A slowing of spike development, when present, suggests some interference with the increase in P_{Na} as well. Recent work by Grundfest and his associates (113, 114) on invertebrate muscle, as well as the calculations already alluded to that show the importance of a delayed increase in P_K for prolongation of the spike, have substantiated these conclusions. Thus, Ba^{++}, tetraethylammonium, and related compounds are particularly notable in having a marked effect on P_K and especially its changes.

Besides the rate coefficients, the maximum amplitude of the permeability changes can be modified, thereby also affecting excitability and the generation of the spike. This has been found to be the case in studies by Shanes *et al.* (87), and Taylor (104) on the action of stabilizers (e.g., the local anesthetics procaine and cocaine), on the voltage-clamped and on the conducting giant axon of the squid, and by Hagiwara & Saito (43a) on the action of urethane on the giant cells in Onchidium. As known earlier (83, 84), the resting characteristics of the excitable membrane may be little affected

by the anesthetics at blocking concentrations, whereas the ability of both P_{Na} and P_K to increase is depressed (87). Reduction in *excitability* will be governed, of course, chiefly by the effect on the ability of P_{Na} to increase. Hagiwara & Saito (43a) find that the change in P_{Na} is affected much more by urethane than that in P_K. The action of local anesthetics and other "stabilizers" in such terms was predicted by Shanes (83) from evidence by Skou (90) that the effectiveness of local anesthetics, now more recently confirmed for alcohols and other blocking agents (93), parallels their ability to penetrate and, thereby, to increase the surface pressure of monolayers of myelin extract. The view has been further developed and supported by the kinetics of the alteration of ion fluxes by cocaine [Shanes & Berman (86)], as well as by a review of earlier observations that clearly points to membrane action (83, 84). The outermost lipoidal layer of the bimolecular leaflet constituting the membrane appears to be affected by the local anesthetics by their entering primarily in the membrane regions between the sites of ion passage. This need increase but slightly the resting surface pressure (thereby affecting but little the resting ion permeability), whereas it could raise appreciably the resistance encountered by the molecules around the site of ion passage when, as a result of excitation, they tend to separate to admit Na^+ or K^+. At higher drug concentrations even ion passage at rest would be affected, as shown by electrical measurement (87), and by ion transfer studies (76, 79, 81). In keeping with this view, studies by La Mer and his associates (3, 8, 72) with monolayers of aliphatic fatty molecules have shown that increase in the surface pressure can indeed reduce penetration of the monolayers by gases.

It is surprising that more than 30 years after Gasser & Erlanger (38) pointed out the unacceptability of a hypothesis of blocking based on the reaction of anesthetics with axoplasm because of the slower unblocking, as well as blocking, of smaller fibers, and after the considerable evidence acquired since that local anesthetics act at the membrane level (see summary reference 86, also 83, 84, 87, 104), the earlier observations on the increased effectiveness of local anesthetics at higher pH still are ascribed to an effect on the *penetration* of the drugs as the base rather than on the greater reactivity of the base with the excitable membranes (69). Since a number of studies establishing greater potency at higher pH utilized critical blocking concentrations acting for long periods on desheathed nerves as a measure of effectiveness (e.g., 74, 89), rate of penetration could not have been a pertinent factor, especially in view of electron microscopic evidence by Nelson (66) and Robertson (71) as well as the physiological evidence by Dettbarn (22) against a diffusion barrier at least at nodes of Ranvier. A reverse effect of pH on the block of single nodes of Ranvier by procaine, tetracaine, and dibucaine is reported by Dettbarn (23) for between pH 7 and 9. Below pH 7 a decrease in effectiveness is again observed. On the basis of this study the cation rather than the base appears to be the effective agent. To what extent a difference in the membranes of fibers from different species may explain the difference in results remains to be determined. It has long been known

in studies with monolayers on water that interaction with substances dissolved in the underlying solution will depend, among other things, on the interfacial activity of the solute (i.e., its accumulation at the interfaces, because of repulsion by water molecules, which would increase with the amount of base and hence with pH in the case of local anesthetics), and on coulombic interactions (which would increase with the amount of cation, and, hence, with a decrease in pH with local anesthetics if the polar groups of the membrane are anionic and not neutralized by the lower pH's). Thus, the possibility cannot be ruled out that in the species used in the later study coulombic forces predominate over interfacial activity in the interaction of the local anesthetics with the nodal membrane, whereas the reverse would seem to be true in the earlier work. However, before this is seriously considered, it should be shown, as in the earlier study, that a pH insensitive stabilizer, e.g., butyl alcohol, is equally effective at all pH's. A decrease in pH generally raises threshold (25, 84), so that this could conceivably have lowered the safety factor sufficiently to give an apparent reversal of the pH action on the effectiveness of local anesthetics. A similar demonstration is desirable for the recent study by Ritchie & Greengard (69) on the C-fibers of the rabbit vagus, according to which dibucaine, tetracaine, chlorpromazine, imipramine, and procaine incr
decreases. The unusual technique employed in the last study also requires evaluation. The increase and decrease of the multifiber spike obtained during the washout of local anesthetic (to which the fibers were previously exposed) in an anesthetic-free solution, with increase and decrease in pH, was attributed to changes in the relative proportion of cations in the axoplasm. The unlikelihood that the intracellular anesthetic affects conduction has already been noted. Consideration should be given to alternative possibilities to account for the pH effect, such as alteration in the amount of anesthetic trapped between the axolemma and the inner myelin layer of the surrounding Schwann cell by changes in the permeability of the myelin (see reference 97 concerning the labile properties of myelin). Studies by Ritchie & Straub (70), and by Greengard & Straub (41) on the after-potentials of C-fibers have given strong evidence for a marked barrier to diffusion around the axolemma. It may be recalled that giant axons exposed to local anesthetics for sufficient periods of time to allow entry into the axoplasm recover quickly upon being washed in anesthetic-free solution. When then elevated into a moist chamber they block as the anesthetic diffuses from the axoplasm into the surrounding fluid, and recover again on washing—a process repeatable many times (108). Such extracellular effects by changes in anesthetic concentration, but induced by the diffusion barrier, could be involved in the pH study.

The demonstration that stabilizers interfere with the transitory increase in P_{Na}, and hence with an adequate rate of Na^+ entry to achieve the depolarization of excitation, provides a relatively simple basis to account for the ability of elevated $(Na)_o$ to counteract the stabilizers and, conversely,

for the enhancement of stabilizer action by lowered $(Na)_o$ (84). Earlier observations to this effect with n-hexanol, cocaine, benadryl, amylcarbamate, diisofluorophosphate, and physostigmine (14, 15, 68) were repeated recently by Condouris (12) with cocaine. Since from current views a given depression in the change in P_{Na} could be compensated for by a suitable increase in Na^+ entry achieved by raising $(Na)_o$, the conclusion of a direct antagonism between drug and Na^+ drawn by Condouris seems quite unjustified; moreover, such an antagonism would certainly be difficult to visualize between n-hexanol and Na^+.

In addition to block by stabilizers, which leave the resting potential little altered because of minor or equivalent changes in the resting permeability to Na^+ and K^+, block results from depolarization, whether it be caused by a directly produced diffusion potential (e.g., by K^+ or Rb^+), by an increase in the resting permeability to Na^+ (as caused by veratrine or its components, veratridine and cevadine), or by a reduction in ionic gradients (e.g., through action of metabolic inhibitors, such as azide, dinitrophenol, cyanide, and oxygen substitutes) (83, 84). Until Bishop's (7) critical survey of the effect of blocking agents on the resting potential, it had been generally assumed that all blocking agents act by reducing the membrane potential. He also pointed to the relative smallness of the depolarizations—a fact now understandable in terms of inactivation and its sensitivity to depolarization (84). By a mechanism that has not been established, although a new hypothesis has recently been proposed (85), a sustained lowering of membrane potential limits the ability of P_{Na} to undergo the large transitory increase of excitation (84). In keeping with this, any procedure that restores the membrane potential despite the presence of a depolarizing agent will restore conduction. Thus, anodal currents suffice to counteract metabolic inhibitors, veratrine, and K^+; moreover, local anesthetics, which reduce the ion leakiness caused by veratrum alkaloids, also restore the membrane potential and conduction at least temporarily (84). The restoration of conduction in veratrine blocked fibers has been confirmed by Herr & Akcasu (44) for both mammalian and amphibian nerve with procaine, cocaine, dibucaine, lidocaine, benzyldibucaine, benzyl-cocaine, Ca^{++}, and Mg^{++} under conditions more strictly defining the rates and concentrations required. Also they point out that at lower concentrations of veratrine the block, instead of being alleviated, may be accentuated by the stabilizer—a result suggestive of a stabilizing action by low veratrine concentrations. A stabilizing action was noted much earlier with respect to the ability of both veratrine and local anesthetics to depress the hyperpolarizing action of CO_2 on frog nerve (78). This two-fold action of veratrine is of interest in view of the studies by Shanes & Gershfeld (88) with stearate monolayers with show (*a*) the stabilizer type of interaction, namely an increase in surface pressure of the monolayer, and (*b*) the "labilizer" interaction, that is, a gradual decrease in surface pressure, apparently due to a withdrawal of stearate from the monolayer and which, like the permeability increase induced in excitable membranes by veratrine and its components (84), is antagonized by procaine, calcium, and low pH.

The labilizer-stabilizer antagonism illustrated by veratrine and local anesthetics may underlie the depolarization (and reduced action potential) described by Armett & Ritchie (4, 5) to occur in C fibers of the rabbit vagus exposed to high concentrations of acetylcholine and carbachol, and the reversal of these effects by agents known to have stabilizing action (83, 117), such as physostigmine, prostigmine, diisopropylfluorophosphate, tubocurarine, hexamethonium, atropine, and tetraethylammonium. The mechanism whereby acetylcholine and carbachol depolarize was not defined. At myoneural junctions they increase P_{Na} as well as other permeabilities (83). A lowering of potential caused by reduced resting P_K would merely be accentuated by stabilizers—that brought about by increased P_{Na} should be prevented by stabilizers, as observed. If an increase in P_{Na} is involved, acetylcholine depolarization should also be prevented by low $(Na)_o$ and restored by returning to high $(Na)_o$, as occurs in frog nerve with veratridine (96), and as more recently reported for analogues of acetylcholine: noracetylcholine 12, pyridinium dodecyliodide, and pyridine aldoxime dodecyliodide (21). The depolarizing action of the last compound is also prevented by physostigmine (20).

The antagonisms demonstrated between acetylcholine and anticholinesterases in C fibers show the accessibility of the excitable membrane to the latter, yet no effect (e.g., depolarization) indicative of acetylcholine accumulation was observed in the anticholinesterases alone. This and other data lead Armett & Ritchie (5) to remark that, while their findings suggest the presence in attenuated form in the C fiber membrane of a receptor mechanism similar to that in end-plates and ganglion synapses, "there seems no reason to infer from the present experimental findings that it plays a role in conduction along the nerve membrane similar to its well established role in neuromuscular and synaptic transmission." Other observations also appear to render less certain the role of acetylcholine in the increase in P_{Na} in nerve conduction as proposed by Nachmansohn (59) in a recent summary of his views. Thus, Friess and his colleagues (37, 115, 116), studying series of and optical antipodes of blocking anticholinesterases on desheathed frog nerve and single nodes of Ranvier, found no correlation between functional activity and the anticholinesterase activity of these agents. Moreover, Brady *et al.* (10), with careful injections into squid giant axons of high concentrations of cholinesterase, physostigmine, diisopropyfluorophosphate, and acetylcholine found no effect on conduction. Wright (118) has shown, too, that, contrary to the acetylcholine hypothesis, physostigmine concentrations which block conduction in single crustacean motor axons do not alter the time course of the subthreshold response.

Workers in Nachmansohn's laboratory have attempted to verify their proposal, that extracellular lipid barriers cause the ineffectiveness of many compounds that should modify conduction by virtue of interaction with the acetylcholine system, by adding agents that might disrupt such barriers. Cobra venom and cetyltrimethylammonium were found by Rosenberg & Ehrenpreis (73) to enhance the blocking effectiveness of d-tubocurarine on

squid giant axon; very high concentrations of other agents (acetylcholine, benzoylcholine, carbamylcholine, choline, prostigmine, decamethonium, chlorisondamine, protamine) were still required to block conduction. The usual objection to experiments with "specific" inhibitors remains that has been posed for experiments where access is not a problem, viz., the absence of evidence for the actual specificity of the interactions assumed to take place when conduction block is the sole criterion of action. It was pointed out some time ago that the process of stabilization is so characteristically lacking in specificity that any substance, including acetylcholine, can at high enough concentration exhibit this property (83, 84). In addition, it is difficult to accept the view of an effective barrier extrinsic to the membrane itself in the giant axon in view of the speed with which K^+, a very polar ion, can pass through the surrounding layer of Schwann cells. This is in keeping with the large channels estimated and seen passing through this layer (109, 110). Phospholipase A, a major component of cobra venom, and phospholipase C attack the excitable membrane—presumably the lipid and especially the lecithin component—of the lobster giant axon and lead to depolarization and irreversible conduction block (66, 106, 107). Such action, which apparently occurs in giant squid axons with five-times higher concentrations of cobra venom and cetyltrimethylammonium within a half hour (time used by Rosenberg & Ehrenpreis to presoak squid giant axons in lower concentrations of these agents) suggests that a rise in threshold and hence a lowering in safety factor—not evident in spike height determinations—could have occurred. This would accentuate the effectiveness of compounds like curare which were able to block at higher concentrations without presoaking (73). Similar objections can be raised against the conclusions of a related study on sheathed frog sciatic nerve in which cetyltrimethylammonium hastened the block induced by curare, mecostrin, prostigmine, succinylcholine, carbamylcholine and acetylcholine (111). At least part, if not all of this action is, however, more likely to have been caused by a reduction of the now well established barrier action of the perineural sheath (83), especially since Dettbarn (22) has confirmed experimentally the ready accessibility of nodal membranes to curare. Hence, until it can be shown that the lipases and detergents did not raise threshold and lower the safety factor of giant axons, or merely increase the penetrability of the perineural sheath of sheathed vertebrate nerve, the enhancement of the effectiveness of curare and other compounds produced by these agents cannot be safely attributed to weakening of pericellular barriers.

Energetics.—No energy, other than the potential energy inherent in the concentration gradients established previously by the work of ion transport, appears necessary to explain spike production. The older strong evidence favoring this view has been summarized repeatedly (e.g., 80, 84): (*a*) Action potentials continue to be produced long after the extra oxygen consumption of activity is suppressed; (*b*) they are maintained or restored despite strong metabolic inhibition by preventing the decline of, or by restoring the mem-

brane potential with an electric current; and (*c*) severe curtailment of the active transport of the unexcited fiber leaves the ion interchange during excitation unaltered. These facts make it unlikely that the permeability changes themselves are directly linked to metabolism. Furthermore, drug effects by metabolic inhibition are in many instances ruled out (*a*) by the important difference between the functional effects of metabolic impairment and drugs, and (*b*) by the obvious action of metabolic inhibition through secondary alterations in membrane potential and ion gradients (83, 84). Moreover, the action of many drugs in terms of *measurable* modifications of the related electrochemical membrane properties—membrane potential, resistance, and permeability to ions—is now too well established to be discounted (83, 84).

Measurements of initial heat production lend little support to the view that metabolic reactions occur that are directly concerned with spike production. The earlier estimates of the initial heat of activity, which were too small to account even for that to be expected from the physical (e.g., thermal heating by ionic currents) (47) or physicochemical events of conduction, have recently been increased five- to ten-fold for invertebrate nerve by correction for a secondary negative heat that recent measurements in Hill's laboratory (2) show had obscured a major part of the initial heat in the early measurements. Half of the larger initial heat appeared to be accounted for by the non-ideal mixing of Na^+ and K^+ in the medium and axoplasm as a result of the ion interchange during the spike (2). However, replacement of the medium Na^+ by Li^+ failed to achieve the reduction in initial heat to be expected if mixing were its source (44a). Hence, at least part, and perhaps all of the negative heat, may be linked to the irreversible thermodynamics of the membrane permeability changes. Certainly part of the remaining initial heat must be attributed to the heat resulting from the ionic currents during conduction; this follows from Abbott's finding (1) that the ideal situation expected of concentration cells, viz., the uptake of heat equivalent to the energy liberated by current flow, does not apply to the biological situation.

On the basis of available data, then, it remains likely that a major part of the energy turn-over of resting nerve fibers is concerned with the maintenance of the potential energy stores in the form of the potassium and sodium ion gradients, the former gradient contributing largely to the resting potential and both to the action potential (13, 49, 83, 84). Metabolism therefore compensates not only for the leakage at rest, which is essential to the origin of the resting potential, but for the increased leakage occurring during activity. After-potentials, in particular the positive after-potentials, have been linked to metabolism and restoration of ion gradients.

After-Potentials

Negative after-potential.—By the end of 1957 only two situations were recognized to give rise to this after-potential. A transitory accumulation of

released K^+ around giant axons of the squid, due to diffusion limitations imposed by the surrounding Schwann cells; this principle was found to be the normal situation following spikes in this preparation; this principle had previously been demonstrated with repetitive stimulation of crab nerve (84). Evidence has recently been presented by Straub and his associates (41, 70) for a similar process in C fibers of mammalian dorsal roots and of sympathetic trunks, and by Narahashi & Yamasaki (63) for cockroach giant axons. A second process underlying the negative after-potential was suggested for that induced by veratrine and its components, viz., a delay in the shutting off of the increase in P_{Na} during the spike (in reference 84). More recently Narahashi & Yamasaki (64) provided evidence that the negative after-potential produced in cockroach giant axon by the insecticide, DDT, is more closely related to the action of tetraethylammonium, viz., a delay in the turning on of the increase in P_K during the spike. Much earlier work on DDT in crab and amphibian nerve (75, 78) found no evidence for a negative after-potential, although a repetitive response was obtained as in cockroach axons.

It is probable, according to Meves (57, 58) and others [e.g., Dettbarn (22)], that at the node of Ranvier diffusion limitations are inadequate to give rise to sufficient K^+ accumulation to produce a negative after-potential by this process. Under the special conditions of K^+-treated nodes repolarized electrically, Meves (57, 58) finds that a large negative after-potential appears which is caused chiefly by a slow second stage in the decline in P_K following the spike. The latter may underlie the normal negative after-potential at nodes if a process other than potassium-sodium diffusion, as suggested by Stämpfli (95), sets the potential higher than to be expected from a simple steady state diffusion potential. Böhm and Straub (9a) have recently presented indirect evidence, based on the action of lithium on the resting potential of bundles of myelinated fibers, that the membrane potential is a consequence of active sodium transport which is not coupled with potassium uptake. In keeping with this, the extrusion of sodium in unpoisoned frog nerve, and during recovery following azide inhibition, appears to be independent of external potassium (49); however, the ratio $[K]_i/[K]_o$ is much too large to be explainable by this mechanism, hence further study appears desirable.

Positive after-potential.—By 1958 this was viewed as arising primarily in association with the active transport processes of recovery (84). One mechanism demonstrated previously in crab nerve—the depletion of potassium from a restricted extracellular space because of diffusion limitations and of active potassium reabsorption (77)—has been invoked by Straub and his associates (41, 70) to account for the positive after-potential in mammalian C fibers. This accounts qualitatively and quantitatively for findings such as the dependence of this after-potential on external K^+ and its abolition by metabolic inhibitors, by ouabain, and by replacement of external sodium by lithium. However, Connelly (13) has pointed out that a mechanism of sodium extrusion without coupling to K^+ uptake would increase membrane

potential, and, thereby, could give rise to the same phenomenon. Demonstration of sodium extrusion without external potassium or without potassium uptake following a period of activity would be needed to establish this view. This demonstration has actually been made in unstimulated fibers upon termination of azide inhibition (49), but not during post-anoxic recovery (82), or after other conditions that stimulate sodium extrusion (13); hence, the situation following activity remains to be evaluated. Another mechanism of active sodium transport has been demonstrated in frog nerve—sodium exclusion—which requires external potassium (83); this mechanism is capable of raising membrane potential (but not above the potassium equilibrium potential), and has been postulated as an alternative basis for the metabolism-dependent after-potential (84). Therefore, analytical data should be able to determine whether uni-ionic transport or sodium exclusion is the mechanism underlying the positive after-potential at nodes of Ranvier and whether either may be significant for C fibers.

The action of yohimbine, quinine, and other substances which induce or enhance the positive after-potential (83, 84) remains to be more clearly delineated. Recently the analgesics morphine, acetylsalicylate, codeine, dihydromorphinone, levorphanol, and meperidine have been described by Krivoy (54) as causing a gradually increasing, greatly prolonged, positive after-potential in frog nerve (54); strychnine and methadone were found by the same author to differ in inducing a rapid development of "positive after-potential"; but this more likely reflects the positive overshoot (positive potential), hence is the result either of a delayed decline in the elevated P_K terminating the spikes or of a prior lowering of resting potential, which accentuates or makes apparent the positive potential (84). The enhancement of the positive potential by depolarization has been noted again at frog nodes of Ranvier (57, 58), and in mammalian C fibers (4).

Refractoriness and Repetitive Response

A number of studies continue to utilize changes in the ability of nerve to follow repetitive stimulation as an index of drug action, but no analysis of the bioelectrical events or underlying permeability processes seems to have been attempted in current research. The duration of the absolutely refractory period must depend in large measure on the duration of the spike (inclusive of the plateau when present), and also on the rate of disappearance, following the spike, of the inactivation and the augmented P_K that developed during the spike. Both of the latter raise threshold; the first, by slowing the rate of active (i.e., sodium dependent) depolarization during an applied test stimulus, so that inactivation or the rise in P_K during the test depolarization prevents excitation; the second, by hastening repolarization (by potassium exit) so that active depolarization fails to achieve the necessary rate or level for excitation during the test stimulus. In more general terms, as brought out so clearly by Tasaki & Takeuchi's graph of the time course of threshold and bioelectrical response of a control and urethane-

treated node of Ranvier (103), the time for the threshold to decline and for the (initially subthreshold) response to rise to the same amplitude sets the critical time at which conduction becomes possible. The blocking agent delays the recovery of both the threshold and the subthreshold response, and thereby lengthens the minimum interval possible between successive impulses. This provides one clear-cut basis for the decrease by drugs of the maximum frequency of stimulation that can be followed by fibers; to this must be added the possibility of a lengthening of spike duration. The decrease of the maximum frequency by cocaine—a stabilizer—has again been noted recently by Condouris (12); the depolarizing blocking agent veratrine acts similarly (44). Of interest is Herr & Akcasu's demonstration (44) of the temporary partial reversal of the veratrine action by the stabilizer procaine; the antagonism between veratrine, a labilizer, and stabilizers has already been pointed out to be understandable in terms of the restoration of membrane potential. Krivoy (54) has shown that analgesics also decrease the maximum frequency of conduction in frog nerve.

Molecular Models of Drug Action

The success or the promise of success with which the action of many pharmacological and physiological agents on nerve (and muscle) can be understood in terms of alterations in the resting permeability, in the transitory permeability changes of excitation, and in the metabolically dependent transport of ions (83, 84) has encouraged a renewed attack on molecular mechanisms through models. If correct concepts are eventually to be achieved, the model selected must first of all be a reasonably close approximation of the composition and organization of the actual structures affected in the living system (e.g., the excitable membrane), and show a lability in properties that can be readily related to permeability. Secondly, the pharmacological agents selected for study must have been thoroughly characterized in terms of their detailed action in living systems by actual measurement (e.g., the specific permeabilities or changes in permeability affected), rather than in terms of hypothetical or vague ideas of mode of action yet to be demonstrated. Watson (112) has recently reviewed many of the hypotheses as well as modifications of these hypotheses on the mode of action of local anesthetics. Here only three particularly recent models will be considered.

Monolayers.—Skou's utilization (90, 93) of lipoidal monolayers, of which the living membrane consists, to study interactions of well characterized stabilizers provided a particularly fruitful approach. He succeeded in demonstrating that the blocking potency—varying by a factor larger than 13,000—of a wide variety of related and unrelated compounds closely parallels the ability of these agents to interact with and to penetrate the monolayer, as indicated by the rise in surface pressure—a spreading pressure—measured in the Adam-Langmuir film balance. Inhibition by local anesthetics of acetylcholinesterase in solution or *in situ* in erythrocyte membranes was found by Skou (91, 92) not to exhibit this parallelism with blocking potency. Subse-

quently, Shanes (83, 84) called attention to the possiblity that the development of increased pressure by penetration of stabilizers into the regions of living membranes immediately around the sites of ion passage might be expected to interfere more markedly with the opening of such channels during excitation than with the resting permeability. Still higher concentrations could cause the now well-known interference with ion passage in the resting state by compressing the regions of ion passage. As already noted, subsequent study on giant squid axons (87) confirmed the interference with the increase in P_{Na} and P_K by stabilizers at low concentrations of anesthetic, and the decrease in the ion permeability at rest at higher concentrations; the former result has been reconfirmed (104). La Mer and his colleagues (3, 8, 72) have actually demonstrated that the passage of gas molecules through monolayers of aliphatic fatty acids is reduced by increased surface pressure; protein monolayers, on the other hand, exhibit no appreciable interference with gas passage.

Further critical evidence for the validity of using lipoidal monolayers as a model for understanding drug action in living systems was provided by Shanes & Gershfeld (40, 88) when they demonstrated that agents acting oppositely to stabilizers, e.g., veratrine and its components, which increase membrane permeability, decrease the surface pressure of stearate monolayers (apparently by removal of monolayer molecules); and that, as demonstrated for the permeability increase in living systems, the effect of these labilizers is antagonized by a local anesthetic, Ca^{++}, and low pH. As in the studies with stabilizers, effective concentrations are the same as employed for living systems.

Important quantitative support for the generality of the interfacial film approach for an understanding of the action of anesthetics or narcotics has just been provided by Clements & Wilson (10a). They find that, with "inert gases" as well, "there is a systematic relationship between anesthetic potency of an agent and its affinity for" films of lipoprotein extracts from beef lung. To a lesser extent a relationship was noted with films of defatted plasma albumin and of crystalline ribonuclease. They also note that energy considerations favor lipid-anesthetic interactions over aqueous clathrate formation, proposed by Pauling (67a), as the basis for anesthesia.

The lipoidal monolayer model requires much further development, but it has already served to clarify the significance of a number of old as well as new findings. For example, lipid solubility as a factor in drug potency can be seen to reflect two factors in actuality, (*a*) interfacial activity (the tendency to accumulate at the outermost boundary of water because of hydrophobicity, which is inversely related to lipophilicity), and (*b*) the strength of van der Waals and other related forces which govern in part the ease with which a molecule will interact with the non-polar portions of other organic molecules. It is generally assumed in pharmacological studies that when a minor structural change in a compound markedly increases potency, enhanced specificity of interaction is involved; but measurement of the lowering of surface tension

should invariably be checked as a rough index of interfacial activity, since this may well indicate a greater tendency to accumulate at the membrane-water interface solely because of repulsion from the water phase. Hence, comparison of potency should be based not on relative concentration in solution but rather on relative surface concentration or activity. In the veratrum alkaloid studies (88) the relative potency of procaine, cevine, and veratridine (which differs from cevine merely by the addition of a small molecule—veratrate) parallels their large differences in surface activity, but their activity (i.e., effective concentration) at the surface is also dependent on (and enhanced by) more specific interaction with the non-polar moiety of the lipoidal molecules and also with the polar (carboxyl) groups facing into the water. The latter is made evident by the reduced interaction of veratridine with stearate monolayers in the presence of Ca^{++} and at low pH, which neutralize the COO^- charge of the stearate molecules. The relative sensitivities of different nerve fibers to a given drug, after allowance is made for such physical factors, could well be related to the different relative amounts of specific lipoidal components in their membranes, and the relative contribution of coulombic and various other forces to their interaction with the drugs. It is noteworthy that the relative content of lecithin in the ghosts of red cells from different species parallels the rate of hemolysis in glycol and hence is inversely related to the rate of glycol penetration into these red cells (19).

Thick lipid membranes.—Recently, a paper appeared by Tobias *et al.* (107a) describing relatively thick membranes of cephalin, or of cephalin and cholesterol. These membranes exhibit a much lower resistivity in solutions of alkali metal ions than in solutions of alkaline earth ions—a difference attributed to alterations in the state of hydration of the membranes. The effects of drugs in this type of system remain to be examined.

Receptor protein.—A protein (actually a lipoprotein) has been extracted from the electric organ of the electric eel (28) which, by virtue of its precipitation by nerve blocking agents that have anti-acetylcholine action at junctions, is considered by Nachmansohn (60, 61) to be the acetylcholine receptor he has postulated in nerve membranes. Support for this view has been withdrawn by Ehrenpreis (29), who originally contributed to this work. Ehrenpreis mentions, among other things, (*a*) that the interaction of the protein with acetylcholine is weak, whereas that with acetylcholine analogues (noracetylcholine-12, norcholine-12, and pyridinealdoxime dodeciodide) is strong—the reverse of their effectiveness on the living cell—and (*b*) that the antagonism to be expected of carbamylcholine against curare binding by the protein, in pharmacologically effective proportions, is not obtained. The conclusion is drawn that the protein is a more general constituent of excitable membranes, and that its degree of interaction with the various compounds related to the acetylcholine system explains their blocking potency quite independently of their action at end-plates. This is in keeping with the general principles of stabilization previously elaborated for nerve fibers and with monolayer models. Nachmansohn provides a rebuttal (61) the adequacy

of which will be judged in part by one's own prejudices, and in part by other available incongruous facts such as were mentioned earlier, including those presented by Brady *et al.* (10), by Friess & associates (37, 115, 116), and by Armett & Ritchie (4, 5). As yet the "receptor protein" model does not meet the criteria of thorough characterization suggested earlier as essential for acceptability. Moreover, certain aspects of the inferences drawn seem questionable. Thus, how certainly can a gross extract from electric organs, which are modified muscle cells with one membrane having many junctions, be related to the electrically excitable nerve or even muscle membrane? Why may not the interactions observed be with the lipoidal component of the protein, or merely be similar to the actual reactions with identical groups (carboxyl, phosphate, etc.) in the lipoidal element of excitable membranes? And how would alcohols and cocaine, which block nerve in a manner indistinguishable from agents presumed to act on the acetylcholine receptor, react with the protein?

Conclusion

Technical methods which still are generally ignored have been available for some time for evaluating the action of drugs on nerve. The same equipment used to supply solely the height of a multifibered action potential could be used to measure at least threshold changes and demarcation potential alterations—data that would help enormously for a proper interpretation of effects. With little additional trouble, external electrodes, and better still internal electrodes, can provide data on configuration changes of the spike from large single fibers of readily obtainable invertebrates, and, thereby, can offer still more information for the understanding of mechanism. And, of course, with considerably more effort, the use of voltage-clamp methods offers the ultimate at present in the analysis of alterations in the electrophysiological or, more properly, in the electrochemical events of conduction. Until the maximum possibilities of such analysis, including electrolyte studies, are explored, attempts at interpretation, especially in molecular terms, or of extrapolation to the central nervous system, can be regarded at best as tentative. In the meantime, those agents which have been well characterized provide an important set of data that can serve as criteria for judging the validity of molecular models. As additional drugs are carefully studied, they will undoubtedly also serve as powerful tools for probing the mechanisms in excitable cells and as tests of the validity of molecular models.

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