NEUROPHARMACOLOGY OF PERIPHERAL NERVE*

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I. INTRODUCTION

Active research in peripheral nerve physiology is now over a century old. Like the centenarian, it tends to become set in its ways. The vast body of accumulated experience is often hard to translate for the benefit of those in other fields, who grow impatient with the language, the compulsions and the scholastic disagreements of the axonologists. But present needs, on the part of the neurophysiologist who requires chemical tools for his work as well as on the part of the pharmacologist who wishes to find mechanisms of action, demand that someone build a neuropharmacological bridge. The present reviewer, poorly equipped for this pontification, will try to assemble the most relevant data and opinion from the works of those neurophysiologists who have contributed most heavily to the pharmacological field in the past two decades—in particular, Lorente de Nó and his colleagues (98–100, 165, 166, 182–189), Gerard's group (32, 33, 45, 73, 74, 103–107, 175, 176, 210, 236, 265, 266), Nachmansohn and associates (41, 42, 120–122, 159, 205–207, 234), Bronk's laboratory (34–37, 52, 167, 168), Prosser (221–223a), Rosenblueth *et al.* (177, 229–234), Welsh and others (114, 239, 240, 287, 288), Höber (135–139), Crescitelli (56–60) and Shanes (245–247). It will be impossible to cite everyone who has worked on drug action on nerve, and some contributions, although important, will be passed by to permit space for discussion of certain key problems in neurophysiology.

It is the purpose of this review first to survey those properties of peripheral nerve which may be studied to the advantage of pharmacology, and then to enumerate the actions of specific drugs of interest—particularly those which have actions of relevance to normal neuronal function. Unfortunately, except for local anesthetics, little of this information has practical significance as yet for the

clinical applications of pharmacotherapy. It will have to justify itself at present for whatever theoretical overtones it can offer.

II. PROPERTIES OF NEURONES

This section is not planned as a consistent summary of theoretical neurophysiology. Instead it is intended to outline those properties which can easily be measured and which are potentially modifiable by drug action. For theoretical summations on the relationships of properties of nerve fibers, a number of reviews and monographs, old and new, should be mentioned (12, 20, 23, 24, 26–28, 36, 67, 75, 82, 101, 105, 106, 120, 133, 135, 137, 146, 152, 164, 179, 184, 190, 202, 206, 214, 218, 223, 223a, 225, 238, 251, 269, 291). The following subdivision of properties into "resting" and otherwise is made with some hesitation, because modern investigations show beyond a doubt that the non-conducting fiber is considerably more than an osmotic sausage-casing. Indeed it can be said that those same processes which enter into propagation and recovery are most probably operative but in balance in the "resting" state. However, it is somewhat convenient to differentiate between steady states on the one hand and explosions on the other.

A. *"Resting" Properties*

1. *Structural features.* Only sporadic attention has been paid to visible alterations of axone structure as a function of experimental procedures, but recently there has been an increasing interest in this field. Following Flaig's report (95) of opacity changes associated with stimulation of large invertebrate fibers, Tobias (253, 265, 266) and Hill (134) have studied light-scattering and volume changes, and Tobias has related these to effects of cations. Lorente de Nó (184) has made occasional observations on frog axones, noting, for example, the swelling and disintegration of the myelin sheath with high calcium concentration, and the tendency of myelin to flow and surround the nodal incisures in old preparations. Spectophotometric studies during activity have been made by Minz (200) and von Muralt (280) for the detection of liberated substances. The tissue culture methods of Pomerat (219, 220, *cf.* also 15), which have now been applied to adult neurones and glia of brain and cord, offer sensitive preparations for visual study of drug action on regenerating cells. It would be worth while to repeat some of the gross observations by Marinesco (see 13) on such preparations. In view of the increasing interest in neuro-regenerative substances, and the demonstration by Weiss (286) that there is a perpetual normal flow of axoplasm from the cell body, it would be important to gather information on the pharmacology of isolated intact neurones in culture.

The variations in size and type of axones offer a perplexing choice of material for studies in pharmacology, but certain fundamental qualities are universally present. Although the fiber diameters may vary from a micron in vertebrate unmyelinated fibers to a millimeter in invertebrate giant fibers (48, 223, 288), all have in common a complex lipoprotein surface, whether vanishingly thin or measurably thick, and densely or loosely packed (48, 67, 241). Both the lipoprotein surface and the gelatinous axoplasm show structural orientation (67, 69,

241). Since all modern theories of excitation demand active physical and/or chemical changes in the membrane, and some require participation of the core as well (184), there is probably much of value to be learned from visual observation during drug application. Certain differential features, such as the nodes of vertebrate myelinated axones or the septa of certain invertebrate giant fibers, appear to be of less functional interest. At any rate, modern microscopic techniques, such as polarization or phase microscopy, spectrophotometric and scatter measurements, and the use of time lapse cinematography, are worth further exploration by pharmacologists.

2. *Permeability.* It is of prime importance to determine whether substances under study can penetrate into the nerve fiber and, if so, whether they in turn alter the permeability of the fiber for other substances. Many controversies have hinged on the question of penetration of an ineffective drug, for example, acetylcholine (33, 106, 184, 206), and permeability changes are often invoked to explain drug actions (*e.g.*, 97). To perplex the situation further, drugs are sometimes assumed to act by adsorption upon a surface membrane (23, 114), in which case evidence concerning penetration into the axoplasm would be irrelevant. There are available a number of excellent reviews dealing with permeability (39, 67, 135, 137, 160, 214, 238, 275).

Actually it is difficult to define the site of the supposed surface where critical functions are thought to occur in neurones. Lorente de Nó (184) has given reasons for considering that there are three such sites, that the axoplasm itself is involved in depth, and that the myelin-extracellular interface is not critical. For the present, therefore, it might be better to think of a cortex rather than an interface, and to assume that agents which act upon nerve must penetrate some distance toward the interior.

Earlier studies seemed to indicate that neurones were impermeable to anions and to sodium (19), and passive penetration of ions has been assumed even recently to determine the distribution of electrolytes, with potassium inside and sodium outside (53). However, investigations with radioactive ions have made it clear that there is a metabolic pumping action in operation to separate ions. Krogh (160) and Ussing (275) among others have given evidence for the existence of active transport of substances in cells, particularly in the case of sodium. Since the transfer rate of radioactive sodium appears to be even faster in nerve than that for potassium (284, 292), it is apparent that an active mechanism, dependent upon metabolic processes, is necessary for the maintenance of the observed electrolyte distribution. The high temperature coefficient for penetration of phosphate (93) would also seem to indicate active transport. In brief, even where a particular substance seems to penetrate very slowly if at all, as judged by gross chemical methods, there exists the possibility that it does indeed penetrate but is pumped out at a rate which keeps the intracellular concentration low or constant.

For any drug group there appear to be a number of factors determining penetration rate into nerve, as into other tissues. In general, nonelectrolytes are taken up more easily than electrolytes, smaller more easily than larger ions or molecules,

undissociated more easily than dissociated molecules, tertiary more easily than quaternary ammonium compounds (137, 223a). Nonpolar compounds which are more lipid-soluble tend to penetrate more readily than polar, but there are special considerations concerning the long-chain fatty acids and other polar-nonpolar compounds which are thought by some to play a special role in nerve (135–138). It is chastening to note that in some cases even proteins, such as botulinus toxin (6, 126), with a molecular weight of a million and bristling with polar groups and free valences, nevertheless manage to penetrate nerve fibers and exert characteristic actions on enzymes that must lie deep below the surface.

In view of the ease with which many substances can now be prepared with radioactive or heavy isotopic tracers, and the encouraging results which have been obtained by tracer studies in nerve (68, 93, 117, 121, 155, 156, 204, 234, 284, 292), it seems that much of the older information on permeability, based on gross chemical analysis or even on indirect estimation by observation of ultimate effects of drugs, will be in need of revision.

3. *Resistance of membrane and core:* Membrane resistance is reciprocally related to permeability for ions, and thus has significance for practical determination of drug effects, as well as for a study of the basic electrical properties of axones. Two general methods have been evolved for measuring membrane resistance. The method of Curtis and Cole requires the actual penetration of the axone by a microelectrode and the use of alternating current as the signal in a bridge circuit, for which the membrane serves as one arm (49–51, 63, 285). The other utilizes external electrodes and the passage of a steady subthreshold current through the fiber or nerve; the necessary data are derived from measurements of the rate of fall of the steady electrotonic potential in the extrapolar region and the current flow in the interpolar region (140–144, 153, 184, 284). The basic theoretical discussions necessary for an understanding of the latter method will be found in the works of Hodgkin and Rushton (144) and Lorente de Nó (184).

The estimated membrane resistance varies widely among axones, from 15,000 ohm cm^2 in myelinated peroneal fibers of the bullfrog (184) to 300 ohm cm^2 for giant fibers of the squid (51). Strangely enough, if one multiplies the membrane resistance by the axone diameter for several types of fiber, one obtains an approximately constant value, the resistance dropping regularly with increasing diameter over a fifty-fold range. The relationship is such that the flow of ions across the membrane tends to remain constant per unit volume of axone regardless of size. Since nerve metabolism per unit volume does not vary widely among various fibers, and membrane voltages are similar despite size, the significance of the diameter-resistance relationship becomes obvious. However, such a relationship tends to set a limit to fiber size, for above a millimeter the membrane would have to approach axoplasm in permeability, and below a micron there would be little room for axoplasm, in order to satisfy the above conditions.

Axoplasm itself has a relatively low resistance, although several times greater than that of the usual extracellular fluid.

It is disappointing to note that this methodology and theory have received practically no pharmacological application except insofar as potassium has been

found to decrease membrane resistance (141, 142, 156). For ordinary purposes a relative estimate, based on the measurement of extrapolar electrotonic potential at a few points, should suffice to indicate the general direction of change with drug action. In view of the importance attributed to the axoplasmic core (184) and the previously discussed physical changes during activity, estimates of drug-induced changes in axoplasmio resistance might also be useful.

4. *Capacity and inductance.* All electrical models of nerve demand, as one of the circuit elements, an equivalent capacity—that is, the property of storing a quantity of electrical charge. The membrane capacity can be estimated either by phase analysis, using the frequency method of Cole and Curtis (50), or by the method of Hodgkin and Rushton (144) and Lorente de Nó (184), based on the time rate of rise or fall of the electrotonic potential in conjunction with the method for determination of membrane resistance. Myelinated vertebrate fibers have an equivalent capacity of approximately 0.01 microfarads/cm^2 (184), while most other fibers are reported to be of the order of 1.0 microfarad/cm^2 (49–51, 140, 144, 284). The difference is presumably related to the depth of the myelin layers in vertebrate fibers, giving a series arrangement of equivalent condensers.

There is less agreement on the presence or significance of an equivalent inductance in nerve models. An inductive reactance may be assumed in a circuit element when a potential appears across it which is proportional to the rate of change of current. Such an effect appears in the overshoot of the action potential of squid fibers, and in various rhythmic phenomena. Cole (49) has estimated the apparent inductance of squid fibers, but Lorente de Nó denies the validity of such estimates on theoretical grounds (184). Practically, an inductive reactance assumes importance only when the membrane resistance is low and damping therefore minimized.

Since estimates of both capacity and inductance are dependent upon measurements of resistance, it is not surprising that drug effects have been neglected.

5. *Characteristic length, time and frequency.* The passive electrical properties already described may be manipulated to evaluate certain quantitative features of nerve which are of importance to excitation theory. The natural unit of length, λ, is the distance over which a steady electrotonic potential falls to $1/e$ in the extrapolar region. It is simply related to the internal, external and membrane resistances by the relation $\lambda^2 = r_m/(r_e + r_i)$. For frog nerve its value is given by Lorente de Nó as 3 mm, and others have reported similar values (152, 184, 238). For invertebrate preparations the values are lower since the membrane resistance is lower (144, 284), and one must assume that the same would hold true for the unmyelinated soma and processes of vertebrate central neurones, where a high value would lead to serious problems in neuronal interactions.

The natural unit of time, τ, is estimated from the time required for the electrotonic potential to reach 85% of its final value at a polarizing electrode. For frog nerve it is reported by Lorente de Nó as 0.2 msec (184). It corresponds to the product of the membrane capacity and resistance, $c_m r_m$. Since invertebrate fibers have in general a larger capacity, and a resistance reciprocally related to size, one finds values for giant fibers approaching in brevity those of vertebrate nerve.

A combination of inductance and capacity imparts a characteristic frequency to a circuit. Brink *et al.* (34) have estimated a basic local oscillatory period of 3 msec in calcium-free squid axones, which agrees well with the characteristic undamped frequency derived by Cole (49) from inductance and capacity measurements. Brink found a similar periodicity of 6 msec for frog myelinated fibers. It would be of interest to determine drug effects not only on characteristic frequency but also on damping, since the latter is related to membrane resistance.

6. *Electrotonic potentials and polarizability.* Lorente de Nó (184) has used the measurement of electrotonic potentials extensively in studies of drug action. The method is simple in that one records the voltage developed between two widely separated electrodes while applying rectangular pulses of known current through another pair. The resultant record of voltage exhibits three distinct components, corresponding presumably to three polarizable layers within the fiber, and related to three differentiable components of the membrane resting voltage. Although such measurements are among the oldest in neurophysiology, Lorente de Nó has developed them to a high state of perfection. The method was primarily focussed on the solution of basic neurophysiological problems, but the variety of drugs used as tools attests to the sensitivity of this method for pharmacological study. The slowest electrotonic component is particularly vulnerable to chemically-induced changes. Since changes may be observed under conditions of conduction block or depolarization, the range is less restricted than for methods involving propagation of impulses.

7. *Membrane voltage.* Over a century ago Matteuci (197) discovered that voltage differences could be measured along the length of dissected muscle, and DuBois-Reymond (75) showed that an injured portion of nerve was electronegative to the intact surface. Helmholtz (129) and later Hermann (131) dealt with the various alternative possibilities of origin of injury potentials. Hermann believed that they were produced at the time of injury, while Bernstein (19) contended that the pre-existing difference of potential across the nerve membrane was conveniently revealed by the injury. Bernstein and later Cremer (55) postulated that the resting membrane potential was attributable to the difference in concentrations of potassium inside and outside the nerve, which was believed to be impermeable to sodium and anions. This passive explanation still finds considerable acceptance, although the evidence against such a simple mechanism has considerably expanded in the last two decades. Koch (157) and Gerard (103) were the first to demonstrate the dependence of the membrane potential upon oxidative metabolism, and it is now generally accepted that, regardless of the precise mechanism of voltage production, it can be maintained by the nerve only through the performance of metabolic work.

The method of measurement of membrane voltage by direct insertion of a microelectrode was first developed for use in large plant cells (28, 215), and the first such measurements in nerve were made by Cole and Curtis (50) in the giant axone of the squid, over half a millimeter in diameter. For investigations of smaller frog muscle fibers, Ling and Gerard (175, 176) developed an extremely fine KCl-filled glass capillary, less than a micron in tip diameter. The method

was applied to cardiac muscle by L. A. Woodbury and to frog sciatic A fibers by J. W. Woodbury (293). Weidmann (284) has applied this more refined method to squid axones as well as to mammalian heart, and many others are now utilizing microelectrode recording for other tissues. Judging from the stability of membrane voltages recorded by various authors, the technique is relatively non-injurious and gives a prompt and accurate evaluation of membrane potential.

The voltages recorded by puncture methods are in general greater than those previously reported by indirect methods, running usually in the range from 60 to 100 mV for all tissues which have been studied. The technique also has established beyond a doubt that the voltage is developed across something corresponding to a membrane, since the establishment of potential is sharp during the initial puncture and relatively constant on further manipulation, although minor variations with insertion suggest that the voltage-producing layers have a finite thickness.

One disadvantage of the micromethod for pharmacological purposes in procedures on vertebrate nerve is the difficulty in maintaining a given insertion in a particular fiber while altering fluid. For this reason many prefer to continue the use of older gross methods, involving a comparison of treated and untreated surfaces, or measuring against a reference electrode in an area depolarized by injury or by isotonic KCl. Lorente de Nó and his coworkers (98, 100, 182, 189) have made extensive observations of drug actions on frog nerve in this manner, as has Shanes (245–247) with invertebrate nerve. Although Shanes and a number of others (54, 89, 90, 125, 141) place primary emphasis on the role of potassium in maintaining membrane voltage, Lorente de Nó inclines toward the view that depolarizing agents including potassium act upon oxidative mechanisms which maintain voltage in some other manner. Hodgkin (141) assumes that the metabolic work is done in pumping out sodium ion. The experimental fact remains, as shown by Lorente de Nó (184), that in frog sciatic nerve the membrane potential is maintained for long periods of time in the absence of any inorganic ion species in the external fluid, and that the nerve requires only oxygen and its own consumable substance for voltage production.

Lorente de Nó has identified experimentally three distinct fractions of the membrane voltage, the Q, M, and L fractions in order of increasing sluggishness, and corresponding to polarizable systems previously mentioned in relation to electrotonus. They are more or less independently modifiable by stimulation or drug action. The slow L fraction is most nearly related to changes in threshold.

Even though the precise mechanism of membrane voltage remains in controversy, the measurement is often desirable for analysis of drug action, and the equipment is relatively simple. There has been little excuse in the past century for assuming depolarizing actions of drugs before making actual determinations (*cf.* 205, 272).

B. *Properties Associated with Impulse Propagation*

1. *The local excitatory state.* The nature of the process by which electrical or other stimulation gives rise to a propagated disturbance has been the subject of

many investigations (19, 20, 24, 27, 55, 75, 109, 120, 129, 131, 133, 142, 151, 152, 164, 170, 184, 190, 202, 205, 211, 218, 223a, 225, 232, 235, 238, 251, 260, 280, 283, 289).

Following Hill (133), most modern theories are based upon the relationship between a hypothetical local excitatory state (l. e. s.) and an equally abstract threshold state. A stimulus, no matter how brief or small, sets up l. e. s. which thereafter subsides exponentially at a rate dependent upon the electrical characteristics of the fiber. The rate of rise of l. e. s. is likewise determined, and therefore, at least for short stimuli, the state is a function of the duration-voltage product of the stimulus. If the state exceeds threshold, impulse initiation occurs. The limiting stimulus strength to attain threshold with prolonged stimulation was called by Lapique (164) the "rheobase." The time required for a stimulus of twice rheobasic strength to reach the same threshold was called "chronaxie." These two types of measurement, by whatever name, set the time and current characteristics of the l. e. s. It is generally implied in these formulations that the l. e. s. is synonymous with an actual partial depolarization of the membrane, and that the process of depolarization goes to completion when a critical value is exceeded. Since the threshold is itself altered by the stimulus, but more slowly and in a direction to negate the effect of stimulation, a wide variety of phenomena of excitation can be explained by modifications of the time and intensity dimensions of the two factors (*cf.* 27, 146, 152, 225, 232, 238, 251).

It will be obvious from this brief summary that a drug which appears to modify excitability to brief stimuli might do so by altering any or all of four excitation constants. Thus an agent might raise the voltage required for a brief shock, but lower the requirement for a long pulse, if for example, the drug lowered rheobase but increased membrane resistance. Therefore it is of importance to define the stimulating conditions and preferably to use a range of duration before making conclusions concerning pharmacologically-induced "threshold" changes.

2. *Accommodation and the nerve "reaction."* The most general form of two-factor theory assumes an interplay of two processes, one favoring impulse initiation and the other opposing it. Excitation occurs when the first process outruns the second, which is made possible by the relatively greater speed of adjustment of the excitatory process. "Accommodation" is the term ordinarily used for the adjustment of the more sluggish opposing process. If the adjustment did not occur, it would be impossible to account for such phenomena as post-cathodal depression, anodal "break" excitation, etc. There are a variety of methods for the estimation of accommodation, including the use of slowly rising as against rapidly rising pulses, the voltage required for anodal "break" as against cathodal "make" stimulation, or variants of these (27, 133, 218, 232, 251).

From what has been said already, it should be clear that no single accommodation "constant" (251) can describe adequately both the time and voltage course of the process, and the derivation of any single value for drug action will be misleading. Furthermore, asymmetrical effects of anodal and cathodal pulses and anomalous changes with high voltage (184, 232) militate against any simple evaluation. To make matters worse, Lorente de Nó (184) has posed three such

processes, or nerve "reactions," corresponding to active adjustments tending to negate the polarizing effects of applied currents, and related in turn to the three basic fractions of the membrane potential. Despite this welter of complexity, it seems important to make at least qualitative pharmacological observation on these processes, since they are intimately concerned with rhythmic behavior of neurones, are labile to drug action, and probably play a vital role in central nervous function.

3. *The action potential spike and the nerve "alteration."* The culmination of all the processes already discussed, and the most obvious function of nerve, is the propagation of a disturbance, the impulse, and its electrical signal, the action potential. DuBois-Reymond (75) first detected the action potential and defined it as a negative variation of the resting potential, and Bernstein (1919) demonstrated that it travelled with a velocity corresponding to Helmholtz' (129) indirect determination of the speed of the nerve impulse. The form and speed of the action potentials of vertebrate mixed nerve were finally determined with great precision by Erlanger and Gasser (82), following the advent of cathode ray oscillography.

Although long considered to be a wave of depolarization, the peak of the action potential was found by Cole and Curtis (49–51, 63) to exceed the membrane voltage by a considerable amount in squid nerve, and all subsequent investigators using microelectrode recording in various tissues have made similar findings (143, 148, 284, 285, 293). The reported amount of overshoot ranges from a few millivolts to almost a complete reversal of sign of the membrane voltage. The external concentration of sodium ion is directly related to the amount of overshoot (143, 148). The phenomenon of overshoot has been variously viewed as the effect of an inductive membrane component (63), a sodium diffusion potential associated with breakdown of the pumping mechanism (143), or the chemical alteration of organic membrane constituents (135).

The form of the action potential in single myelinated fibers has been analyzed by several investigators (82, 184, 233, 243, 263). It is apparent from theoretical consideration (233) that a number of separable processes must be taken into account. There is an initial passive depolarization produced by spread of the electrotonic wave ahead of the advancing spike; a more rapid and relatively linear depolarization associated with the active local process; a slowing of the latter process as the recovery phase takes over even during the rising limb of the spike; a linear recovery of potential almost to the original membrane voltage; a slower recovery phase, the negative after-potential; and finally a prolonged hyperpolarization in the form of a positive after-potential. Extrapolating from data on cardiac muscle, which shows a long phase of relative stability between the depolarization and repolarization waves (285, 293), a similar but brief period of "active" stability is to be expected in other tissues on theoretical grounds, and appears with drugs which retard recovery (270). From these considerations it can be seen that pharmacological studies would profit from a careful measurement of action potential form, preferably made monophasic by crushing between the recording electrodes, since the various components are separately variable.

The height of the action potential is not constant but varies with previous activity and chemical changes in the medium. Lorente de Nó (184) uses the term "nerve alteration" to describe the variable process resulting in the spike, and relates it to active changes in the several components of membrane potential.

The form of the action potential recorded from a nerve immersed in a conducting medium is more relevant to physiology than is the form recorded in air. Lorente de Nó (184) has shown from volume conductor theory and demonstrated experimentally that a positive variation precedes the negative spike, and recordings both from the brain (62) and from peripheral artificial synapses (9, 195, 229, 250, 261) indicate that the initial effect of such an approaching wave upon another separate neurone must be inhibitory.

One of the most striking events associated with the action potential is the fall in membrane resistance, demonstrated by Cole and Curtis (50) for squid fibers, by Hodgkin (141, 142) for crustacean fibers, and by Tasaki (262) for myelinated frog fibers. The membrane resistance drops to about 2% of its resting level at the height of the spike, and recovers slowly during the refractory period. Hodgkin attributes the effect to outward leakage of potassium. Regardless of mechanism, it is obvious that there are profound changes in the physicochemical state of the membrane during activity. It is interesting to note that in heart (285) the resistance fall is associated only with the beginning of the action potential, depolarization persisting long beyond the recovery of resistance. Voltage and resistance are therefore not reciprocally related.

What are the minimum requirements for initiation of a nerve impulse? Lorente de Nó (184) has recorded action potentials after complete anoxia plus poisoning of all known metabolic pathways, simply by elevating the potential of the depolarized membrane above a critical level by means of anelectrotonus. Sodium ion in relatively low concentration (0.015 M) is important for the maintenance of excitability (184), but can be replaced in part by lithium (99, 100), certain quaternary ammonium ions of the type of tetraethylammonium (185, 186), and even transitorily by cocaine (188). Thus only a voltage source and one of several cations for transport are necessary for impulse formation—the rest of the process depends upon chemical interactions in the substance of the fiber.

It should be pointed out that several non-living inorganic systems have been shown to produce action potentials, for example, the iron-wire model of Lillie (174); some of these systems rival in complexity the neurone itself (16, 30). Eyring *et al.* (85) have attempted to set down the requirements for excitation in terms of the theory of absolute reaction rates. Unfortunately they constrict the generality of their treatment by postulating a separation of hydrogen and bicarbonate as the source of voltage.

The traditional controversy between chemical and physical explanations of the action potential continues, but ultimately there must occur some fusion of the two. On the one hand the high temperature coefficients and pharmacological vulnerability of excitation processes speak for the making and breaking of chemical bonds and for enzymatic mechanisms. On the other hand chemical reactions, by their very nature, may be made to produce electrical differences of potential,

and there is great convenience in electrical circuit theory both for mathematical handling of data and for practical experimentation. It is of course tempting to assign a vital role in excitation to specific chemical substances, such as acetylcholine (41, 122, 205–207), but the supporting evidence is slim, as will be seen later. If there are trigger substances of low molecular weight which are essential to excitation, they may very well serve as coenzymes in the sense of the formulation given by Welsh (287). In the search for trigger processes, pharmacological research can be of preponderant value. The nature of the membrane change associated with excitation is the core problem of neurophysiology today.

4. *Chemical correlates of impulse propagation.* "Resting" neurones consume oxygen and produce carbon dioxide and water as well as more complex metabolites. A number of investigators have studied changes in metabolic rate during activity and as a result of drug action (28, 32–37, 52, 73, 74, 88, 91, 105, 107, 167, 168, 249). Normally there is an increase of about 20% in oxidative metabolism during propagation of action potentials. Doty and Gerard (73–74) have recently shown that this extra respiration can be abolished by azide, yohimbine, and hydroxylamine, without detriment to conduction of impulses. Conversely, the "resting" metabolism may be separately reduced by methyl fluoroacetate without loss of function. Thus, there is no simple relation between metabolism and activity, nor is it clear why one should be expected. Oxidative metabolism is normally essential to the maintenance of membrane voltage, which is in turn a requirement for excitation. During depolarization or partial reversal the metabolic requirement would actually be lower if it were not for the continuation of processes restoring voltage. Yohimbine actually slows the recovery process, and some investigators (*cf.* 74) have even reported a decrease in metabolism during conduction.

Many investigators have noted an outward flow of potassium ion during activity (54, 89, 142, 155, 156, 246–248, 284, 298), and some have observed a reciprocal inward flow of sodium (121, 156, 284). The actual amounts exchanged are relatively small, but may be of great significance for the mechanism of development of the action potential. Hodgkin and Huxley (142) take the view that the membrane becomes highly and specifically permeable to sodium during the rise of the action potential, and that potassium is passively lost. The inward migration of sodium would be adequate to account for the membrane voltage drop, and the outward flux of potassium for the recovery, according to their view. The ability of released potassium ion to reduce membrane resistance and depolarize could be included as part of the mechanism of propagation. The hypothesis is attractive, but it would be desirable to have suitable data on vertebrate nerve, as well as a repetition of some of Lorente de Nó's extreme tests for dispensability of sodium and potassium before attributing any universality to this mechanism.

Conduction of impulses is associated with liberation of acetylcholine from cholinergic fibers and sympathin from adrenergic fibers, according to the investigations of Lissak (177). Minz (200) and Von Muralt (280) report the liberation of thiamine from sensory fibers during activity, and there has long been conjecture concerning histamine release from unmyelinated dorsal root fibers. To

attribute to any of these a vital role in the conduction process in the fibers in which they happen to appear, in the absence of any direct evidence of action of comparable amounts applied externally or injected into fibers, would be to stretch considerably the meaning of chemical mediation. It is of course possible, as has been argued for acetylcholine (205–207), that the important processes occur internally, but more direct proof would be in order. Meanwhile it might be important to search for other organic constituents of nerve which are capable of exerting consistent actions upon nerve (*cf.* 185, 186). There must be many more substances released during activity than have hitherto been accounted for, and modern chromatographic methods might be of assistance in separating them.

5. *Structural changes.* As previously mentioned, Flaig (95), Hill (134) and Tobias (253, 265, 266) have noted visible changes in axones during activity. Hill attributes the decrease in opacity of crustacean nerve fibers following stimulation to an associated swelling, since the two effects have the same course, and can be reproduced in osmotic experiments. There appears to be an initial small and rapid shrinkage and increase in opacity prior to the major change in the opposite direction.

6. *Conduction velocity and failure of conduction.* Once a nerve impulse has been initiated, there arises the problem of its propagation along the axone. This is essentially an explosive process, in the sense that more energy must be liberated by the development of an impulse than is needed for the activation of the next region of nerve. When this condition is fulfilled, conduction occurs without decrement. It is "all-or-none" in the sense that it is independent of the original stimulus, and dependent only on the explosive conditions which prevail locally. The ratio of voltage output to voltage input is called the "factor of safety" for conduction (120), and is in part related to the ratio of total height of the action potential spike to the local critical depolarization required to produce it.

Originally measured for frog myelinated fibers by Bernstein (19) directly and by Helmholtz (129) indirectly, conduction velocity was investigated intensively for vertebrate nerve by Erlanger and Gasser (48, 82, 102) who related fiber size and myelination to function and in turn to speed of propagation. The simplest division of vertebrate nerve groups on this basis is: A fibers: large myelinated somatic motor axones and sensory fibers for touch, pressure and proprioception, of the order of 10 micra in diameter, and conducting with speeds of or up to 100 meters per second in mammals; B fibers: small myelinated preganglionic autonomic fibers predominantly with intermediate conduction velocities; C fibers: unmyelinated post-ganglionic sympathetic and unmyelinated sensory fibers including those for pain, of the order of one micron in diameter and one meter per second velocity of conduction. There is considerable subdivision and overlap within these groups, but the main divisions are of pharmacological importance, particularly with regard to the selective blocking of the smallest fibers by local anesthetics. There are a number of useful reviews which deal with conduction in vertebrate and invertebrate nerve (82, 102, 120, 152, 190, 223, 223a, 238, 288), including theoretical and methodological considerations.

One controversy which has plagued the literature in recent years concerns

saltatory conduction in axones with nodes—that is, in vertebrate peripheral myelinated fibers. Bethe (20) first demonstrated that conduction could occur across an injured inexcitable gap, and a number of investigators (81, 101, 151, 260–263, 254) have subsequently presented evidence that the excitation process jumps from node to node, but others (165) find smooth and linear conduction without discontinuities unless the fibers are injured. The question of whether drugs must act on the fiber as a whole or only on the relatively unmyelinated internodes is of course important to pharmacology. Saltation can hardly be a general phenomenon, since central vertebrate myelinated fibers, as well as all unmyelinated and invertebrate fibers in general, are lacking in nodes. But it is important to note that Bethe's original phenomenon, conduction across a non-responding gap, is of greatest theoretical significance; many studies on transmission across blocks, or between apposed cells, show beyond doubt that the action potential is adequate to excite without the intervention of a mediator (9, 81, 101, 118, 120, 147, 150, 151, 197, 215, 229, 250, 254, 261).

Factors determining conduction velocity have been worked out by a number of investigators (1, 85, 131, 144, 152, 153, 184, 209, 243, 267, 270, 272, 289). Other things being equal, larger fibers conduct faster than smaller, myelinated faster than unmyelinated. The reason for this is implicit in the electrical characteristics of core conductors. Lorente de Nó (184) and Hodgkin and Rushton (144) have proved from cable theory and demonstrated in nerve that the equivalent conduction velocity for a decrementing electrotonic wave is $2\lambda/\tau$, or twice the characteristic length per characteristic time. Actual conduction velocities tend to be of the same order of magnitude. From a glance at our previous discussion on passive properties, a simple relation between characteristic velocity and the membrane capacity and resistance and internal and external resistances can be derived: $V^2 = 4/c^2_m r_m (r_e + r_i)$. Thus a larger fiber will conduct more rapidly both because of smaller physical internal resistance and the physiological drop in membrane resistance; a more myelinated fiber will conduct more rapidly because of the preponderating drop in capacity. A non-electrolyte medium of high resistance should and does slow conduction (153, 184). Agents which increase or decrease membrane permeability should have corresponding effect on velocity, but these are still to be reported.

Since the above factors concern only the passive aspects of conduction velocity we must deal also with the local explosion, the ratio between input required and output obtained. Hermann (131) predicted that there should be an inverse relationship between threshold and conduction velocity, and Werigo (289) demonstrated it, although the function is not a simple one (267, 270, 272). Eyring *et al.* (85) have dealt with the underlying chemical dynamics of conduction velocity. The size of the action potential spike must also be considered, since an increase in spike amplitude will lead to increased velocity, other things being equal (1, 209, 243). This may be important in relation to sodium lack (148, 184). It is interesting to note that Lorente de Nó (182, 185) obtained extremely low conduction velocities with some quaternary ammonium salts in place of sodium.

From the foregoing, it can be seen that much information relevant to drug

mechanisms might be obtained by combining measurements of conduction velocity with other key determinations.

So far we have dealt only with factors altering velocity. What are the limiting circumstances that can result in conduction failure? It is inadequate to describe a drug as blocking conduction without further examination of mechanism. In general it can be said that conduction will fail if the threshold rises high enough or the spike amplitude falls low enough to reduce the factor of safety below 1.0, or if the membrane potential falls below a critical value. Thus, as we shall see later, many local anesthetics, anticholinesterases, centrally acting drugs and other agents may block by threshold increase, after an initial slowing of conduction, while agents affecting the sodium transport system might act by reducing the spike amplitude. Depolarizing agents, such as KCl, various metabolic poisons, and anesthetic gases, can block without producing an initial threshold rise. A depolarizing block might be identified without membrane potential measurement by the unblocking action of anelectrotonus (10, 184, 294–296).

Hyperpolarizing blocking agents are also known, including calcium (184) and very high concentrations of CO_2 (270). Although electrical anodal block is well-known (184, 218, 232, 294–296), hyperpolarizing agents have not been often described. In part the mechanism is probably increase in threshold by increase in the L fraction of the membrane potential (184), more than sufficient to offset the corresponding increase in action potential amplitude. Catelectrotonic relief of block would assist in identifying a hyperpolarization, as would also the relief of block by rapid repetitive stimulation (184).

Other types of blocking mechanisms which are indirectly related to threshold or polarization alterations include prolonged delay in recovery time and failure at high frequency stimulation, as with yohimbine (74), and possibly hyperaccommodation, as with prolonged phosphate treatment (270).

For differentiating the mechanisms of various unknown conduction-blocking agents, it can be seen that several measurements at the time of onset of block are important. With conventional stimulating and recording equipment they include measurement of threshold with long and short pulses, estimation of conduction velocity or at least of stimulus-response latency, tests with slow and fast stimulus frequency, measurement of spike height of individual fibers as well as of total action potential in a mixed nerve, and observations on the relief or exacerbation of block by electrotonus at anode or cathode. In addition, it is sometimes valuable in a blocked nerve to record at the stimulating cathode for evidence of abortive decrementing impulses (152), which are frequently found with threshold-raising agents.

7. *After-potentials and recovery of excitability.* Several investigators (7, 104) had previously noted the existence of prolonged electrical changes after impulse conduction, but Erlanger and Gasser (82) were the first to establish the relationship between these after-potentials and the recovery of excitability. Lehmann (171, 172) and Graham (115, 116) are among those who have studied the effects of pH alterations and inorganic ions on these related functions in vertebrate nerve, while Shanes (246, 247) has made pharmacological observations in in-

vertebrates. Lorente de Nó (183, 184) has reinvestigated the relationship with the aid of drugs in frog nerve.

The classical excitability cycle (152, 190, 238) was considered to consist of an absolutely refractory period concomitant with the depolarization process, and a subsequent relatively refractory period during which threshold recovered toward normal. But the latter period may be very short (one or two msec. in frog sciatic) and continuous with a rapidly developing period of supernormal excitability which reaches a peak at 5–10 msec. in frog sciatic A fibers in air, and more slowly subsides. Certain drugs, particularly veratrine, markedly increase and prolong it. Following this there may be a much longer period of subnormal excitability, more prominent in smaller and unmyelinated fibers, or after repetitive stimulation, and persisting even for minutes under the action of some drugs such as yohimbine. Associated with the supernormal phase is the negative after-potential, and with the subnormal phase, the positive after-potential (82).

Lorente de Nó (184) has reinterpreted these changes in the light of his investigations on polarizability of nerve. The three fractions of the membrane potential, which collapse to varying extents during the action potential spike (nerve alteration), are actively restored in the same general manner as if a cathodal pulse alone had been applied (polarization) and actively opposed (nerve reaction). The time lag of the reaction processes and the tendency to overshoot, multiplied by the interrelations between the three polarizable layers, gives rise to a variety of oscillatory changes in membrane potential. However, it is notable that in general the threshold and the membrane potential behave in roughly parallel fashion in Lorente de Nó's observations as in those of others; and also these changes show great liability to drug action, which increases their interest for pharmacology. Although post-impulse changes may not be of great significance for function in peripheral nerve, they must become of great importance in spontaneously active nerve-nets, particularly in the cerebral cortex.

One interesting aspect of the recovery process is the tendency of spike amplitude to increase during repetitive stimulation, a result of the cumulative loss of membrane potential fractions (184). This effect appears to be of importance for facilitating effects of repetitive sensory root stimulation in the spinal cord (178). It can be imitated in peripheral nerve by strychnine (173, 281) and may account in part for the central excitatory effects of that drug.

8. *Rhythmicity and spontaneous firing.* Rhythmic activity, spontaneous or evoked, is more the rule than the exception in neurones which are left intact within the body, whether they be peripheral or central. Sensory and motor information are communicated usually in trains or bursts of action potential spikes. Most investigators find some spontaneous activity in motor or sensory nerve fibers, and isolated nerve under supposedly optimal conditions usually shows a few discharging fibers. It is probably fair to say that activity, rather than quiescence, is the mode of existence of living tissues, and it is notable that both neurones and effectors, deprived of afferent connections, tend to approach a level of resting spontaneous activity. Therefore a few words might be well spent on such phenomena.

Rhythmicity and the factors concerned in adaptation have been studied in single fibers from sensory receptors (2, 154, and many others). Among the highest frequencies of discharge known are those recorded in pyramidal fibers during convulsive seizures, produced electrically or by convulsant drugs (3). Spontaneous rhythms are characteristic of some invertebrate ganglion cells (221–223) and rhythmicity would appear to be more common in general in invertebrate than in vertebrate neurones in response to stimulation (223, 223a, 238, 288). Rhythmic activity following the use of various "decalcifying" and other agents have been widely studied (8, 34, 37, 42, 114, 171, 184, 189, 193, 203). Among the agents which have been used to produce repetitive firing, Gordon (114) lists: fluoride, carbonate, phosphate, oxalate, citrate, tartrate, malate, acetate, lactate, pyruvate, formate, nitrite, sulfite, thiocyanate, thiosulphate, tetraethylammonium, DDT, naphthalene, hexachloreethane, veratrine, quinoline. To these should be added increase in pH and decrease in calcium ion.

There has been a strong tendency to equate all of these excitatory drug actions with reduction in or competition with calcium (34, 35, 114), but Lorente de Nó (184) attacks this thesis in characteristic fashion, and attributes the actions in most cases to more specific effects on metabolic processes producing the various fractions of membrane voltage.

The occurrence of rhythmic local potentials as a precursor to firing of repetitive spikes has been noted by many (8, 34, 35, 171, 184). Brink *et al.* (34) have correlated these rhythms with the basic electrical characteristics of nerve.

In general, two types of mechanism would seem important for the development of rhythmicity. One is a fall in threshold, which permits small oscillations in membrane potential to become adequate for excitation. The second is a fall in membrane resistance, which by loss of damping would tend to oscillatory phenomena in the equivalent circuit of nerve. The former effect is frequently but not always found. The latter has not been sufficiently studied. Neither explains why local oscillations occur in the first place, although many types of nonliving models may be constructed which oscillate when a continuous source of energy is provided.

One point of pharmacological interest in drug-induced neural rhythms is the great sensitivity of such preparations to various depressant drugs (159, 267–271), in comparison to normal "resting" nerve. Taking advantage of this sensitivity, one can often demonstrate consistent actions at dose levels which approach those for central nervous effects.

9. *States of excitation.* In concluding this general discussion on properties of nerve and effects of drugs thereupon, something should be said about the variations in status of nerve which may cause wide variance in pharmacological studies. Lorente de Nó (184) has taken this variance into account and described several alternative states of excitability, based on the appearance of their recovery curves and after-potentials, and related to differences in the fractions of the membrane potential. These include, for frog nerve, a "resting" state, corresponding to that *in vivo* when 5% CO_2 is supplied or the membrane potential raised by anelectrotonus; a "pseudo-resting" state after a period of time in air

without CO_2; an "exalted" state immediately after dissection; a spontaneous "rhythmic" state seen particularly in mammalian nerve in oxygen without CO_2; and a "depressed" state resulting from fatigue or anoxia. These studies emphasize the important role of CO_2 in neuronal function and the desirability of imitating *in-vivo* conditions for a proper evaluation of drug actions.

III. ACTIONS OF DRUG UPON NERVE

A. *Local Anesthetics*

1. *General considerations.* Cocaine, which had been isolated from coca leaves in 1860 by Niemann, became the first widely used local anesthetic shortly after its introduction in 1884 by Carl Koller. Two decades later the synthesis and clinical trial of "novocaine" (procaine; diethylaminoethyl p-aminobenzoate) by Einhorn and his collaborators in 1905 opened a new era in the search for better local anesthetic agents. Of the hundreds of active compounds which have been tried, procaine still remains the most widely used. Although far from the most potent, it has a high margin of safety between local anesthetic and irritant concentrations.

The desired property of a local anesthetic is its ability to reduce pain by its local effect in blocking impulse conduction preferentially in the pain fibers along a nerve trunk or at their terminations, without the production of irritation, nerve or tissue damage, and without systemic effects of absorption from the local site of administration. By an extension of local anesthetic action, regional nerve block, diagnostic ganglionic block, and caudal or spinal anesthesia are also made possible. In recent years the local anesthetics, particularly procaine, have been found to give analgesia upon intravenous administration, but it is still not clear to what extent this represents an ability to block conduction in small fibers.

One of the most complete studies of chemical and physical properties of the local anesthetics is that of Löfgren (181). Taking xylocaine (α-diethylamino-2,6-dimethylacetanilid) and a number of related substances, Löfgren has examined their physicochemical characteristics as well as various features of their clinical and experimental actions. By determining molar refraction, ultraviolet absorption spectrum and other properties, he demonstrates the effect of alterations in the aromatic ring upon such features as resonance through the ring-attached nitrogen, and of this factor in turn upon thermodynamic ionization constants. He presents data showing the importance of this latter factor on local anesthetic activity, demonstrating that it is the free base which determines the activity. From measurement of the oleyl alcohol/water distribution coefficients he demonstrates that local anesthetic activity does not follow the Meyer-Overton rule. He also derives relationships between chemical structure and rate of diffusion into a nerve trunk, a factor of clinical importance in establishing nerve block. He concludes from these studies that while indifferent narcotics, which adhere to the Meyer-Overton rule, probably act through van der Waal forces rather than chemical combination, the amino groups of the true local anesthetics interact with polar groups in the lipo-protein-metal film of the nerve membrane.

The mechanism of local anesthetic action seems to be essentially an elevation

of threshold, the smaller fibers being the most vulnerable. Gasser and Erlanger (102) demonstrated that conduction block produced by cocaine appeared first in the small unmyelinated fibers of a nerve trunk, including presumably those chiefly concerned with the modality of pain, and last in the large afferent and efferent myelinated fibers of fast conduction, the motor fibers being the most resistant of all. This was not the same as the order of block produced by mechanical pressure. Evidence from the laboratories of Bishop and others (17, 25, 184, 272) indicates that nerve block is produced by concentrations below those which cause depolarization of the nerve membrane, and that threshold elevation is the principal factor in blocking conduction. Shanes (246, 247) has presented evidence that the local anesthetics stabilize the nerve membrane against potassium loss. Löfgren (181) has proposed that local anesthetics, in contrast to many depressants which in large concentrations have local anesthetic effects, act by polar association between the amino group of the local anesthetic and suitable polar group in the lipo-protein film of the nerve membrane.

Whether local anesthesia involves only a stabilization of the nerve membrane, without modification of the mechanism by which electrical energy is stored and released by the membrane, is a problem still to be decided. There is evidence that local anesthetics alter nerve metabolism. For example, Sherif (249) reported that procaine and cocaine inhibited respiration of the sciatic nerve of the rabbit. On the other hand, the studies of Larrabee *et al.* (167, 168) seem to indicate that conduction may be blocked by cocaine in concentrations which do not alter oxygen uptake.

For details concerning structure-activity relations and action mechanisms of various local anesthetics, several works should be consulted (80, 112, 169, 181).

2. *Procaine.* Although procaine is capable of reducing membrane potential in high concentrations, it seems clear that the major mechanism of block is an increase in threshold, without a notable change in membrane potential (17, 267). Block occurs in both A and C fibers when the threshold is approximately doubled and the conduction velocity halved. Some investigators still imply that hyperpolarization is a factor, on the basis of antagonistic effects of supposed depolarizing agents (14, 96, 97, 212). Shanes (246) has found that in squid nerve procaine suppresses oscillatory behavior and the negative after-potential before block occurs. In crab nerve (247) he noted that concentrations up to 0.02% (0.8 mM) stimulated oxygen uptake, while higher concentrations inhibited Q_{O_2} up to 50% at blocking levels (about 10 mM). In frog sciatic nerve (270) as little as 0.05 mM can be shown to abolish phosphate-induced hyperexcitability and rhythmicity, as compared with the usual full blocking concentration of 10 mM for the same fibers. For comparison, the customary 1% solution of procaine is about 40 mM, and the dilution used for differential spinal block in man (237) is 4 mM. For intravenous analgesia, plasma levels are of the order of 0.001 mM (35a).

Concerning the differential effects of procaine, although a preferential attack on small fibers is generally assumed (237), the rule is not universal. In rabbit vagus nerve it can be demonstrated regularly that a 1.0 mM solution depresses

A fibers first and C fibers last (84). Qualitatively, procaine is a depressant for all peripheral excitable tissues (112), and may be even more effective on skeletal muscle conduction than on nerve (252). It is therefore not surprising that death from accidental overdosage usually is the result of cardiac arrest (112). The ability of procaine to produce central convulsions is paradoxical, since central neuronal thresholds are found to be increased by procaine, while radiation of evoked discharge is also increased by unknown mechanisms (270). It has been shown that the products of procaine hydrolysis, para-aminobenzoic acid and diethylamino ethanol, oppose convulsant actions but not central depressant or peripheral local anesthetic actions of procaine (226, 227).

3. *Cocaine.* Cocaine differs from procaine in possessing more excitatory components, particularly on adrenergic effector systems (112), but differs very little in its action on peripheral nerve. Gasser and Erlanger (102) showed that it first affected small fibers in peripheral nerve trunks, in contrast to pressure and asphyxia. All investigators seem to be in agreement that depolarization is not a factor in blocking with concentrations at least up to 20 mM, but that threshold increase is the primary mechanism (17, 25, 184, 238). There is probably no direct effect of cocaine on oxygen consumption at local anesthetic doses (249), but both anoxic depolarization and subsequent recovery are delayed (184, 247). In squid nerve cocaine suppresses oscillatory phenomena and the negative after-potential (246). In frog nerve it has little effect on electrotonic potentials or other phenomena which can be electrically measured (184), except as noted above. An interesting and paradoxical action of cocaine is its ability to restore conduction transiently in frog nerve in a sodium-free medium; however, it ordinarily summates with partial sodium lack to abolish conduction (188). Lorente de Nó considers the possibility that cocaine may compete in the chemical processes by which sodium ion maintains excitability, while Shanes (246, 247) postulates a stabilizing mechanism opposing potassium loss.

4. *Xylocaine.* Because of the many pertinent observations on physical properties and dynamics of local anesthetic block which have been made with xylocaine and its congeners, the literature is of importance for pharmacological methodology (80, 111, 181). However, there is little in the published reports to indicate any unusual deviation from the mechanism of action reported for other agents of the same root-structure.

B. *Centrally-active Agents*

For the most part those drugs which are used in therapy for their effects upon the central nervous system are not known to have important effects on peripheral nerve at the doses customarily employed *in vivo*. The study of their *in-vitro* actions on nerve in higher concentrations is of pharmacological significance insofar as such measurements may provide clues to central mechanisms, since basic neuronal properties are more difficult to isolate within the brain and cord. Therefore some passing attention will be given to centrally active agents, with a word in advance that there are few examples as yet of meaningful observations on peripheral actions which have helped to explain the differential effects observed

centrally. It is of great theoretical interest that some drugs may alter synaptic transmission by a direct action upon axones rather than upon synaptic endings (35, 231).

Except for the central excitants (Section 9, below), all of the agents described in the ensuing discussion can be characterized as central depressants, in the sense that they diminish some aspect of central nervous function, normal or abnormal. Of these, the most heterogeneous group includes the general anesthetics.

1. *General anesthetics.* So diverse in chemical structure are the many substances which produce central depression or complete anesthesia that it is hard to imagine any single mechanism of action common to all. Indeed, there is no reason to believe that a common action is essential, since there must be as many different ways of reducing or blocking central nervous activity as there are independent physiological parameters necessary for the normal maintenance of function in the central nervous system. However, there has been no dearth of attempts to find a common denominator, and certain physical and chemical features appear with sufficient frequency in anesthetics to merit some discussion. The reader is referred to recent reviews by Butler (43) and Gerard (107).

The Meyer-Overton theory (199) depends upon the rule, particularly applicable to alcohols, that the anesthetic potency is correlated with the oil: water partition coefficient. The theory assumes an ability of lipid-soluble substances to produce changes in permeability and electrical properties within the lipid phase of nerve cells. However, since the critical lipid phase of neurones is still relatively unknown insofar as either electrical properties or affinity for narcotics is concerned, and since many single anesthetics and groups of agents fall out of line, the theory does not have great generality.

Other common physical properties have also been found for certain of the central depressant drugs. Thus Traube (*cf.* 137) advanced the theory that anesthetics alter cell surface properties by their absorption thereupon, as suggested by the correlation between anesthetic potency and ability of many drugs to lower surface tension. This concept has become deeply imbedded in pharmacological literature and is often assumed as a mechanism of action, although it has not been subjected to a rigorous experimental test.

A more recent attack upon the problem of the common narcotic action of widely different molecular species has been made by Brink and Posternak (35). They enlarged upon the investigations of Ferguson (92) who had demonstrated that for equally effective doses of many substances the thermodynamic activities lay within a narrow range of values in contrast to a more than thousand-fold range in aqueous concentration. (Thermodynamic activity is an estimate of the work per molecule required to transfer the narcotic from the pure liquid phase to the unknown phase of locus of action in the narcotized cell, and is derived from vapor pressure determinations.) The Brink-Posternak treatment is more general than the Meyer-Overton theory, in that no assumptions concerning the lipid constitution at the site of narcotic action are required. These authors speculate that equal degrees of narcosis are caused by equal numbers of narcotic molecules in those portions of cells in which narcosis occurs. Unfortunately their

rule does not cover all of the experimental situations studied or reviewed; for example, the thermodynamic activities of substances producing nerve conduction block in non-synapsing preganglionic fibers of the perfused stellate ganglion in the cat are not constant, but increase with the activity coefficients. Their work has recently been subjected to critical review by Butler (43). The significance of their work, as of Ferguson's, is that it makes possible a comparison of narcotic activities based on a simply measured property of molecules, and suggests a common but non-specific action of a large number of dissimilar compounds. It also stresses the need for a search for more specific enzymatic or other actions of substances which do not fall within the general group on the basis of thermodynamic activity.

If no common physical feature can be held to account for all anesthetic actions of diverse drugs, the same may be said for chemical aspects, in the sense of actions upon enzyme systems or upon any process of bonding of the drug with any constituent of the cell. In recent years particular attention has been given to the action of depressants upon cellular respiratory enzyme systems of the brain. For a general discussion on the present status of the field of general anesthetic effects upon cellular metabolism, the reader is referred to the review of McElroy (198) who defends the thesis that a relatively non-specific mechanism of reversible denaturation of protein may have relatively critical effects upon certain metabolic processes *in vivo*.

Gerard (107) has reviewed the several theories of anesthetic action, and examined critically the evidence for a blocking action of narcotics on specific respiratory enzyme systems. He marshals the evidence which points to an action of several narcotics on cytochrome b or on an intervening flavoprotein, but warns that this site has been identified solely by exclusion of other portions of this sequence, not by positive evidence at the suspected links.

Even if the metabolic steps affected by general anesthetic agents could be well delineated, it is important to know the consequences of this metabolic interference upon particular functions of neurones in order to know the mechanism of action by which anesthesia is produced. At this level of investigation, the drugs which have been most extensively studied are ethyl ether, ethyl alcohol, the carbamates and the barbiturates, particularly phenobarbital.

Ethyl ether was first studied extensively on nerve by Biedermann (24) who found that it abolished slow electrotonus and the polarizability of the axoplasmic core. Bethe (20) made the interesting observation that certain reactions between dyestuffs and axoplasm which were normally altered by current flow were no longer sensitive to electrotonus after ether had been applied to the nerve. Alcock (5), who made the first modern quantitative determinations on membrane potential, confirmed the depolarizing action of ether. Heinbecker and Bartley (128) found evidence for an increase in accommodation in frog sciatic nerve, which should be properly correlated with a transient initial increase in slow electrotonus, especially at the anode, as described later by Lorente de Nó (184). The latter investigator found that eventually the slow electrotonus decreased, concomitant with a fall in membrane potential. When potential fell below about

15% of resting value, conduction failed. It could be restored by anodal electrotonus. Lorente de Nó found a small increase in excitability prior to block, but Wright (297), working with mammalian nerve, found only that excitability decreased as depolarization progressed. Naess (208) has noted a small stimulating effect of anesthetic concentrations of ether *in vivo* in rabbit motor nerve. Whatever the preliminaries, it is clear that ether must be classified as a depolarizing agent.

Chloroform has been less studied in nerve than ethyl ether, but probably works by the same mechanism. It is said (238) to raise rheobasic threshold with no initial effect on chronaxie, and to slow conduction velocity in addition to its depolarizing action.

2. *Ethyl alcohol.* Schaeffer (238) describes ethyl alcohol as increasing membrane potential while increasing rheobase and chronaxie and decreasing conduction velocity. However, most authors have found alcohol to be a depolarizing agent. Feng (90) showed that the course of depolarization was different from that of potassium, involving a sharp fall at a critical concentration. Wright (297) found that the threshold was progressively increased during the course of depolarization but Gallego (98) has observed an increase in excitability during the early course of depolarization. He finds that block occurs at alcohol concentrations of 1.0 to 2.0 M, when membrane potential has dropped by 12.5 mV. At this point conduction can be restored by anodal polarization, but if the membrane potential loss is permitted to go beyond 20 mV, there is an irreversible loss of conduction and polarization.

3. *Carbamates.* Lorente de Nó (184) found that ethyl urethane (40 mM) had little effect on membrane potential and somewhat less effect than cocaine on slowing of anoxic depolarization and recovery. Crescitelli (56, 58), however, noted that a small but significant initial hyperpolarization (actually a millivolt or less) arose with low doses of ethyl, propyl, butyl and amyl carbamates, and that higher concentrations eventually depolarized. Since the hyperpolarization effect did not change with temperature, he attributes it to a physical surface-action effect. Depolarization rate increased with temperature, in a manner to suggest a metabolic action. It appears from his data that block occurs during the hyperpolarization phase (but not that there is a causal relation). Crescitelli favors a decrease in permeability as the mechanism of block. His records are compatible with a threshold increasing mechanism. The order of conduction block is similar to that of anoxia, with B fibers most sensitive, A intermediate, and C least. Equivalent blocking concentrations (ethyl 210 mM, propyl 70 mM, butyl 18 mM, amyl 6.5 mM) give approximately equivalent reductions of air-water surface tension.

4. *Barbiturates.* The effects of pentobarbital (Nembutal) on peripheral nerve fibers have been studied by Heinbecker and Bartley (128). In frog sciatic nerve the changes included increase in threshold, reduction in amplitude of action potential spike and negative after-potential, prolongation of absolute and relative refractory period, slowing of conduction velocity, and a slight decrease in accommodation. In turtle vagus, the small unmyelinated fibers were most easily

blocked while the large myelinated fibers of fast conduction were most resistant. The concentrations required for these actions on nerve were apparently high in comparison with those needed for central nervous depression, but the findings are compatible with central effects of lower dosages. With smaller doses temporal summation and facilitation were reduced in the excised superior cervical ganglion of the turtle. In this connection Larrabee *et al.* (167) have more recently observed that synaptic transmission is more easily suppressed than fiber conduction in the branches of the stellate ganglion of the cat, and that oxygen consumption is decreased even by non-blocking doses.

Eccles (78) has made precise studies on changes induced by pentobarbital in spinal motoneurones. The threshold is increased in the sense that greater depolarization is required in motoneurone soma for the evocation of a propagated response. This action seems to be associated with an increase in membrane resistance. Similar effects are apparent in Curtis' (62) studies on callosal projections. The principle actions of barbiturates on higher centers seem to be a mixture of increase in threshold and prolongation of recovery time (269).

In our own experience (268, 270) barbiturates including phenobarbital, barbital, pentobarbital and Mebaral produce conduction block in frog sciatic nerve in concentrations of 15–30 mM. However, phenobarbital, in contrast to others, has a distinct protective action against electrically evoked hyperexcitability at concentrations of 1.0 mM, a matter of interest in connection with its anticonvulsant action (see below). Pentothal i. v. may increase the direct electrical excitability of cortical neurones in the rabbit at a time when synaptic transmission is depressed (270).

(For consideration of the possible enzymatic actions of barbiturates on neurones, *cf.* 107, 119, 269.)

5. *Relaxants.* Drugs which produce skeletal muscular relaxation through their action upon the central nervous system have recently received considerable clinical attention. Myanesin (mephenesin) is perhaps the best known of these agents; its central action has been related to a preferential block of small internuncials (*cf.* 269 for literature and discussion). Finkelman and Arieff (94) found no direct action of systemically administered relaxant doses of Myanesin on peripheral nerve or muscle in animals.

The author and his colleagues (270, 271) have looked for common effects of relaxants on properties of peripheral nerve and attempted to relate these quantitatively to clinical and experimental observations. The studies were restricted to three representative "antispastics", Parpanit, Myanesin and benzimidazole. These drugs were found to block conduction in frog sciatic nerve by raising threshold rather than by depolarizing the nerve membrane. Increase of threshold, decrease of conduction velocity and spike amplitude, and prolongation of recovery were found within the concentration range for lethal effects in experimental animals *in vivo*. In lower concentrations all three drugs gave protection against hyperexcitability induced by excessive stimulation or by phosphate treatment. These effects were produced at concentrations equivalent to the relaxant dosages for experimental animals. The absolute potency for any of these

effects was greatest for Parpanit and least for benzimidazole. The blocking doses and minimally detectable doses, respectively, for these three agents were: Parpanit 0.10 and 0.007 mM; Myanesin, 5.0 and 0.10 mM; and benzimidazole, 6.5 and 0.30 mM. Although these observations reveal the expected order of potency, they are of little help in interpreting the differential manifestations of action seen clinically and in experimental animals.

6. *Anticonvulsants.* Some interesting actions of anticonvulsants upon peripheral nerve have been described by the author and his colleagues (93, 113, 258, 268–270, 292).When untreated frog sciatic nerve is stimulated excessively and repetitively, the threshold falls to about half and recovers over a period of a minute. Concomitantly, repetitive discharges of A fibers are produced by single shocks. The phenomenon is of interest because of its resemblance to similar effects described by Adrian and Morruzzi (2) in pyramidal cells of the cat during seizures. Pretreatment with a number of anticonvulsant drugs can abolish these effects in relatively low doses. In the cases of diphenylhydantoin the action on nerve is apparent at concentrations of 0.04 mM, and can be demonstrated in limb nerves dissected from mammals which have been pretreated i. p. with an effective dose of an anticonvulsant. In addition to these actions, the excitatory effects of phosphate, citrate and oxalate on frog sciatic are opposed by many of the anticonvulsants in low dosage. Korey (159) has observed this effect of diphenylhydantoin and Mesantoin in squid nerve. It has been shown (93, 292) that in the case of diphenylhydantoin these effects are not directly attributable to permeability changes, since there is no marked effect of the drug upon the exchange rate of radioactive phosphate, sodium or potassium. The protective actions of the clinically effective anticonvulsants against excitatory phenomena in nerve occur at doses which have only trivial actions upon normal properties of nerve, except perhaps for a reduction in polarizability and extent of accommodation (270). Effective doses of various barbiturates, hydantoins, oxazolidine-2,4-diones and acetylureas lie usually in the range of 0.1 to 1.0 mM for those compounds containing a phenyl side-group and unsubstituted ring nitrogens; there is also a rough correlation between these structural features and the clinical effectiveness of the same drugs against grand mal epilepsy. Trimethadione, for example, is ineffective by various tests on nerve, while diphenylhydantoin, phenobarbital, Mesantoin, phenacetylurea (Phenurone) and others are active at 1.0 mM or less.

Conduction block does not occur with the anticonvulsants or their homologs until concentrations of approximately 20 mM are achieved, and many anticonvulsants are not soluble at this level. Saturated solutions of diphenylhydantoin have little consistent effect upon threshold or conduction (0.5 mM), but saturated Phenurone solutions (3.0 mM) raise threshold somewhat. Those agents which block conduction at higher concentrations probably do so by threshold increase.

It should be pointed out that many other drugs which are not clinically useful anticonvulsants also have protective actions against chemically or electrically induced hyperexcitability and hyper-responsiveness, but the margin of safety be-

tween these effects and actions upon normal parameters of nerve appears particularly high among the anticonvulsants which are clinically useful against grand mal.

7. *Analgesics.* The peripheral nerve actions of analgesics have not been adequately examined by modern techniques. Schaeffer (238) reports that morphine reduces both chronaxie and rheobase in low dosage, and raises rheobase at higher levels, which would be compatible with the mixture of excitatory and depressant effects manifested by this drug on brain (112). It would be of interest to determine the relative sensitivity of various fiber groups to analgesics.

8. *Cannabinols.* Natural marihuana derivatives and their synthetic congeners are of interest because of their mixture of excitant and depressant properties (112), and the very low effective dosage of some of the synthetic compounds (down to 10–30 micrograms/kg), which is the more surprising in that they are non-nitrogenous substances (see 269). Data on peripheral nerve are lacking except for two highly potent synthetic preparations, which were found to be quite without effect on frog sciatic nerve even in saturated solution (270).

9. *Central excitants.* Of those drugs which produce signs of excitement or frank convulsions by central nervous action, Adrian and Morruzzi (3) found that strychnine, picrotoxin, nikethamide, absinthe, and Metrazol all produced high frequency discharges in pyramidal cortical cells of the cat. However, the response seems to be characteristic of seizure discharges however produced, and when we turn to peripheral nerve we find little evidence for anything that can be construed as an excitatory action, except perhaps in the case of strychnine.

Strychnine. Heinbecker and Bartley (127) found decreased threshold and a reduction in degree of accommodation in strychninized frog sciatic nerve. According to Schaeffer (238), chronaxie and rheobase are reduced (but the latter is increased in higher dosage), conduction velocity is reduced and membrane potential is unchanged. In our own experience (270), strychnine produces a moderate increase of threshold and slowing of conduction velocity at a concentration of 30 mM, and no consistent action at 6 mM or below. Wall (281) and Lettvin (173) emphasize the ability of strychnine to increase the amplitude of the action potential spike. This is an interesting example of the possible role of responsiveness rather than of threshold, and the occurrence of such an effect at neuronal terminations on nerve cell bodies would help to explain the known selective excitatory central actions of strychnine (76, 77) in the absence of any apparent direct effect of strychnine on central neuronal thresholds (270).

Metrazol. Eyzaguirre and Lilienthal (86) have seen what appear to be excitatory effects of Metrazol on mammalian nerve and muscle. However, in frog sciatic nerve we have seen no excitatory actions up to 20 mM concentration, at which point depolarizing block begins to appear without lowering of threshold (270).

Caffeine. According to Schaeffer (238), caffeine initially decreases chronaxie and increases conduction velocity, but detailed studies are needed.

Amphetamine. Despite its central excitatory effects, which are usually not attended by a reduction in neuronal thresholds, amphetamine appears to be

primarily depressant in frog sciatic nerve (270). In 2 mM concentration it stops multiple firing evoked by phosphate, and at 10 mM it begins to elevate normal threshold.

Ephedrine. Chronaxie and rheobase are both increased by ephedrine, according to Schaeffer (238).

Lysergic acid diethylamide. This substance, related to the ergot alkaloids, is remarkable for the low dosage in which mild central excitatory effects have been reported in psychiatric patients (less than 0.1 mg. orally). However, up to 20 mM there does not appear to be significant action on frog sciatic nerve (270).

C. *Autonomic Agents*

1. *Acetylcholine and congeners.* In spite of the claims which have been made for acetylcholine as a mediator of conduction in nerve fibers (205–207), the results of acetylcholine application to peripheral nerve have been most undramatic. Lorente de Nó (182, 184) demonstrated that isotonic acetylcholine chloride could be used in place of Ringer's solution as an inert medium, that membrane potential was maintained in this solution if small amounts of anticholinesterases were added to prevent hydrolysis, and that with the addition of a small amount of sodium ion, 0.022 M, excitability was also maintained. Without esterase inhibitors, acetylcholine produced irreversible deterioration of the nerve, suggesting that acetylcholine ion could penetrate to some extent. Nachmansohn (205–207) contends that acetylcholine and other quaternary ammonium compounds do not penetrate, so that the internal role of acetylcholine in conduction can not be imitated by external application. In this respect the nerve membrane would appear to be a one-way street, because appreciable amounts of AcCh are found in the perfusate of stimulated motor nerve fibers (177). Acetylcholine is also released (by DDT) from various insect nerve cells (267), but does not appear to have a direct effect upon conduction in cockroach axones (228).

Some authors report neuronal actions of AcCh. According to Schaeffer (238), chronaxie is reduced, conduction velocity increased and membrane potential lowered by acetylcholine. Nordqvist (212) states that 0.5% AcCh can relieve a procaine block. It might be supposed that fibers rendered sensitive by exciting agents would be useful indicators of an AcCh effect; this does not appear to be the case with either citrate (34) or tetraethylammonium (270).

Choline and methacholine are also said to be without effect on nerve, and can be used in isotonic solution as Ringer's substitutes (184). Schaeffer (238) states that pilocarpine decreases chronaxie. It might be of value to study this and other muscarinic acetylcholine-like agents on peripheral nerve with modern methods, if only for the sake of completeness.

For critical discussions of the controversy concerning acetylcholine as a universal mediator, the publications of Feldberg (87), Gerard (106), Gilman (110), Lorente de Nó (182, 184), Nachmansohn (206, 207), Prosser (223, 223a) and Welsh (287, 288) are suggested.

2. *Anticholinesterases.* Much of the argument on acetylcholine mediation has centered around agents which inhibit cholinesterase, since in some cases they

have quite dramatic actions upon neural properties. The case is particularly crucial with regard to DFP (di-isopropylfluorophosphate) which is capable of destroying cholinesterase activity irreversibly. The chief disagreement on fact concerns the extent to which axones can behave normally when most or all of their cholinesterase is destroyed.

DFP. The Nachmansohn group (41, 122, 206, 207) first described conduction block in vertebrate and invertebrate fibers treated with DFP. They attributed the block to a failure of recovery mechanisms requiring the destruction of acetylcholine, and assumed a depolarizing mechanism of block. They found that conduction could be restored by washing, and have demonstrated partial recovery of cholinesterase activity under these conditions. They contend that cholinesterase activity is essential to conduction, but that the cholinesterase activity need be only a fraction of normal.

Crescitelli and Gilman (60, 110) showed that, after conduction block by DFP, propagation of impulses could be restored simply by exposing the nerve to air, presumably due to the rapid hydrolysis of the DFP itself. They indicated that under these conditions the cholinesterase activity of frog nerve was zero. Boyarsky *et al.* (33) found that conduction could continue for 6 hours in 3 mM DFP, at the end of which time cholinesterase activity was absent by the most careful tests. We found (267, 269, 272) that with concentrations of DFP high enough to block conduction promptly (25 mM), there was no fall but sometimes a slight increase in membrane potential. The mechanism of block was shown to be a doubling of threshold in both frog and earthworm fibers. (This effect is clearly seen in the prolongation of latency and action potential in the records of other investigators.) The membrane time and length constants of blocked frog sciatic nerve remain normal, indicating that there is no important change in membrane resistance (270). At a much higher concentration than required for block, 125 mM, depolarization begins (272). Although studies with arthropod nerves and synapses are sometimes difficult to interpret, it would appear from the data of Roeder (228), Schallek (239, 240) and Wiersma (290) that DFP blocks both conduction and transmission by a threshold-raising mechanism.

From these considerations one is forced to conclude that nerve conduction can occur normally in the presence of remarkably little cholinesterase, and also that DFP must have effects over and above its anticholinesterasic action. Heymans (132) has given evidence for effects on other systems, and it is of course no revelation to pharmacologists that drugs which have "specific" selective effects in their lowest effective dose range may have a variety of actions when the dose is increased.

Eserine. Bullock *et al.* (41) also found that eserine blocked conduction in parallel with its ability to depress cholinesterase. Lorente de Nó (184) noted that 0.1 mM was sufficient to prevent the slow irreversible depolarization which occurred in frog sciatic in isotonic acetylcholine chloride. Higher concentrations up to 2.0 mM had no further effect. At 10.0 mM, there was a slight hyperpolarization, followed by depolarization associated with conduction block. The block could be relieved by anodal current. In our observations (272) we found that 25 to 50 mM

blocked rapidly without depolarization, while hypertonic solutions finally depolarized. The time factor obviously accounts for the difference between these results and Lorente de Nó's. The mechanism of block, when carried out rapidly, consists in a critical increase in threshold. In later studies (270) it has been noted that threshold increase begins to be evident at 1.0 mM, and that 3.5 mM can block conduction when permitted to act for several hours. An interesting phenomenon encountered at much lower dosage is the prevention of spontaneous firing by tetraethylammonium. This protective action of eserine can be seen with concentrations as low as 0.05 mM and may possibly bear some relationship to anticholinesterase activity.

Neostigmine. Prostigmine was found by Lorente de Nó (184) to prevent acetylcholine depolarization in concentrations of 0.1 mM, but apparently by itself it produced depolarization and block after many hours. Both effects are of interest in that they would seem to indicate that neostigmine can penetrate the nerve membrane (*cf.* 11).

3. *Cholinergic blocking agents.* If nerve conduction were based upon one of the mechanisms of transmission which prevail at various synaptic regions, one should expect to find blocking agents for nerve paralleling those for some particular synapse, and the most likely parallelism would be with autonomic ganglia or neuromuscular transmission. Unfortunately the full gamut of blocking agents has not yet been studied, but it can already be concluded that nerve does not show the expected parallelism. However, the nature of the discrepancy is of considerable theoretical importance, as may be seen from the following studies of quaternary ammonium ions.

Tetraethylammonium is a useful and effective autonomic ganglionic blocking agent. Its pharmacology has been reviewed recently by Moe and Freyburger (201). Among its side-effects are stimulation of peripheral nerve, as noted by Brink *et al.* (34) and others. In our experience this effect is seen clearly at 10 mM and is evident even at 1 mM concentrations. The effect consists of lowering of threshold and the appearance of rhythmic evoked discharges and spontaneous firing, together with slowing of velocity and broadening and blunting of the spike, in A fibers of frog sciatic nerve. The effect can be abolished by relatively low doses of eserine (270).

Lorente de Nó (185, 186) observed that isotonic tetraethylammonium maintained the excitability of most of the B and C fibers of frog sciatic nerve, in addition to maintaining the membrane potential of the nerve trunk, in sodium-free solution. Furthermore, tetraethylammonium could restore excitability of most B and C fibers when conduction had previously been lost, for example, in choline chloride solution. Those fibers which could be restored, designated as "Et" fibers, could also be affected in similar manner by certain other quaternary compounds.

Other quaternary ammonium compounds. Lorente de Nó (185, 186) studied the structural requirements for tetraethylammonium-like compounds capable of replacing sodium. He concluded that quaternary ammonium derivatives having two alkyl substituents ranging in length from ethyl to butyl were adequate. He

also concluded from these studies that chemical reactions resulting in a change of tricovalent to tetracovalent nitrogen play an important role in the establishment of electrical double layers in the nerve membrane. Ethylated quaternary ammonium derivatives of piperidine, *l* (+) lysine and histamine were found to play the same role as tetraethylammonium. Quaternary bases extracted from ox brain were even more effective in that they effected a partial restoration of A fibers.

The implications of this work are so important that it would be well to keep in mind the relatively high concentrations of sodium-substituents used, and the need for more convincing demonstration that organic ions rather than sodium itself are primary in the excitation process.

As anticlimax, it might be pointed out that the bis-trimethylammonium compounds *C10*, a neuromyal blocking agent, and *C6*, a ganglionic blocking agent, are without apparent effect on frog sciatic nerve in concentrations up to 30 mM (270).

Of other related blocking agents *curare* has been known since the time of Claude Bernard to be without direct effect on nerve (238), although it may modify nerve excitability curves dependent on muscular responses *in vivo* (257). Nicotine has interesting actions on invertebrate ganglion cells (221, 239, 240, 290); it is not clear whether fibers are directly affected. *Atropine* has mild local anesthetic actions, which is not surprising in view of the resemblance of its structure to that of cocaine (112). The blocking action of atropine is said to be of the hyperpolarization type (97), but it is also reported to have no effect on membrane potential or, for that matter, on threshold (238). Since atropine, in addition to its antimuscarinic actions, has been repeatedly shown to antagonize the effects of cholinergic drugs on the central nervous system (87), it would be of interest to study some of the aforementioned effects of cholinergic agents on peripheral nerve in the presence of non-blocking concentrations of atropine.

4. *Inhibitors of acetylcholine synthesis. Botulinus toxin*, which produces a curariform paralysis of slow onset, is one of the most potent neurotropic substances known. Taking the molecular weight as approximately one million and the lethal dose in rabbits as about 0.05 microgram per kg. (6), the equivalent concentration would be 5×10^{-14} M. Guyton and McDonald (126) estimate the lethal dose as one molecule per 10 neuromuscular end plates. Either the molecule must make many trips, or more probably it sets up a chain of antibody reactions which produce the same end result. In any case it qualifies as a "destructive enzyme". It is not a lecithinase, as are some of the potent neurotropic poisons (6). Both Ambache (6) and Guyton and McDonald (126) have presented convincing evidence that botulinus toxin does not act upon the endplate region of the muscle to produce a curare-type of neuromuscular block. Neither does it block conduction along the nerve trunk. Since the cholinergic effector cells, somatic and visceral, still respond normally to injected acetylcholine, these authors conclude that there is inhibition of acetylcholine synthesis or release from nerve. They also believe that *tetanus toxin*, although it produces predominately opposite clinical manifestations related to acetylcholine overproduction, is

fundamentally related in mechanism of action to botulinus toxin. It is notable that neither of these toxins, in spite of profound intraneuronal effects on acetylcholine metabolism, alters conduction in nerve fibers.

5. *Epinephrine.* Epinephrine has not received attention in axonology comparable with acetylcholine, although an adrenergic substance is known to be released from sympathetic post-ganglionic fibers throughout their length (177). It is said to reduce chronaxie of peripheral vertebrate nerve (238). Since it has both depressing and facilitating actions in invertebrate ganglia (221), and a universal excitatory action on cardiac tissue including that of invertebrates (223a), it has at least as much claim to universality as acetylcholine.

6. *Histamine and antihistaminics.* Barany and Nordqvist (14) have reported that nerve block due to antihistaminics and to procaine can be relieved by histamine. Crescitelli and Geissmann (59) have repeated their observations with a group of ten assorted antihistaminics and other related agents. They found no relation between antihistaminic potency and blocking dose, which varied from 0.8 to 10.0 mM. The mechanism of block was apparently threshold increase without depolarization, although all could depolarize in high concentration. Histamine was without effect up to 30 mM at which concentration it showed slight depolarizing action. They attribute the results of Barany and Nordqvist to acidification by histamine. Nordqvist (212) states that both acetylcholine and histamine (in relatively high dosage) relieve procaine block, and that the effect is independent of pH. Fleckenstein and Hardt (97) discuss the resemblance between anodal polarization and block by calcium, antihistaminics and atropine.

From the foregoing discussion of autonomic agents, it appears safe to conclude that nerve conduction, although undoubtedly dependent on chemical processes, can not be fitted into any of the schemes of chemical mediation which have hitherto been devised for ganglia and neuroeffector junctions. Much of the data suggesting particular systems is apparently based on diffuse effects of high drug concentrations, and does not meet the rigorous criteria previously required for transmission at other sites. Nor is there any *a priori* reason why all fiber types should operate by precisely the same chemical mechanisms, provided only that they are equipped with some general explosive system. The search for new types of systems is still in the embryonic stage.

D. *Metabolic Agents*

Although the purpose of this review does not extend to a discussion of nerve biochemistry and metabolism, it is appropriate to summarize some of the changes which are brought about by excess or deficiency of metabolites or by metabolic poisons.

1. *Oxygen.* It now appears that oxygen is the one substance which is completely indispensable to nerve for the maintenance of its membrane potential, the necessary metabolites being included in the structure of nerve (184). Anoxia slowly decreases the membrane potential after a preliminary period in which already oxidized reserves are apparently utilized (45, 88, 91, 103, 106, 157, 158). The oxygen tension must fall to a very low value (approximately 0.5%) before

depolarization appears (184). Diffusion of oxygen is not the limiting factor in determining rate of oxygen utilization, but rather an internal regulatory process which varies with activity and treatment (91). The oxygen utilization is low compared to most tissues—for frog nerve, values of 0.10 cal/gm/hr. at rest and up to 0.18 cal/gm/hr. for maximum activity are given (106). The values appear to be of the same order of magnitude for various fibers at the same temperature (88). The oxygen requirements in activity may be the same or less than at rest (74). In vertebrate nerve the low oxygen utilization of axones may be related to the relative lack of synthetic processes since the bulk of material required seems to be manufactured in the soma and transported down the axoplasm (286). In fact, a calculation of the power dissipation across the membrane resistance at normal membrane voltage would account for the entire metabolism of nerve (270).

Associated with anoxic depolarization, the polarizability of the various components of the membrane potential is altered (184), the action potential may be reduced to half, and the conduction velocity also halved without great change in threshold until the critical point is reached (297), ordinarily at about 15% less than the normal membrane voltage (184). The conduction process is said to be more resistant than the excitation process to anoxic depolarization (40). The rate of depolarization, as well as the rate of recovery in oxygen, is sensitive to a great many substances; many depressant agents actually slow both processes (184, 247).

All the effects of anoxia can be reversed temporarily by anodal polarization (184), indicating that the existence of the membrane potential is the basic work requirement for excitability.

Older methods of measurement of heat production and O_2 consumption (88) have been improved (74) and new methods devised (34–37, 167–168) to the point where there is now little reason for estimating drug effects on Q_{O_2} by indirect means.

2. *Metabolic poisons.* Practically all the known metabolic poisons have been tested in nerve for one purpose or another. The *carbamates*, of which ethyl urethane probably acts as an antidehydrogenase, have already been mentioned (56, 58, 184). They initially hyperpolarize, presumably by a surface action mechanism, and then depolarize by metabolic inhibition. *Azide*, which acts on the cytochrome system, at concentrations of 0.05 to 0.3 mM inhibits "activity" respiration without abolishing conduction (34, 73, 74), and *hydroxylamine* at 1.0 mM has the same enzymatic and nerve actions. *Yohimbine*, at 0.01 to 1.0 mM, similarly affects "activity" respiration (74) presumably through the same system. More important, it brings about a great lengthening of refractoriness (259) and a corresponding increase in positive after-potential (246, 247), and blocks by threshold increase (194). The relationship of these effects to its known adrenergic blocking action is unclear. *Cyanide*, another agent working on the cytochrome system, depolarizes reversibly in concentrations from 1.0 to 50.0 mM in frog nerve (184, 279). It has an initial excitant action on crayfish ganglion cells (222). *Carbon monoxide*, an oxygen competitor in the cytochrome system, in high con-

centrations depolarizes frog nerve (297), and in crayfish ganglia produces a reversible inhibition of spontaneous activity which can be partly abolished by light (222).

Of the glycolytic inhibitors, *iodoacetate* has no effect on crayfish ganglion cells in air (222), blocks glucose protection against anoxia in crab fibers, but by itself reduces the rate of anoxic depolarization (247). However, in frog nerve it is said to increase anoxic depolarization (45). Depolarizing concentrations of 1.0 mM *iodoacetamide* are reversible by anelectrotonus (184). *Fluoride*, which presumably blocks both aerobic and anaerobic glycolysis, at first excites in 20 mM concentration (possibly by its action on calcium), but eventually blocks by depolarization (184). It may account for the irreversible block noted after long exposure to DFP (272).

Methyl fluoroacetate, which acts early in the tricarboxylic acid cycle to cause accumulation of citrate, acts in a manner opposite to azide, reducing "resting" respiration by half while leaving "active" respiration and conduction intact, in a concentration of 5.0 mM (32, 73, 74). *Dinitrophenol*, in line with its general ability to stimulate metabolism, shows only excitatory effects in crayfish ganglia at low doses, but finally depresses in high (222). *Phlorizin*, presumably active on phosphoryllation mechanisms, has a hyperpolarizing action at 10 mM and does not depolarize frog nerve even at 30 mM; at 20 mM it blocks A fibers before C, and the hyperpolarization block can be relieved by cathodal stimulation or anoxia (184).

Oxine (8-hydroxyquinoline), a metal chelating agent especially for iron, produces hyperpolarization and conduction block at 3 mM; *Carbostyril* (2-hydroxyquinoline) and *quinoline*, which are less effective in formation of metal chelate complexes, produce the same effects at 7.0 mM (57). In crustacean nerves, however, quinoline produces spontaneous firing and possibly partial depolarization, reversible by calcium (114) *Thiocyanate* combines hyperpolarization with spontaneous firing, the latter supposedly by the establishment of a sharp voltage gradient (32). *Arsenite* at 10 mM produces depolarization (184), possibly by action on sulfhydryl groups. *Cupric salts* produce irreversible depolarization in frog nerve in doses of 5 to 10 mM.

In general, it can be said that the differential metabolic blocking agents have interesting but sometimes unpredicted actions on the function of nerve cells, and that only to a limited extent do the data suggest roles of particular metabolic enzyme systems.

3. *Metabolites.* *Glucose* and other sugars in isotonic solution containing adequate sodium ion were found by Lorente de Nó (184) to have no important effects on nerve except those which could be accounted for on the basis of a combined longitudinal and membrane resistance increase due to reduction in conductivity (increased apparent membrane potential and spike height, decreased conduction velocity). However, glucose as well as *levulose* delayed anoxic depolarization, but the nerve suffered an irreversible loss of excitability in spite of restoration of oxygen. The effect was shown not to be due to accumulation of lactate. It could be prevented to some extent by adaptation of the nerve to anoxia prior to glucose

treatment. In contrast, *galactose* and *sucrose* had no such effect. The mechanism of this phenomenon is obscure, except insofar as it illustrates both the utilizability of glucose and levulose and the distortion of metabolic processes when excessive use is made of anaerobic pathways.

Insulin in low concentration is said to excite, and in high concentration to to depress, firing in crayfish ganglion cells, presumably by action on hexose metabolism (221).

Glycerol appears to have no effect, and *acetone* inconstant depressant actions, in isotonic solution on frog nerve during exposure for several hours. *Acetoacetate* prolongs refractoriness but has no other important effect in isotonic concentration (270).

A number of the short-chain acids produce spontaneous firing in frog sciatic nerve, including *acetate*, *lactate*, *oxalate* and *citrate; valerate* does not, and it produces a moderate initial increase in membrane potential (184). The possible role of the longer-chain acids in nerve function is a matter of controversy (135, 136, 184).

Various *alkyl amines* appear to produce a depolarizing type of block in crustacean fibers (290). In ability to depolarize frog nerve, the longer-branched alkyl amines approach potassium, while the shorter have little effect (291).

Thiamine is of some interest because of its apparent release from sensory fibers during conduction (200, 280). Isotonic solutions eventually depolarize irreversibly (184), but the onset of the block and depolarization is so slow as to merit some caution concerning the assignment of a role to thiamine in the conduction process.

In summarizing the actions of some metabolites, one is again struck by the high concentrations required to produce any notable effect. Since it cannot easily be denied that they play a functional role, at least in the processes required for maintenance of membrane potential, their very inertness may give comfort to those who contend that other important cell constituents may not have easily demonstrable effects of direct application in excess of normal requirements.

4. *Carbon dioxide.* Waller (282) first noted the effects of carbon dioxide on nerve and called attention to the importance of CO_2 for normal function. Davis *et al.* (66) observed that CO_2 increased electrical threshold, decreased the conduction velocity, and increased amplitude and duration of action potential. Gerard (104) observed prolongation of the positive after-potential, and Lehmann (171) demonstrated the importance of CO_2 in mammalian nerve for maintenance of viability and exhibition of properties equivalent to blood-perfused nerve. Graham (115) studied the relationship between after-potentials and excitability in relation to CO_2.

Among additional actions of 5% CO_2 summarized by Lorente de Nó (184) are: enhancement of action potential amplitude and excitability after activity; prevention of decrease of membrane potential and spike height following tetanic stimulation; prevention of spontaneous firing induced by many drugs; restoration of conduction after critical depolarization in KCl or veratrine; and moderate increase in membrane potential. Lorente de Nó points out the resemblance of all of these actions to anodal polarization, and shows that they are attributable

primarily to an increase in the L fraction of the membrane potential. He presents convincing evidence that the effects are due to CO_2 *per se* and not to a fall in pH or to an alteration of calcium complexes. He shows also that the effects can occur in the absence of oxygen.

Lorente de Nó intimates that even 100% CO_2 does not impair nerve, but this has not been our experience; pure CO_2 rapidly doubles the threshold and the conduction time, and the effects are not always reversible even after a moderately short exposure (270). To attribute these actions to anoxia rather than to excessive CO_2 requires some rearrangement of time scales, in view of the known slow course of anoxia.

Laget and Legouix (162) have recently made a systematic study of differences in response to CO_2 among various nerve types, using an indirect method for estimation of the L fraction of the membrane potential. While motor roots are relatively invulnerable to CO_2 lack, certain peripheral mixed branches in mammals soon go into spontaneous firing and deteriorate irreversibly in air. Gernandt (108) has illustrated the exquisite sensitivity of post-tourniquet paresthesias in man to changes in CO_2 tension.

Davenport (65) has demonstrated that drugs which suppress carbonic anhydrase activity have no effect on function of either central nervous system, where the enzyme system is present, or of peripheral nerve, where it is negligible.

5. *Hydrogen ion concentration.* Lorente de Nó (184) has indicated that nerve is relatively unresponsive to changes in pH between the limits of 5.5 and 8.0, and that therefore the effects of CO_2 and of many acids within this range are specific rather than related to hydrogen ion concentration. Below pH 3.0 there is conduction block (184), and above pH 8.0 spontaneous firing develops (171). Although this range offers considerable latitude, to be on the safe side we have found it convenient to use a small amount of phenol red in all working solutions and to maintain pH at approximately 7.3 for most observations unless specifically desired otherwise (270).

E. *Inorganic Ions*

1. *Monovalent cations. Sodium* plays a vital role in the maintenance of excitability of nerve, as has already been discussed. For frog nerve, a concentration of 17 mM is adequate to maintain normal function as long as the osmolarity (110 mM) is maintained by such "neutral" ions as acetylcholine, choline or methacholine (184, 187). Many quaternary ammonium compounds having at least two ethyl groups (*e.g.*, tetraethylammonium) can substitute in part for sodium (185, 186). In myelinated nerve the size of the action potential spike varies in a fairly direct manner with sodium concentration (148). Sodium entry into invertebrate nerve during stimulation can be demonstrated (121, 155, 156). Metabolic work is probably required directly for the extrusion of sodium, and the mechanism of extrusion may break down concomitantly with the rise of the action potential (143).

Lithium is able to substitute temporarily for sodium as far as conduction is concerned, but it has different effects on electrotonic potentials and accommo-

dation; lithium also has an initial hyperpolarizing action on the membrane potential, but eventually depolarizes, in part irreversibly (99, 100, 238).

Ammonium ion is unable to substitute for sodium (99, 100, 184); it depolarizes but also may to a limited extent delay the onset of nerve block in the absence of sodium.

Potassium has probably been studied more than any other single agent for its effects on nerve (2, 10, 19, 24, 32, 44, 45, 54, 89–91, 99, 116, 137, 139, 141, 142, 148, 155, 156, 163, 172, 184, 191–193, 221, 238, 246, 247, 270, 272, 275, 276, 298, and many others). The amount of attention is attributable to the universal depolarizing action of potassium, and its unique role as the major intracellular cation. Bernstein (19) postulated that potassium was the only ion of importance that could diffuse freely through the membrane, and therefore establish a potential by concentration difference. Bishop (26) and Höber and Strohe (139) were among the first to study KCl block, and Biedermann (24) had observed that during KCl block excitation could still occur at the anode (break-shock). Various authors (54, 89, 90, 246, 247) have described a simple logarithmic relation between membrane voltage and the inside/outside K ratio, but the ratio is not constant, since inside K tends to vary with the outside concentration (91, 184). The leakage of potassium from nerve during activity has been frequently noted (141, 142, 155, 156, 234, 298).

Potassium causes a fall in membrane resistance (141, 142), and therefore has been implicated in conduction. It has been postulated as a mediator between end organs and sensory nerve terminations (2). It has been reported both to inhibit (45) and to increase (32) nerve respiration. Mild excitatory effects prior to block are probably seen more in vertebrate (44, 116, 238, 270, 272) than invertebrate nerve (270). The normal ratio in frog nerve is better maintained in Ringer's with 5 mM KCl than the usual 1.3 mM (91). In the course of blocking, A fibers are normally affected first and C's last (184), but the relations are somewhat complicated for various nerves (163). Depolarization is not complete below 50 mM (184). The actions of potassium can best be summarized as depolarization, membrane resistance reduction, and obliteration of electrotonic potentials, all of which effects are reversible by anodal block.

Rubidinium depolarizes about as fast and to the same extent as potassium, while *caesium* is somewhat less effective (90, 99, 184).

Although at first glance the series seems erratic, it will be noted that there is a regular trend in the increasing series of monovalent cations from lithium, which transiently hyperpolarizes, through sodium, which is without action, to a peak depolarizing effect with potassium and rubidinium, tapering off with caesium.

2. *Polyvalent cations. Calcium* has also been extensively studied with regard to its actions on nerve (34, 116, 172, 184, 193, 203, 238, 246, 251, 255, 256, 270, 295), and very often effects of other agents have been ascribed to alterations in the state of, or competition with, calcium (34, 114, 171–172). Woronzow (296) noted that a calcium conduction block could be reinforced by anodal or relieved by cathodal current. The general effects of calcium are equivalent at least initially to those of anelectrotonus, including threshold increase, spike increase, and

slowing of conduction velocity. The block can be relieved at first by anoxia, cathodal current or a train of impulses (184). However, concentrations as low as 10 mM eventually produce depolarization, the A fibers being first affected and the C fibers last. The effect may be irreversible, due to acute swelling and damage to the myelin sheath.

Magnesium acts in a manner similar to calcium, but much less markedly (45, 184, 238). The effects of 10 mM concentration are comparable to those of 5% CO_2, including a small increase in threshold and membrane potential. Magnesium also reduces oxygen consumption (103).

Barium (189) was found to have primarily a depolarizing action in concentrations from 3.7 mM to 43 mM. However, the L fraction is increased, causing abnormally large after-potentials and electrotonic changes. Rhythmicity occurs when the L fraction falls sufficiently. A fibers are much more susceptible than C fibers to barium block (125, 294, 295).

Effects of various metals have been described by Guttman (125) and Woronzow (294, 295).

3. *Monovalent anions. Chloride* is probably not indispensable for nerve function, since it can be replaced by acetate, lactate and other ions with no notable difference except for the tendency toward spontaneous firing in some of these solutions (184).

Bromide has minimal effects against electrically or chemically induced hyperexcitability after as much as ten hours in isotonic solution (270). *Fluoride* has marked effects at 20 mM, causing at first spontaneous firing (possibly related to calcium binding) and later an irreversible depolarization in line with its general inhibitor effect on metabolism (184, 270, 272).

4. *Polyvalent anions.* The most generally reported feature of the polyvalent anions has been production of spontaneous firing, usually associated with an increase in oxygen consumption. The action is usually attributed to precipitation of calcium, although this would not explain all of the cases observed (184). In fact, the number of anions of all types which have been listed as exciting substances (114) lead to the impression that chloride is in a class by itself, a sort of normal "depressant" of nerve function.

Citrate and *oxalate* at 10 mM typically produce a fall in threshold, repetitive spikes following single shocks, or spontaneous activity; a fall in membrane potential is not necessary to the phenomenon. Azide, concomitant with a reduction in Q_{O_2}, can depress the spontaneous activity without blocking conduction; KCl, which may increase O_2 consumption but depolarizes the nerve, also stops the discharge; thiocyanate, which increases membrane potential, may increase the discharge rate (34). Carbon dioxide abolishes the excitatory effects of citrate or oxalate (184). One mechanism projected to explain these effects is a lowering of the Q/L fraction of the membrane potential, which would favor oscillatory phenomena (184). Oxygen consumption is increased during citrate or oxalate induced hyperexcitability. *Sulfate* (184) and *phosphate* (268) are able to induce repetitive firing. Phosphate (isotonic) can be opposed by a variety of drugs, particularly the anticonvulsants, in relatively low dosage. Phosphate hyperexcitability can still be demonstrated with concentrations as low as about 10 mM.

Phosphate hypersensitivity is of some interest because of the possibility that metabolic phosphorylation mechanisms are concerned. Furthermore, since anticonvulsant drugs can block the action of phosphate (268), the question arises whether they do so by altering the permeability of the nerve for phosphate. Since P^{32} uptake into various fractions of nervous tissue can conveniently be followed (29, 117, 204, 236), the question was examined in frog sciatic nerve, diphenylhydantoin being used as a convenient blocking agent (93, 292). It was found that diphenylhydantoin had only minor effects on either total uptake of P^{32} or integration into the protein, lipid, organic acid-soluble or inorganic fractions, despite complete protection against the phosphate-induced hyperactivity, so that changes in membrane permeability or synthesis into the major nerve fractions could not be invoked as blocking mechanisms. Therefore, the phosphate hypersensitivity must be both induced and blocked at a point in metabolic processes beyond the initial active transport across the membrane and synthesis into tissue constituents. Further studies with sodium and potassium exchange indicate the possibility that the crucial point might be related to the system involved in the active extrusion of sodium.

F. *Miscellaneous Substances*

1. *DDT and other insecticides.* DDT (2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane) has been shown to produce persistent repetitive firing in crustacean axones in concentrations as low as 0.014 mM (114). The firing is suppressed by increased calcium, magnesium and potassium ion, the latter apparently by a depolarization mechanism. Repetitive firing due to DDT and other agents is inferred to result from a delay in restoration of calcium ion to a surface complex, following the breaking of the chelate linkage of calcium ions to surface polar groups by the initial exciting impulse. That some deeper mechanism may be involved is suggested by the finding that acetylcholine is released by DDT from various neurones, an action not duplicated even by anticholinesterases (267). DDT repetitive firing is not seen in squid nerve, in contrast to crab nerve (246, 247), but excitatory effects have been found in mammalian motor nerve (86).

2. *Veratrine.* Veratrine has long been of interest to neuropharmacology because of its ability to produce vascular hypotension of central nervous origin, and tetanic neuromuscular and ganglionic responses followed by block. On closer inspection these effects appear to be intrinsic in the axone (231) and muscle membrane (161) functions rather than peculiar to neuromyal or ganglionic synapse. The chief effects noted have been a large increase in negative after-potential (145, 161, 184, 231, 242, 246, 247), particularly after repetitive stimulation, and finally a depolarization (90, 96, 184, 238). The negative after-potential may develop a distinct rising phase of its own (161, 184, 242). The blocking effect can be increased by potassium (184, 247) or antagonized by carbon dioxide (184), anodal current (96, 184), calcium (247) and local anesthetics (96), but the unblocking agents also tend to increase oscillatory phenomena. Veratrine increases nerve oxygen uptake (242). Lorente de Nó (184) has given approximately

the following interpretation of veratrine action: Initially or in low dosage (1:200,000) the rate of recovery of the Q fraction of the membrane potential is slowed because of an enzymatic action leading to wasteful diversion of oxidative metabolism; the effect becomes cumulative with repetitive stimulation, and can lead to a depolarization block by loss of Q fraction. Unblocking agents such as CO_2 increase primarily the L fraction, increasing the L/Q ratio and favoring slow oscillatory phenomena. At higher concentration (1:50,000) direct depolarization intervenes.

3. *Antibiotics. Streptomycin*, which has been reported to produce specific destructive effects on the eighth cranial nerve and cortical convulsions on direct application, produces a depolarization type of block in frog nerve, but only at very high concentrations, approximately 40 mM (270). The inhibitory effect of streptomycin on neural and glial elements in tissue culture has been studied (15), a matter of interest because of the use of antibiotics to maintain asepsis in neural cultures (219, 220).

Sulfanilamide and *thiophene-2-sulfonamide* are reported to delay anoxic depolarization and recovery in frog nerve and to interfere with CO_2 effects on membrane potential (245). The latter agent is a specific inhibitor of carbonic anhydrase, but has no effect on normal properties of frog nerve which is relatively free of this enzyme system (65).

4. *Others. Menthol* is reported to have a specific sensitizing effect on the end organs of fibers receptive to cold, but not on other types, thus explaining the well-known subjective cooling effect of this agent (130). *Chloropicrin* has interesting veratrine-like effects on nerve at a concentration of about 1.0 mM whereas *bromopicrin* has an excitant action on ganglia while blocking nerve conduction, apparently by a depolarization mechanism (12).

IV. DISCUSSION

A. *Principal Actions of Drugs on Nerve*

There is a large gap between our original outline of basic properties of neurones and our recital of the empirical findings with particular drugs. In most cases it has been almost impossible to draw the proper conclusions from the data concerning anything more than the main mode of production of block or of excitatory effects. For example, the only agent concerning which a confident statement can be made regarding effects on membrane resistance is potassium, even though we would strongly suspect that citrate, oxalate, phosphate, etc., could be shown to decrease membrane resistance, and calcium at least to raise it initially.

Since we cannot make an ideal classification, it seems useful to set down an empirical grouping based on the typical actions of well-known agents. Such a catalogue has the practical advantage that in laboratory work the standard compounds can be carried along in the same experimental design, for close observation of similarities and differences (a useful rule in any field of pharmacology where new drugs are to be explored or old ones reexamined with new techniques). With this practical consideration in mind, certain main classes of drug action on peripheral nerve come into focus:

1. Depolarizers

a. *Potassium-like:* by virtue of ability to depolarize and to reduce membrane resistance, diminish threshold moderately, produce loss of slow response to anelectrotonus, etc.

b. *Anoxia-like:* because of ability to depolarize slowly in a manner that can be strictly reversed by anelectrotonus.

c. *Ether-like:* with respect to rapid depolarization which can be carried to a point of irreversible loss of electrotonic changes.

d. *Veratrine-like:* with regard to preliminary prolongation of negative after-potential, especially after rapid stimulation.

2. Threshold-raisers

a. *Procaine-like:* in that membrane potential is essentially unaltered, and large fibers of limb nerves are most resistant.

b. *Carbon-dioxide-like:* by reason of moderate increase in membrane potential, increase particularly of the slow electrotonus, and post-tetanic increment of action potential and excitability.

c. *Calcium-like:* because of producing both hyperpolarization block and ultimate irreversible changes, with preference for large fibers.

3. Excitants

a. *Citrate-like:* on account of threshold decrease, evoked rhythmicity, spontaneous firing, etc.

b. *Tetraethylammonium-like:* specifically through ability to substitute in part for sodium.

4. Inert

a. *Choline-like:* electrolyte which does not alter normal properties in isotonic solution in presence of adequate sodium, oxygen and CO_2.

b. *Sucrose-like:* non-electrolyte, otherwise maintaining normal properties as above.

The classification is incomplete and possibly in error in some respects. However, it might have some real value in improving the lines of communication between investigators, supplementing the extremes of endless recital of protocols by some, of mathematical preciosity by others, and of single-measurement decisions by most. Whether it would be of any value in relating axonological data to fundamental processes or in alerting the research worker to useful pharmacological tools is another matter.

B. *Drug Interrelationships*

The discrepancy in many cases between drug actions on specific systems, such as brain or ganglia, and their lack of action, or inverse or irrelevant action on axones is a strong temptation toward eclecticism in pharmacology. Nevertheless there is no dearth of attempts to develop unifying concepts of drug action (4, 18, 36, 47, 92, 107, 112, 114, 137, 149, 180, 184, 196, 205, 269, 287). The author

finds it convenient to classify substances on the basis of the following major considerations:

1. *Non-bonding agents*, of which the heterogeneous general anesthetics and other chemically sluggish or inert compounds form the main bulk. These constitute a group of relatively low potency, acting by Van der Waals forces rather than formation of chemical bonds, concentrating in lipid phases, or at interfaces, cluttering the living space of important enzyme systems and thus disrupting physiological regulations by their physical presence. By their very nature they must have diffuse and universal, but not necessarily common effects. Here will be found particularly those agents whose important effects are upon permeability.

2. *Single-bonding agents*, having at least one reactive group capable of forming one bond at a time with tissue proteins including enzymes, disrupting reactions somewhat indiscriminately, but showing broad groupings based on their reaction types and therefore having physiologically qualitative actions by quantitative preference for certain enzyme types. Such agents as anticonvulsants and sedatives might fall into this class, where considerable variation in non-reactive structure has only trivial effects on potency. Here will be found many agents of intermediate potency capable of altering metabolic mechanisms which maintain or restore membrane potentials and thresholds.

3. *Multiple-bonding activating and blocking agents*, including all those agents related to chemical mediators, known and unknown, which have a spatial relationship of reactive groups guaranteeing the formation of several bonds at a time with very specific tissue constituents. If these constituents are portions of enzymes which activate important reactions only in the presence of a coenzyme, and if the drug fits the description of the coenzyme, then the drug itself may substitute for better or worse as the mediator or activator in processes which transfer energy out of all proportion to that of the bonds formed. If by a small change in structure the drug can still bond but now stands in the way of substrate-enzyme linkage, it must inhibit rather than activate. Since the structural requirements for activation will necessarily be more rigorous than for blocking, probability considerations will favor the decision that a potent new drug will eventually be accounted for as a blocking agent. Since tissues have a common chemical origin, ontogenetically and phylogenetically, it will not be surprising to find, on the one hand, that there are many related enzyme systems, differing in apparently trivial respects in their optimal requirements for coenzyme structure; on the other hand, the minor difference may determine that at one site or in one species a particular drug will imitate a chemical mediator, that at another it will be a powerful blocking agent, that at a third intermediate site it will be weakly activating in low doses and blocking in higher and that at another site it will be relatively inert. With peripheral nerve the issue is forced by the problem of the inertness of acetylcholine, and the excitatory role of quaternary ammonium compounds. If there are indeed chemical mediation steps in some nerves, the mediators may not be far removed in structure from common agents which are active at other sites but inert with respect to axonal function.

4. *Antigens*. By multiplying the size and complexity of a substance to the point

where it begins to resemble an enzyme in specificity of pattern, there is achieved a structure capable of diverting the prevailing synthesis of enzymes, causing new structures to be formed about it, which in turn serve as templates for the building of facsimiles of the intruding substance, and so on in reciprocal fashion far beyond the effect of the original strange molecule. This process comes close to the central avenue of growth itself, and it is probably not completely absent in axones of nerve cells, low though their level of synthesis may be. By looking in this direction we can accept the fact that a substance like botulinus toxin can alter or abolish acetylcholine synthesis in nerve out of all proportion to the original quantity of toxin present. But the possibility is also implicit that by developing new toxins in favorable hosts to chosen fractions of nerve it may be possible to test in a highly specific way the functions of neuronal constituents.

C. *Translation from Peripheral to Central Neurones*

At the moment, most axonology seems considerably removed from problems of central nervous function, except by broad analogy. However, the application of microelectrode stimulating and recording to spinal motoneurons has already settled questions which could have been definitively answered in no other way (79). Neuropharmacology should be able to exploit these advances at other levels of the central nervous system. The beauty of the method is that it permits examination of a single cell in its natural society of cells. With certain technical refinements it should be possible to make measurements of membrane resistance and threshold, as well as of membrane resting voltage and action potentials, at many sites and with many drugs. Except for estimation of cell size, most of the advances of peripheral neurophysiology can be carried to the central nervous system. It is probably by this direct attack, rather than by constructing model nerve nets from peripheral systems or rationalizations of discrepant drug actions such as we have just concluded, that axonologists can contribute most to an understanding of brain pharmacology and physiology.

D. *Perspectives*

The discussion has already indicated a number of directions in which peripheral neuropharmacology is likely to go, both methodologically and theoretically. There is one more which seems pertinent to a conclusion, and that is the need for a more dynamic view of neural mechanisms than now prevails.

Of the two most complete theories available, that of Lorente de Nó (184) fails to account adequately for the phenomenon of action potential overshoot, now known to be present in all excitable cells which have been studied. That of Hodgkin (143) permits a mechanism of overshoot but does not account adequately for either the recovery or for the maintenance of a membrane potential in the absence of sodium. Both theories suffer from a lack of continuity between the "resting" and "active" states.

Now we are forced by certain facts to wonder about the relationship between these states. The data of Doty and Gerard (74) indicate that there need be no greater utilization of energy in one than in the other. Furthermore, we have the

example of the long period of hyperdepolarization in the heart (293), and the fact that the heart can be shocked from "activity" into "rest" as well as the other way around (285).

To some extent these facts could all be reconciled if it were assumed that in excitable tissues there are two oppositely directed processes capable of establishing voltage differences across the membrane, and that the products of each are in part the reactants of the other. An observed steady voltage across the membrane would then indicate that their rates were equal, but would give no indication of the absolute rates. If there could exist, as there should in such an autocatalytic system operating between limits, two possible states where the rates were equal and where a forced increase in one process could not be sustained by a corresponding increase in the other (that is, two different states of regulation), then there would have to exist an intermediate state where rates were also equal but an increase in one process led to a greater increase in the other. This intermediate state would in effect be the threshold and, above or below it, the system would have to gravitate to either extreme.

If the membrane voltage in turn were a determinant of the rate of each component process, it becomes possible for an extreme state to develop progressively the conditions for its own abrupt disappearance. Some such mechanism must be taken into account in any theory of recovery or of oscillatory behavior.

Without pursuing mechanisms further, the point to be made is that it is possible to conceive of a reciprocating system in which the same processes which appear so dramatically in the explosive phase and recovery are also operative at all other times, but are in balance.

The value of such a formulation for neuropharmacology would be chiefly this: that it would encourage the search for substances and key reactions involved in voltage production both at "rest" and in "activity" and would relieve the search for elusive "trigger" substances which appear and play a role only during the action potential spike.

Whatever view may be taken of the nature of fundamental processes in nerve, it seems to this reviewer that the continued investigation of effects of drugs on axonal properties, and the use of drugs to assist in the search for basic mechanisms of neural behavior, can continue to be of value even though the principles and practice thus derived will probably find application in more important tissues than peripheral nerve.

V. SUMMARY

1. Some basic properties of peripheral nerve are discussed, together with notes on their measurement and on some typical effects of drugs.

2. The actions of a variety of drugs on peripheral nerve are described.

3. A suggested empirical scheme is presented for the tentative classification of drugs acting on peripheral nerve.

4. Interrelationships between drugs in several categories of mechanism of action are discussed, with particular attention to the contradictory actions of presumed chemical mediators and blocking agents.

5. The applicability of modern peripheral nerve methodology to brain neuropharmacology is noted.

6. Some speculations are presented on the continuity between "resting" and "active" functions in nerve.

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