ELECTROCHEMICAL ASPECTS OF PHYSIOLOGICAL AND PHARMACOLOGICAL ACTION IN EXCITABLE CELLS

PART II. THE ACTION POTENTIAL AND EXCITATION[1]

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I. Notation and Special Terminology[2]

A	Transmembrane action potential (Fig. 1)
E	Intrinsic e.m.f. of the membrane (Fig. 3)

[2] Definitions are given on the indicated pages.

E_m	Membrane potential as a variable (relative to inside) (Fig. 1)
E'_m	Resting potential or membrane potential just prior to the spike (Fig. 1)
E_K, E_{Na}, E_{Cl}	Equilibrium potentials of K^+, Na^+, Cl^- (p. 190)
E_T	Critical firing potential (p. 237)
G_m (also G_K, G_{Na}, G_{Cl})	Membrane conductance (also potassium, sodium, chloride conductance) (Part I and p. 202)
I	Membrane current
I_K, I_{Na}, I_{Cl}, I_{Ca}	Current (net flux) of K^+, Na^+ *etc.* (p. 202)
I_K, I_{Na}, I_{Cl}, I_{Ca}	Influx (of K^+, Na^+, Cl^-, Ca^{++}) (Part I)
λ	"Time constant of accommodation" (p. 238)
λ_x	Characteristic length (p. 247)
NAP	Negative after-potential (Fig. 1)
NAP_m	Transmembrane negative after-potential (= E'_m − NAP; see Fig. 1)
nodose fiber	"Myelinated" nerve fiber (p. 172)
non-nodose fiber	"Unmyelinated" nerve fiber (p. 172)
O_K, O_{Na}, O_{Cl}	Outflux (of K^+, Na^+, Cl^-) (Part I)
P_K, P_{Na}, P_{Cl}	Ionic permeability (to K^+, Na^+, Cl^-)
PAP	Positive after-potential (Fig. 1)
PAP_m	Transmembrane positive after-potentia l(= E'_m + PAP; see Fig. 1)
PP	Positive potential (Fig. 1)
PP_m	Transmembrane positive potential (= E'_m + PP; see Fig. 1)
Q	Charge (= I × time)
R_m (also R_K, R_{Na}, R_{Cl})	Membrane resistance (also resistance to K^+, Na^+, Cl^-)
R_i	Protoplasmic resistance
ρ	Rheobase (p. 238)
S	Spike (Fig. 1)
S_m	Reversal potential (Fig. 1)
t	Utilization time (p. 239)
τ_m	Membrane time constant (= $R_m C_m$)
V	Change in membrane potential (= $E_m - E'_m$; see Fig. 1)
V_T	Critical change in potential needed for stimulation (= $E_T - E'_m$; see p. 237)
[ ], *e.g.* $[K]_o$, $[K]_i$, $[Na]_o$, *etc.*	Concentration, *e.g.* of extracellular potassium, of intracellular potassium, of extracellular sodium, *etc.*
$[\ ]_m$, *e.g.* $[Ca]_m$, $[Na]_m$	Membrane content (of ionic or bound calcium, sodium)

II. INTRODUCTION

This part of the review[*] is concerned with substantially faster bioelectrical phenomena than were considered in Part I (200), namely, the components of the action potential and the related processes of excitation. Their possible relationship to contraction will also be considered briefly. References given in Part I are not duplicated in the bibliography; citation of these papers is by the numerical designation in Part I followed by the Roman numeral *I*.

Again, the main interest is to determine how far available qualitative and quantitative data can be correlated by certain principles and concepts that appear most consistent with research findings of the past decade. Literature available by the end of January, 1958, has been included.

As before, advantage will be taken of the opportunity provided by comparative physiology and pharmacology to determine what phenomena are general and which characteristics special properties. Emphasis will be on *in vitro* studies, largely on single cells or on preparations where events are least likely to be obscured by extraneous factors, some of which were noted in Part I.

The biological systems to be discussed are those for which are available the requisite electrochemical data, such as the changes in potential, conductance, and ion movement, and their modification by drugs, ions, and metabolic alterations. Since the most complete information has been obtained from studies on nerve and striated muscle, vertebrate and invertebrate, and on vertebrate heart muscle, they are the basis of this review.

The general approach is the same as before: First, consideration of the potential changes which compose the action potential, and their alteration by ions and drugs; then, the presentation of an electrochemical hypothesis that has been found to be the simplest in accounting for the facts in one system, the squid giant axon; an examination of its applicability to other systems in the light of electrical and ionic data; and, finally, minor modifications in the hypothesis required to account for observations on other systems.

Certain electrical phenomena outside the scope of the original hypothesis—the plateau and the negative and positive after-potentials—will be shown to conform with the principles elucidated for both resting and action potentials. Attention will also be called to the probable nature of the coupling between contraction and excitation suggested by the current picture of ionic events in bioelectrical phenomena.

An important aspect of the findings and their analysis is that, as found in Part I, metabolism remains in the background as a supportive rather than as a direct contributor to the bioelectrical processes except in one respect, *viz.* in the generation of the positive after-potential. But here, too, its role in the electrical changes appears to be secondary to the ionic shifts it induces.

The general view is developed that electrical changes are primarily due to permeability changes which can be highly specific for individual ion species; that modifications in these electrical changes by ions and drugs are predominantly the consequence of modification of these permeability changes, a modification which can be more marked for one permeability or one permeability change than for another; and that the changes in permeability give rise to the electrical changes through the generation of diffusion potentials. It is pointed out that permeability changes may also give rise to phase boundary potentials, although no data yet available demonstrate such potentials.

A remarkable aspect of the findings is that every ion in the milieu may play an important part in the events. Also, a much more specific picture of membrane structure appears possible in the light of current developments in molecular chemistry and physics. Promise of greater detail is seen in future systematic, quantitative studies of the action of ions and drugs on the permeabilities and permeability changes in unexcited and excited cells.

III. THE ACTION POTENTIAL, A

A. General principles

This section of the review is concerned with the "membrane" action potential, A, *i.e.*, the sequence of more or less rapid *changes* in the potential difference occurring simultaneously across the entire length of membrane of an excitable cell that begins with the familiar spike best known for its all-or-none properties under normal conditions. The spike is followed by other variations in membrane potential which may or may not be present depending on the type of fiber or on experimental conditions. An action potential generalized to include all fluctuations in membrane potential that may be present is shown in Fig. 1. The spike, S, composed of the collapse of the resting potential, E'_m, and a reversal of potential, S_m, is followed during recovery by the positive potential, PP. This is part of a damped oscillation that is shown superimposed on the much slower negative after-potential, NAP, which in turn is followed by the slow positive after-potential, PAP. The latter finally subsides to the original value of the resting membrane potential, E'_m. Henceforth, E'_m will refer to the resting potential just prior to the action potential, and E_m will be used in the more general sense as the transmembrane potential as a variable.

As in the case of the resting potential, one must keep in mind that the component "potentials" of the action potential represent *differences* of electrical

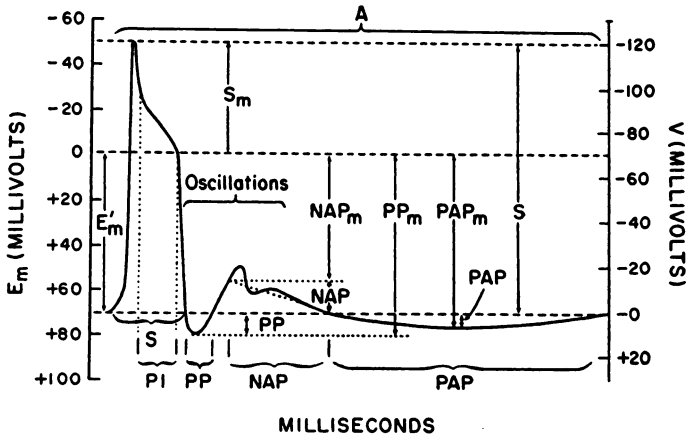

FIG. 1. A generalized action potential, A. The amplitudes are given either relative to the cell interior, which is taken as zero, hence are "transmembrane potentials" designated as E_m, or relative to the transmembrane potential just prior to the spike, E'_m, hence are *displacements* in potential, V. A decrease in E_m is given by a negative value of V; however, signs are usually disregarded and will be in the text. This applies to the negative after-potential, NAP, and the spike, S. The transmembrane reversal potential, S_m, is also negative, but its sign will also be disregarded. The abbreviations are the first letters of the various potentials, *e.g.*, PAP for positive after-potential, PP for positive potential, PI for plateau. The subscript "m" designates the transmembrane equivalent of the displacement potential.

potential. Only the "reversal potential" of the spike, S_m, is referred to the intracellular potential, which is taken as zero, and therefore constitutes a transmembrane potential. The others usually are measured relative to the initial value of E_m, E'_m, the sign being negative if E_m is decreased (as for S and NAP) and positive if E_m is increased (as for PP and PAP). However, analysis of the events occasionally requires consideration of the total transmembrane potential difference during these potentials. These will therefore be designated by the subscript "m," *e.g.*, $PP_m = E'_m + PP$; $NAP_m = E'_m - NAP$.

The terms increase and decrease will refer throughout only to the absolute magnitudes, not to the sign. For example, in some papers "decrease" refers to more negative values, hence when the reversal potential, S_m, increases in amplitude, it would be referred to as decreasing. Here it will be indicated as increasing under these circumstances.

The action potential depicted in Fig. 1 is considered to be that obtained under the ideal situation in which a microelectrode is in the protoplasm of a single excitable cell, perfectly sealed in by the cellular membrane, and the cellular membrane is uniformly and completely excited so that the entire surface undergoes the same electrical changes simultaneously. This is the idealized membrane action potential. As a result, adjacent regions of the membrane are at the same electrical potential at all times and no current flows parallel to the membrane through the external medium or through the protoplasm. Marmont (150) first achieved the equivalent of these conditions with giant fibers of the squid by using guard rings on either side of a segment of uniformly excited axons; with electronic feedback, the potential of the guard rings was made to vary as the enclosed segment, hence no current flowed laterally from the central segment to the adjacent guard rings. As a result, the voltage changes of the central segment, uncomplicated by propagation or by current flow from adjacent unexcited or repolarized regions, were obtained.

In the more usual experimental situation, one end of a nerve or muscle fiber is stimulated and develops a localized action potential which propagates away from the site of stimulation, the "propagated action potential." By virtue of the localized origin and propagation (conduction) of the action potential, electrical currents known as "local currents" flow longitudinally along the fiber, *entering* the active area through the surrounding medium from the as yet unexcited region ahead of the spike (Fig. 2) and from the repolarizing region behind it, and *leaving* the active area in the protoplasm for the adjacent regions. As a result, the tip of a microelectrode located in the protoplasm well ahead of the spike will record a decline in the potential difference (an "electrotonic potential") between the protoplasm and the surrounding medium before the spike actually begins to arise in its vicinity (*e.g.*, 219). This is because the charge on the membrane capacitance is withdrawn by the local currents and because the flow of local current in an outward direction through the membrane may set up a "passive" depolarizing R_mI drop across the membrane. An R_mI drop in the opposite direction may be expected within the spike itself, although this will be small because R_m is very much reduced during the spike (see Section IV A 2 g). The

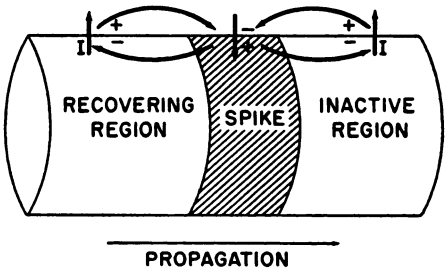

FIG. 2. Elements of a propagating spike. Curved arrows are the "local" currents, the straight arrows the associated transmembrane currents.

potential changes following the spike will also be modified somewhat depending on the extent to which repolarization has occurred and on the distance of the spike, which continues to function as a sink for drawing current.

Systematic observations have not been made of the difference between propagated and idealized membrane action potentials. A theoretical analysis of the difference is possible, but is exceedingly complex because detailed information is needed on the time course of R_m and of A and on the cable characteristics of the fiber. Hodgkin and Huxley have performed this calculation for the squid giant axon based on their analysis of the three processes involved in spike production (table 4 in 105) to be discussed later. They concluded that in the propagated action potential the amplitudes of the spike and the positive potential, and the duration of the depolarization phase of the spike, are less than 10% smaller than in an idealized spike, the duration of the repolarization phase of the spike is only 10% longer, and the duration of the positive phase only 2% longer. In giant axons, then, the error involved in studying the fast components of propagated instead of membrane action potentials is small. The error will be still smaller for the slower and later potentials, partly because so much of the fiber length (given by velocity of propagation multiplied by the duration of the potential) is occupied by them that long distances are involved. Tasaki (214, 477*I*) has noted that the spike amplitude at a node of Ranvier is not affected by activity of an adjacent node under usual experimental conditions.

The majority of observations to be described will be based on propagated action potentials. Current usage also designates these as membrane (transmembrane) action potentials when microelectrodes are employed; this is to distinguish the large, directly measured changes in membrane potential so obtained from the smaller change usually seen when both the recording and reference electrodes are outside but in different regions of individual fibers or of intact tissues. The techniques of extracellular recording, including the use of an active and an inactive region on a multifiber preparation to obtain the equivalent potential changes seen with intracellular recording but on a smaller voltage scale, are too well-known to require elaboration (*e.g.*, 59, 310*I*). It should be noted that, in the case of single myelinated fibers, methods involving air-drying of the internodes (479*I*), potentiometric balancing of potential differences (246*I*), or electronic feedback (155*I*) have been described which increase the effective resistance

of the internodal regions so that almost the whole action potential developed at the node of Ranvier can be obtained with external electrodes. It has been emphasized (*e.g.*, 477*I*, 479*I*) that unless such precautions are taken, the observer measures the potential difference arising in the extracellular fluid or in the input resistance of the amplifier as a result of current flow ("local currents") induced in them by the action potential. Indeed, until recently improved techniques permitted the use of high input resistance without the distortion caused by the input capacitance of amplifiers, low input resistances were deliberately employed with single myelinated fibers to minimize such distortion. As a result, potential differences measured during activity were in direct proportion to the extracellular current flow; these have been usually referred to as action currents (*e.g.*, 110, 477*I*), although the term "action potential" occasionally still is used for this type of recording. Since the time course is similar for both when recording is at the node of Ranvier (214, 479*I*), no distinction will be drawn here. When recording is done at other than the node in large myelinated fibers (110), the modifications of the time course of the spike are consistent with the overwhelming evidence for the development of the nerve impulse only at the nodes and hence for saltatory propagation (71, 457*I*, 477*I*).

It may be noted that the distinction between "myelinated" and "unmyelinated" nerves is arbitrary. Schmitt and his collaborators pointed out some time ago that the decrease of myelin relative to the protein with decreasing size of vertebrate fibers, as measured with polarized light, shows no sudden discontinuity (388*I*). All large "unmyelinated" fibers, under suitable optical conditions, give evidence of a "metatropic" or lipoidal sheath (388*I*). While the term "myelinated" has until now been used to designate the motor and sensory nerve fibers characterized by the presence of nodes of Ranvier, the adjective "nodose" seems preferable. The emphasis is then more properly placed on that feature of structure which underlies the localized origin of the action potential and its saltatory propagation as distinguished from the continuous propagation in skeletal and heart muscle and in invertebrate and smaller vertebrate fibers. All of these can be referred to as non-nodose fibers, but this term will be reserved here for the small vertebrate fibers alone.

We may note, too, that propagation is a complex process involving several factors. From Fig. 2 it can be seen that the intensity of the local current, given by the longitudinal arrows, which determines the magnitude of the current I crossing the membrane at the inactive region in advance of the active region, will depend on (a) the intensity of the reversal of potential (S_m) at the active site as well as on E'_m in the inactive region, since the two are additive, (b) on the resistance of the external medium, if the extracellular volume is limited, and (c) on the resistance of the axoplasm. Membrane resistance may also play a part if membrane capacitance is small, but this is likely to be a factor only in nodose fibers. As will be pointed out in Sections IV A 1 a and V A 3 b, the intensity of I and the magnitude of membrane capacitance determine the rate of discharge of E_m, and when the *change* in E_m, V, called a *prepotential*, attains a critical value, V_T, the spike begins to arise at the adjacent inactive site.

Under ordinary experimental conditions the parameters most likely to alter are E'_m, S_m, and V_T. Consequently, the mechanism of action of agents which block conduction cannot be understood until the effects on these three potentials are known and properly analyzed. Therefore, studies based on the development or reversal of conduction block must be carefully distinguished from the more direct studies at the site of stimulation. In the former case the transmission of the spike depends on its intensity and the threshold, whereas in the latter the intensity of the stimulus is not limited to the spike

amplitude and hence can be much greater, thereby permitting observations impossible in conduction studies (see 477*I*).

Studies of the faster components of the compound action potential of multifibered preparations are of limited usefulness and indeed may be a source of confusion. This is especially true of nerve trunks in which the individual fibers differ appreciably with respect to (a) their delay in response to a stimulus, (b) their velocities of propagation, (c) the site of origin of the action potential, and (d) the relative magnitudes and durations of the various components of their individual action potentials (*e.g.*, 59, 82).

For example, changes in the amplitude and configuration of the compound spike must be considered as possibly due (a) to changes in conduction velocity if observation is at a point other than the site of stimulation and (b) to changes in the range of the latent periods. If the former or the latter increases, the compound spike will be wider and the amplitude smaller because of less complete summation; its rate of rise and fall also will be slower. Obviously, information concerning the behavior of individual spikes cannot be drawn with great certainty, although the significance of effects might be more properly evaluated by studying both the area and amplitude of the compound spike in and beyond a treated area (*cf.* 525*I*).

It was pointed out some time ago (406*I*) that the small but long compound spikes observed in crustacean leg nerves were consistent with the large spikes obtained with the individual fibers (*e.g.*, 101, 217*I*, 228*I*) when allowance was made for the very limited summation of the component spikes. Recently attention was called to an erroneous report of an increase in spike amplitude with cooling which was really due to improved summation by prolongation of individual spikes (175). This possibility should be explored as an explanation of the increase in compound spikes with X-ray treatment (12).

The description of the rapid components of the action potential to follow therefore will be limited largely to studies on single fibers. Data on after-potentials, which because of their long durations are less susceptible to the extraneous factors that have been described, will be drawn from studies on multifiber preparations as well.

B. The spike, S, and factors modifying it

1. General configuration. From Fig. 1 we see that the several features of the spike susceptible to measurement are the rising (depolarizing) phase (expressed as duration or maximum rate of rise), the spike amplitude (expressible as the total, S, or in terms of the individual components, *viz.*, the resting potential and the reversal potential), and the falling (repolarizing) phase, expressed as duration or maximum rate of decline or both when it is uncomplicated by a distinct "plateau". The latter term is applied to the delay in repolarization normally present in some cells (*e.g.*, 89) or brought about by certain drugs. The height and duration of the plateau then become additional parameters susceptible to measurement.

The plateau in all its possible relationships to the total amplitude is most clearly seen in fibers from different parts of the heart, or from the same part of the heart but from different species (21, 37, 57*I*, 89, 512*I*). It can be accentuated

in heart fibers by low temperature (38, 494*I*, 499*I*) and by slowing the rate of beating (493*I*, 499*I*, 523*I*).

The last observation demands that the rate be kept constant if a proper evaluation is to be made of the action of experimental conditions on the spike. Failure to exercise such control (which may be obtained by stimulating at above the natural frequency) accounts for a number of discrepancies in the literature. Reports which fail to indicate whether spontaneous beating was constant or the rate controlled therefore are of questionable significance for careful evaluation of effects. Thus, Trautwein and Witt (225) find that with lowering of the calcium content of the medium the rate of frog hearts increases and the duration of the plateau shortens, while addition of strophanthin restores both characteristics to normal; the effect on the plateau may therefore be attributable at least in part to change in heart rate.

The plateau may arise from the peak of the spike, or at a lower level as shown in the figure; it may sometimes show a distinct rising phase too large to be an artifact (494*I*); and it may be well sustained and then decline suddenly or show a rapid fall from the start so that it almost fuses with the repolarization wave from the peak of spike.

The decline of the nodal spike frequently resembles the plateau in heart fibers (214). High hydrostatic pressure (207), repetitive stimulation (206), and hypertonic solutions (93, 187, 326*I*, 458*I*, 477*I*) accentuate this plateau. Certain quaternary ammonium compounds, such as tetraethylammonium chloride (TEA) prolong the declining phase in skeletal muscle (84), in crab nerve fibers (25), and when injected into giant axons (218); they have an especially striking effect in increasing as well as in prolonging the spike in crustacean muscle fibers (123*I*).

Thus, prolongation of the spike to give a plateau seems possible in all fibers. The extent to which this plateau represents an analogous or homologous situation in the different cells will be considered after additional facts have been presented.

A general feature of the spike is that its amplitude exceeds the resting potential, a phenomenon discovered when measurements of both E'_m and S with intracellular electrodes became practicable, *viz.*, with the giant axon of the squid (70*I*, 100, 101). The amplitude of S is independent of the diffusion potential at microelectrode tips, but the relative proportions of the reversal potential and resting potential are not. Adrian found that KCl-filled micropipettes may give rise to serious diffusion potentials due to factors such as glass charge or protoplasmic plugs at the tips (2*I*, 200), thereby causing readings of E'_m to be too high or too low, especially the latter; consequently estimates of S_m may be too large. Nevertheless, there can be no question of the near-universality of the reversal of membrane potential at the height of the spike. Summaries of the relative magnitudes of E'_m and S of vertebrate and invertebrate nerve and muscle fibers, including vertebrate heart fibers, are given in table 1 in (219*I*), in table 2 in (246*I*), in table 1 in (42), and in table 1 in (512*I*). The only exceptions to the rule of spike reversal are to be found in smooth muscle (25*I*, 26*I*, 27*I*, 514*I*, 520*I*), although occasional reversals have been noted (521*I*), and in crustacean muscle, in which reversal is obtainable by substitution of choline or quaternary am-

monium cations for sodium in the medium (123*I*). The small size of smooth muscle fibers may make them more susceptible to damage by microelectrodes, a possibility suggested by the low values of E'_m in these preparations (Part I); the need for exceedingly fine tips may also have contributed to protoplasmic plugs in them, for Bülbring and Hooton (27*I*) note that the potential frequently did not drop to zero upon return of the microelectrodes from the fibers to the surrounding medium. Systematic checks of this kind should be used to reject values obtained with plugged electrodes. Still another check, necessary to ascertain the significance of the microelectrode measurements, is that on membrane resistance, chiefly to assure that sufficient "sealing-in" of the microelectrode occurs to prevent (a) shunting of the voltages by electrical leakage through the site of puncture (*cf.* 81*I*) and (b) the running down of electrochemical gradients. In the presentation which immediately follows, discussion will be limited to vertebrate and invertebrate nerve fibers and to vertebrate fast (twitch) skeletal muscle and heart fibers.

2. Membrane potential (E_m) and extracellular potassium. From Fig. 1 it is evident that, since the spike amplitude reflects collapse of E'_m as well as reversal of the membrane potential (a single continuous process in all fibers to be discussed), changes in E'_m must of necessity be reflected by at least equal *absolute* changes in the amplitude of the spike. Since many of the experimental conditions to be described alter E'_m, their effects on spike amplitude could be due solely to this; if the absolute change in S exceeds that in E'_m, the experimental agent or the change in E'_m is exhibiting a more specific effect on S.

E'_m may be altered (a) by varying the extracellular potassium concentration, $[K]_o$, (b) by passage of current across the membrane, preferably by changing the potential of an internal electrode running the length of the fiber relative to that of the external solution, and (c) by a variety of conditions which depolarize (*e.g.*, metabolic inhibition, drugs).

Changes in E'_m either electrically or with potassium appear to be equivalent in their effects on the initial part of the spike. Thus, in the squid giant axon, the maximum increase in membrane conductance which occurs during the beginning of the spike is modified by potassium to the degree expected from electrical alterations of E'_m (83). In heart fibers, the spike amplitude and the rate of depolarization during spike development are the same function of E'_m when the latter is altered electrically regardless of an increase of $[K]_o$ by 3- or 5-fold (227, figure 29 in 512*I*).

In general, the decrease in spike amplitude with prior membrane depolarization (E'_m decreased) exceeds that of E'_m alone, which implies that the reversal potential declines; with still larger initial depolarizations (about $\frac{1}{4}$–$\frac{1}{3}$ E'_m) S becomes less than E'_m. These effects require milliseconds to develop (*cf.* 33, 129, 214). They appear with smaller decrements of E'_m in fibers in which the resting potential is normally low, *viz.*, squid giant axons (70*I*, 158*I*, 222*I*) and nodose fibers (53, 117, 210, 221) in contrast to skeletal (16, 92*I*) and heart (Purkinje) (227) muscle fibers (see Part I, Tables 1, p. 71, and 2, p. 72). Conversely, the same studies show that the spike increase with hyperpolarization

exceeds that of E'_m only to a limited extent and only in the fibers with smaller initial resting potentials (see also 210, 247*I*, 393*I*, 481*I*); beyond this range of hyperpolarization S and E'_m will increase only by the amount E'_m increases. With the procedure employed by Schoepfle and Erlanger (195) on nodose fibers, the increase in spike height with hyperpolarization includes the increment in E'_m, whereas the method of stimulation of Tasaki and his associates (477*I*) in effect subtracts this increment in E'_m; consequently, the apparent difference in their results is only one of technique.

Thus, the decline in spike height with a decrease in E'_m involves more than the latter. Studies on whole nerve trunks supplement this, for they have shown that block associated with depolarization (*e.g.*, during action of K^+, Rb^+, drugs, metabolic inhibitors) occurs when the injury potential falls to about ⅔ its original level and can be counteracted in the continued presence of the depolarizing conditions by restoring E'_m electrically or with drugs (see Sections 8 and 9 below).

The rate of depolarization (the rising phase of the spike) is affected by changes in E'_m in the same general direction as the amplitude of the spike, but usually proportionately more. It has been observed to exhibit a maximum for a value of E'_m slightly below the normal resting potential in Purkinje fibers (227) and is not further increased in frog muscle by hyperpolarization above the normal resting level (92*I*). The many available observations on nodal spikes are in general agreement on the effectiveness of depolarization in slowing the development of the spike (53, 117, 210, 221, 326*I*).

Electrical depolarization does not exert its full effect on spike amplitude and on the rate of depolarization immediately but rather after many milliseconds (33, 53, 71, 326*I*). Similarly, the increase in nodal spike height with electrical hyperpolarization takes substantially longer than the hyperpolarization itself (393*I*). These phenomena will be shown later to reflect the kinetics of "inactivation" or of the subsidence of inactivation ("reactivation").

Repolarization, following the peak of the spike, responds differently to changes in E'_m depending on the excitable cell involved; moreover, the effect of an electrically induced change in E'_m can differ from that brought about by altering $[K]_o$. Anodal polarization (hyperpolarization) applied to a node of Ranvier, whether before or after the start of the spike, hastens recovery (129, 214, 221); indeed, a sufficiently intense, brief anodal shock can terminate the spike abruptly (214, 326*I*, 458*I*), a phenomenon also observed during the very much longer plateau of Purkinje fibers (226) and of tetraethylammonium-treated squid axons (218). Repolarization in skeletal muscle (16, 92*I*) and squid axon (160*I*) is not appreciably affected by elevation of E'_m. Repolarization of frog ventricular fibers is prolonged by reduction in $[K]_o$ (18).

Cathodal polarization (depolarization) prolongs repolarization during the nodal spike whether the current is applied before or during the spike; the gradual fall followed by the sudden termination of the spike is replaced by a more continuous graduated decline (129, 214, 221). Repolarization of skeletal muscle (16, 92*I*) and squid axon (160*I*) appears to be unaffected by mild depolarization.

The prominent plateau of Purkinje fibers is unaffected by weak depolarizing (outward) current except as might be expected from the R_mI drop due to the large R_m during the plateau (figure 22 in 512*I*) (see Section IV E 1). However, reduction of E'_m by raising $[K]_o$ before (18) or during (228, 512*I*) the spike hastens the termination of the plateau of frog and turtle ventricles and Purkinje fibers, an effect which cannot be reversed by hyperpolarizing current (230). In this particular case, therefore, a more specific effect of potassium than its action via E_m appears to be involved, in contrast to the situation with respect to the amplitude and rise time of the spike.

3. Sodium and sodium substitutes. The critical importance of the sodium ion in spike generation was recently emphasized by Hodgkin and Katz (221*I*). Their finding of the dependence of the rate of rise and amplitude on extracellular sodium concentration in the squid giant axon has since been verified for the squid axon (28, 83), for skeletal (344*I*, 438*I*) and heart muscle (96*I*) fibers, and for nodose (247*I*) and crab nerve fibers (25). Earlier studies had demonstrated the need for sodium at the node of Ranvier (*e.g.*, 57). This dependence is approximately a logarithmic function of $[Na]_o$ for concentrations below normal and slightly in excess of normal, the slope approaching 58 mV for a ten-fold change in $[Na]_o$. In solutions made strongly hypertonic with NaCl, nodose fibers produce only a highly transitory increase in spike amplitude (93, 326*I*, 458*I*), the secondary decline being accompanied by an increase in duration (see also 213); in muscle the spikes are little changed in amplitude or duration (438*I*).

The greater duration, but not the increase in amplitude of the nodal spike, is apparently a function simply of hypertonicity rather than of the NaCl, for the same effect is obtained with excess sucrose added to Ringer (459*I*). The secondary decline in spike amplitude may be due to a rise in $[Na]_i$ resulting from sodium penetration. This is a definite possibility in the light of the evidence for NaCl movement into and out of nerve and muscle fibers in proportion to $[NaCl]_o$ (Part I) and for the lowering of spike amplitude when $[Na]_i$ is increased by injection or by stimulation of squid axons (186*I*, 227*I*, 419*I*). Increase of $[Na]_i$ in skeletal muscle also reduces spike amplitude reversibly (92*I*). Failure of solutions made hypertonic with NaCl to increase the spike in muscle is attributable to the rise in $[Na]_i$ due to withdrawal of water and to the entry of NaCl.

Na^+ generally is replaced with $choline^+$, leaving the Cl^- level unchanged, or with sucrose, thereby reducing Cl^- correspondingly. As pointed out in Part I, E'_m usually is raised under these conditions. Hence, the fall in S cannot be due to a decrease in E'_m. Rather, it must be due to a decline in the reversal potential, S_m, and, under more extreme conditions of sodium depletion, to the failure of E_m to collapse completely. This, then, is a result more specifically of dependence on Na^+ than on changes in E'_m (see Section 2 above). Nastuk and Hodgkin (344*I*) obtained the same effects of low $[Na]_o$ on muscle when the increase in E'_m in low $[Na]_o$ was prevented by raising $[K]_o$ slightly.

Substitutions for Na_o^+ were shown by Fatt and Katz (123*I*) to have complicated effects in the case of crustacean muscle. Partial sucrose or glucose replacement led to depolarization and to a slower rising phase; in a few instances in

which the rising phase lasted longer, the spike amplitude was either unchanged or even larger. Particularly striking was the finding that choline substitution, (and more so tetraethylammonium, TEA^+, substitution) augmented the rate of spike development as well as the amplitude and the duration of the spike. Relatively small quantities of these quaternary ammonium compounds (10% and 1% of isotonic strength, respectively) appreciably lengthened the spike duration and increased the amplitude.

Indeed, it appears likely that the rapid repolarization seen in normal sea water prevents attainment of the full spike amplitude (it will be recalled that in these preparations the spike is generally smaller than the resting potential), so that the delay in the falling phase brought about by these compounds may permit the initial depolarization to progress further, thereby increasing the spike even to the point of exceeding the resting potential. The stabilizer procaine also seems to act this way. In keeping with this proposal, lowering of the temperature also prolongs the spikes and increases their amplitude, as also seen in heart fibers (*59I*, *499I*) and squid axons (*222I*). Low temperature generally slows the rising phase as well (*e.g.*, *222I*, *499I*), in which respect it differs from choline and TEA^+ but resembles tetrabutylammonium (TBA^+) in its action on the spike of crab muscle fibers.

The studies on crab muscle indicate that changes in spike amplitude may reflect alterations in the rate of either depolarization or repolarization and that these may be altered either individually or together. The fact that choline and TEA^+ lengthen the duration of the spike and hasten the rising phase at higher concentrations may reflect a highly selective retardation of the recovery process, which normally is in operation during the initial part of the spike as well; TBA^+ and low temperature, on the other hand, appear to be less selective and retard the mechanism responsible for depolarization as well as that concerned with repolarization.

Fatt and Katz (*123I*) also provide important data on membrane conductance changes in crab muscle fibers. Thus, the sodium substitutes gradually depolarize and increase membrane resistance, as might be expected from a decrease in P_K.

An important consequence of the crab muscle studies is that they provide the most clear-cut demonstration of a system in which Na^+ can be dispensed with in the production of spikes. Thus, following TBA^+ treatment, spikes were obtainable in a solution containing sucrose instead of NaCl, with only the cations K^+, Ca^{++}, and Mg^{++} present.

Working with sheathed multifiber nerves, Lorente de Nó (141) noted that B and C fibers can conduct in sodium-free TEA^+ solutions; unlike invertebrate muscle fibers, however, these fibers were said not to continue to conduct impulses in choline$^+$ or tetramethylammonium (141). In preliminary notes Lorente de Nó and associates report that onium cations (*e.g.*, guanidinium) enhance the effectiveness of low sodium solutions (but do not act in the absence of sodium) in maintaining conduction of A fibers in frog spinal roots (135, 143).

Lüttgau has substantially clarified the action of guanidine hydrochoride (Gua^+) at the nodes of Ranvier (146a). He finds (a) that spike amplitude varies linearly as the logarithm of $[Gua]_o$ in sodium-free solution, the slope being the same as with $[Na]_o$, *viz.*, 58 mV/10-fold concentration change, but the ampli-

tudes lower by about 30 mV (choline$^+$ served as the substitution cation in both cases); (b) that the rate of rise of the spike is ⅕ as great in sodium-free guanidine solutions as in solutions containing Na^+ at the same concentration; (c) that when $[Na]_o$ is at normal levels, addition of guanidine hydrochloride (31 and 93 mM) reduces the rate of rise of the spike, whereas at low $[Na]_o$ (*e.g.*, at 10 mM and less) the same $[Gua]_o$ increases the rate of rise; and (d) in solutions in which Na^+ constitutes ½ the total cations and either choline$^+$ or Gua^+ the other half (besides K^+ and Ca^{++}), the maximum rate of rise obtainable with hyperpolarization is greater with choline$^+$, but the reduction of the rate of rise by previous depolarization is less with Gua^+ present. The significance of these findings is discussed later (Section IV B 1 b). Here we may note that Gua^+ functions like Na^+ but less effectively; and when both are present at high concentrations, the former interferes with the action of the latter.

Unpublished results from Weidmann's laboratory are reported to show that in Purkinje fibers spikes are not altered by choline chloride solutions whereas sucrose substitution has the usual effect (42).

Apparently, then, the role of Na^+ in relation to the repolarization process, as well as the initial part of the spike, can be assessed properly only if a substitute can be found which does not exert an effect of its own. We have seen above that for most fibers the equivalence of choline$^+$ and sucrose with respect to their action on the spike and its rising phase have sufficed to indicate no specific effect by either as a substitute for Na^+. This also suggests that choride ions are unimportant. In squid axons (221*I*), for which data are most complete, repolarization is delayed as $[Na]_o$ is lowered. The close parallelism between the rate of repolarization and spike amplitude as functions of $[Na]_o$ (table 4 in 221*I*) indicates that the rate of recovery may be dependent on spike amplitude, as controlled by Na^+, rather than on sodium directly. This will be seen later to follow from the dependence of a delayed increase in P_K on spike amplitude. The prolongation of the repolarization phase of nodal spikes by depolarization, which also reduces S, and the reverse by hyperpolarization, noted previously, appears consistent with this view. Results with skeletal muscle are qualitatively similar to those with giant axons (344*I*). We have already noted that the prolongation of nodal spikes by Ringer solution rendered hypertonic with NaCl is not specifically related to Na^+ or Cl^- but rather to the tonicity.

With respect to duration the plateau of heart fibers is differently affected by low $[Na]_o$. Thus, in addition to the usual decrease in spike amplitude, repolarization is greatly accelerated as $[Na]_o$ is decreased (18, 96*I*).

4. Calcium. In nodose fibers (72) and in muscle (118, 250*I*) the rate of rise of the spike is reduced by elevated $[Ca]_o$. Excess calcium (405*I*) increases the amplitude of the spike of squid axons but reduces it at nodes of Ranvier (116). In Purkinje fibers at normal and especially at higher membrane potentials a 4-fold increase in $[Ca]_o$ has an insignificant effect on the rate of rise, but at lower initial membrane potentials it augments the rate as if E'_m had been increased, although E'_m is not markedly affected (511*I*). These results indicate that the effects of calcium are not indirectly by way of its alteration of E'_m and yet can

duplicate those of E'_m. Also, the level of E'_m may be of importance in the action of calcium; it will be noted later that the relative association constant between the membranes and Ca^{++} probably is a factor (Section IV F 1).

In muscle, $\frac{1}{20}$ the normal $[Ca]_o$ does not affect spike height as long as E'_m is not lowered (26a*I*). Complete removal of calcium can cause rapid failure of spike production in squid axons (157*I*, 424*I*), at nodes of Ranvier (72), and in muscle (118). At the node trace amounts of the alkaline earth suffice to prevent the rapid failure, but amplitude and rate of rise are reduced (72). This effect may be progressive (because of increase in $[Na]_i$?) (72, 116). Thus, calcium appears to be essential for spike production, although sufficient elevation of E'_m (by passage of current across the membrane) can compensate for a deficit in $[Ca]_o$ even though the calcium deficit does not appreciably affect E'_m (72, 511*I*). Later this will be related to inactivation. Despite their strikingly different characteristics, crab muscle fibers resemble other excitable cells in being unable to generate spikes in the absence of extracellular calcium (123*I*).

In Purkinje fibers the duration of the plateau is reported to be little affected by increased $[Ca]_o$ (511*I*), but this may be due to uncontrolled frequency (55a). In mammalian auricular and ventricular fibers it causes an initial more rapid recovery followed by a later delay resembling a negative after-potential (figures 75 and 76 in 21); depletion of $[Ca]_o$ which leaves E'_m little changed markedly prolongs the plateau in these fibers (figures 74 and 76 in 21). Other differences in response to $[Ca]_o$ have been reported for cardiac fibers (42); however, these may be the consequence of uncontrolled heart rate. Recovery during the nodal spike is affected in exactly the opposite fashion by changes in $[Ca]_o$ (72).

Barium lengthens the plateau of frog ventricular fibers (128), an effect which might be anticipated from the decreased P_K suggested by the decline in G_m and by the depolarization at nodes of Ranvier and in whole nerve (314*I*, 324*I*) (see Section VI A 2).

5. Other stabilizers. It will be recalled that this classification includes agents (*e.g.*, local anesthetics, antihistaminics, physostigmine, TEA) which affect E'_m either not at all or increase it slightly but reduce or slow *changes* in E_m brought about by alterations in the ionic composition of the milieu, by drugs, or by metabolic inhibition (Part I). In these respects Ca^{++} acts as a stabilizer. The change in membrane potential that constitutes the spike is also depressed and, in fact, until the extension of the concept of stabilization recently, conduction block in whole nerve without prior depolarization was the sole basis for the classification of stabilizers; it was first utilized by Bishop (13*I*) to distinguish such agents from those which block while producing a depolarization. The recent studies have extended the list to include the higher alcohols, additional local anesthetics, antihistaminics, compounds not antihistaminics but related to diphenhydramine (Benadryl), and anticholinesterases such as physostigmine and diisopropylfluorophosphate (DFP) (13*I*, 14, 67*I*, 69*I*, 360*I*, 491a*I*). Calcium differs from these in that, at lower concentrations at least, and in certain preparations, it improves the rising phase of the spike.

Tasaki and his associates have studied the effect of urethane on the nodal

spike in detail. The rate of rise, the amplitude, and the duration are quickly reduced by urethane; the altered duration is due not to a marked change in the rate of repolarization, which is essentially unchanged, but rather to the smaller spike amplitude, as a result of which the repolarization wave reaches the baseline sooner (220, 221, 223). The spike amplitude decreases rapidly with increase in urethane concentration (220, 223). Similar effects on amplitude and duration have been described for cocaine (481*I*). It is of interest that ultraviolet light, at a wavelength of 265 or 280 mμ and at the proper intensity, acts for short periods at the node of Ranvier like a stabilizer, *viz.*, although the resting potential (and membrane resistance) remains constant the rate of rise and amplitude of the spike are reduced (327*I*).

Thesleff (488*I*) finds that in frog muscle fibers a wide variety of blocking agents, including urethane, chloralose, and paraldehyde, slow the rate of rise, the amplitude, and the rate of recovery of the spike. Some, like urethane, decreased the resting membrane resistance; others, like chloralose and paraldehyde, increased it; but E'_m usually was changed but little. It is noteworthy that the modifications in the spike are similar to those brought about by reducing $[Na]_o$.

In frog and guinea pig ventricular fibers and mammalian Purkinje fibers, stabilizers (*e.g.*, quinidine, procaine, cocaine, procaine amide, diphenhydramine) reduce the rate of rise and amplitude of the spike with little change in the maximum diastolic membrane potential (89, 254*I*, 511*I*). Weidmann (511*I*) reports that elevation of E'_m by passage of current through the membrane can in large measure restore the initial rate and amplitude. Some shortening of the plateau in Purkinje fibers is seen with procaine (511*I*). Frog and guinea pig ventricular fibers and rat atrial fibers have their plateaus lengthened by cocaine, procaine, procaine amide, and quinidine (89, 254*I*, 508*I*). Thus, the action of stabilizers is consistent in slowing the rising phase and reducing the amplitude of the spike, but variable on spike duration.

6. Quaternary ammonium cations. It was noted in Section 3 above that crustacean muscle responds to low concentrations of quaternary ammonium compounds by a lengthening of the spike, a result not specific for these since procaine has the same effect. In these fibers the presence of Na^+ or the organic cations was found to be unnecessary for the generation of the spike, a result differing from that obtained with all other fibers which have been studied. Other fibers differ, too, in that fewer organic cations can replace sodium and even these may do so less completely. The most prominent effect in invertebrate nerve (25) and in skeletal muscle (84) is an appreciable slowing of repolarization (prolongation of the spike) when even low concentrations of TEA^+ replace Na^+. In the nerve fibers the spike amplitude is not any higher when TEA^+ rather than $choline^+$ replaces a major part of extracellular Na^+, while in muscle a somewhat larger spike is obtained with TEA^+.

The observation that TEA^+ in the surrounding sea water exerts no action on the spike of the squid axon but greatly prolongs it when injected (218) suggests failure of this compound to reach the site involved in spike production, possibly

because of an extramembranal boundary impermeable to it. The failure of DDT to affect the giant axon of the squid despite its marked effects on other invertebrate nerve fibers (406*I*) was previously considered suggestive of such an extramembranal boundary (405*I*).

7. Labilizers. The effects of low calcium, which is a condition classified in Part I as labilizing, have been described in the section on calcium. Veratrine and its component alkaloids, cevadine and veratridine, have not been carefully studied on the spike proper as compared with the slow, usually smaller component of the action potential known as the negative after-potential. The latter will be discussed later.

No data are available on the effects of the veratrine alkaloids on the rising phase. Spikes at nodes of Ranvier (229*I*) and from squid axons (203) have been reported to be slightly reduced in amplitude by low concentrations of these alkaloids, but since washing of giant axons restores the spike height (203), there is reason to suspect that potassium leakage, such as occurs in veratrine (411*I* 414*I*), leads to potassium accumulation around the fibers that is responsible for the reduction of the spike. In mammalian ventricular fibers veratrine has a slight or no effect on spike amplitude (figure 67 in 21; 154).

Spikes fail to be produced in nerve trunks subjected to the higher concentrations of veratrine which depolarize (148*I*, 310*I*), but this is probably due to the decline in E'_m, for improved maintenance or restoration of spike production occurs under conditions that restore E'_m, *viz.*, anodal (inwardly directed) current (148*I*, 310*I*), local anesthetics (148*I*, 413*I*), and CO_2 (310*I*). The temporary recovery of nerve blocked by veratrine upon application of another blocking agent such as a local anesthetic (148*I*) is a particularly striking example of the importance of E'_m in spike production.

The repolarization phase in all fibers but the heart is not markedly affected by the veratrine alkaloids except close to the end of the repolarization wave, when the relatively small, long, negative after-potential becomes apparent (giant axons: 405*I*, 415*I*; nodes of Ranvier: 229*I*; skeletal muscle: 131, 285*I*). In mammalian ventricle, continued exposure to veratrine leads to a progressive increase in the negative after-potential from the typical small size and upward concavity seen in other fibers, to an amplitude exceeding 50% of the resting (maximum diastolic) potential; as it attains the larger value, it no longer declines smoothly and continuously but rather tends to flatten off with the membrane still partly depolarized and then terminates suddenly in typical plateau fashion (figure 67 in 21).

The behavior of the spike in heart fibers after long exposure to veratrine suggests that the normal resting membrane potential and another smaller potential represent two possible equilibrium values (metastable states) towards which the membrane potential tends to settle when it declines gradually, an effect frequently seen in untreated cardiac fibers. Tasaki has called attention to this in analyzing the action potentials at the node of Ranvier (214) and in TEA-injected squid axons (218), which exhibit similar two-stage recovery (see also 226). Crab muscle fibers treated with quaternary ammonium compounds exhibit the same characteristic decline of the spike (123*I*).

Both low calcium and long exposure to veratrine lengthen the plateau in heart fibers, but the much more prolonged action of veratrine suggests an important difference. Of considerable importance from the standpoint of the mechanisms underlying the plateau and of drug action is the finding that in TEA-treated muscle fibers veratrine also produces a long plateau, as in heart fibers (84). The significance of this is discussed in Section IV E.

8. Metabolic inhibition. Since most early studies on the effects of inhibition were carried out in moist chambers or with sheathed nerves (*e.g.*, 310*I*, 524*I*), potassium accumulation in the interstitial spaces resulting from potassium leakage undoubtedly contributed to the decline in E_m (Part I) and in S. This conclusion is supported by restoration and maintenance of conduction (a) by the washing of nerves under the same inhibitory conditions (*e.g.*, oxygen lack, iodoacetate poisoning) (6*I*, 11, 125*I*, 128*I*, 411*I*, 412*I*), (b) by anodal (hyperpolarizing) current, which restores E'_m despite oxygen lack or the presence of many inhibitors (*e.g.*, cyanide, fluoride, and iodoacetate), even in combination (148*I*, 309*I*, 310*I*), and (c) by drugs, such as local anesthetics at low concentration, and by substrates which also slow the potassium leakage and accumulation (125*I*, 403*I*, 411*I*, 413*I*, 414*I*).

In washed, desheathed toad nerves some depolarization still occurs with anoxia although potassium accumulation in the interstitial spaces is now probably negligible; conditions which prevent this depolarization also block (cocaine, low $[Na]_o$) (Part I and 425*I*). Consequently the failure of propagation in these preparations as well as in single fibers (5*I*) cannot be divorced from the decline in E'_m, although a rise in $[Na]_i$ may also contribute to the failure of the spike.

In washed giant axons E'_m does not decline appreciably in dinitrophenol, cyanide, or azide at concentrations which block metabolically-dependent transport markedly; the spike, also, is little affected (224*I*, 225*I*).

In mammalian muscle fibers ischemia causes the amplitude as well as rate of rise of the spike to fall concomitantly with E'_m; the spike characteristics decline somewhat faster than E'_m (500*I*). In these experiments potassium accumulation in the interstitial spaces may well have been a factor.

Many instances were noted in Part I in which "specific inhibitors" exhibited non-specific stabilization. Here we may note several instances of metabolic inhibitors which may be acting as stabilizers rather than as inhibitors. Thus, methylfluoroacetate, at concentrations which appreciably depress respiration of nerve trunks, either leaves the resting potential unaltered or increases it, depresses spike production, and decreases excitability (17*I*); the homology to local anesthetics such as procaine (Part I) is further indicated by the dependence of fluoroacetate effectiveness on its lipid soluble form (*i.e.*, the methyl ester) and its ineffectiveness as the sodium salt (17*I*). Oxythiamin also exerts effects suggestive of stabilization (290*I*). Brink (20*I*) has suggested that chloretone may also exhibit stabilizer action. Further exploration of whether these agents act by non-specific stabilization appears desirable before more elaborate explanations can be considered.

It will be recalled that, in addition to a gradual decline in $[K]_i$, metabolic inhibition causes an uptake of Na^+ (Part I). Thus, an important increase in

$[Na]_i$ may take place before $[K]_i$ has decreased sufficiently to lower E'_m appreciably. In Section 3 above, it was pointed out that an increase in $[Na]_i$ when $[Na]_o$ is unchanged decreases spike amplitude. Thus, spike production could be altered by metabolic inhibition before E'_m is changed appreciably. Such action would be progressive with time rather than rapid, and recovery also would be slow upon cessation of inhibition, as observed in mammalian heart fibers subjected to excessively low temperatures (63*I*, 497*I*).

In rat atrial fibers anoxia and dinitrophenol leave the resting potential unaltered for 17 min whereas spike amplitude and especially the duration of the plateau decline appreciably (508*I*). Similar results have been described for spikes from heart fibers of the frog (148) and of the cat (495*I*). Trautwein and his associates (495*I*, 496*I*) stress the outstanding sensitivity of the plateau duration to anoxia. They observed an early membrane depolarization of about 10 mV which was maintained thereafter during anoxia; the decrease in spike amplitude had a similar time course but was larger; plateau duration fell continuously. Return to oxygen caused recovery of all characteristics to levels above the original ones, this overshoot being most marked for the duration of the plateau, which developed a clear-cut rise phase of its own.

9. Interactions of ions, drugs, and E'_m in conduction studies. Transmission of the impulse along a fiber has been pointed out to depend on the effectiveness of the spike in activating a region ahead of it, a process dependent on its amplitude and on the "threshold", which is inversely related to the sensitivity of the region ahead (Section A above; see also 95 and 477*I*). Later, evidence will be presented that both the spike and threshold depend on similar processes, hence no great error is introduced by considering the relationships noted in studies of propagation as indicative of the behavior of the spike mechanism itself.

Recent studies are chiefly on multifiber preparations. However, fifteen years ago Takeuchi and Tasaki (210) passed current along a single fiber such that urethane-treated nodes of Ranvier were anodally polarized while untreated nodes beyond were depolarized by the cathode; under these conditions conduction block by the urethane was relieved, not because of the appearance of spikes in the urethane-treated nodes but rather because of the hyperirritability of the nodes under the cathode. With weaker urethane an increase in spike amplitude was seen under the anode which may have improved propagation. Schoepfle (394*I*) now finds that at thiopental (Pentothal) or procaine-blocked nodes the spike is restored in proportion to the intensity of anodal polarization.

Anodal currents of not too great intensity will relieve the block by n-hexanol or cocaine of 1 cm segments of sheathed frog nerve trunks (360*I*). A similar observation was reported earlier for tetracaine, but the effect was assumed to be due to the counteraction of a depolarization (148*I*). Weidmann (511*I*) has shown that single Purkinje fibers treated with a variety of local anesthetics regain a high rate of spike rise when their membranes are hyperpolarized.

Fleckenstein (148*I*) noted that, in general, the block by organic stabilizers (local anesthetics, antihistaminics), measured by the time needed to develop, was weakly augmented by hyperpolarization but greatly enhanced by de-

polarization. With calcium, on the other hand, the relative effectiveness of hyperpolarization and depolarization was reversed. This indicates an important difference between the alkaline earth and other stabilizers, which was also seen in their different effects on the spike (Section 5 above).

In desheathed frog nerve n-hexanol and cocaine are also counteracted by doubling the NaCl content of the Ringer solution, but not by similarly increasing tonicity and conductivity with choline chloride (360*I*). Crescitelli's studies (44, 45) with low concentrations of sodium and of stabilizers (Benadryl, amyl carbamate, cocaine, DFP, and physostigmine) revealed an interrelationship between blocking potency of low $[Na]_o$ and the stabilizers in 2-cm long segments of frog nerve: Stabilizer concentrations too low to affect conduction at normal levels caused block when the sodium level was reduced to a point which would otherwise have sufficed for conduction. The blocking effectiveness of elevated $[K]_o$ was likewise enhanced at low $[Na]_o$ and, conversely, that of low $[Na]_o$ augmented by raising $[K]_o$ (43).

10. Cardiac glycosides. These are of interest because one well-established effect they exert duplicates that of metabolic inhibition, *viz.*, interference with active transport, leading to loss of intracellular potassium and entry of sodium (Part I). Stutz *et al.* (472*I*) maintained a constant rate of beating of dog ventricular strips during application of digitalis. They found as a first event subsidence of the small peak of the spike preceding the plateau, then a shortening of the plateau, which they noted were comparable to the effects of lowering $[Na]_o$. The resting potential did not change. Other studies have appeared, but since rate of beating was not controlled, this may have led to contradictory results on the spike (56*I*, 145*I*, 225, 522*I*). Woodbury and Hecht (522*I*), working with frog ventricles, observed no effect on the rising phase but a marked shortening of the plateau; they note that MacLeod found that digitalis glycosides shorten the refractory period, which is closely related to spike duration (Section 12 below).

Dudel and Trautwein (55a), in an exceptionally careful study, controlled heart rate in papillary muscle and found that fibers in low $[Ca]_o$ underwent a rapid but slight lengthening of the plateau in low concentrations (2.5×10^{-7} w/v) of k-strophanthin with no effect on other characteristics except contractility. Ten-fold higher concentration caused a rapid shortening of the plateau, and a more gradual decline in spike and E'_m. In Purkinje fibers the spike was seen to decline in the glycoside without a change in E'_m; additional important observations were (a) that the rising phase of the spike in 10^{-6} strophanthin is half of that of the controls over a range of E'_m from 60 to 110 mV and (b) that the initial effect of the drug is to double R_m, although later both E'_m and R_m decline.

11. Cardiac transmitters. a. Acetylcholine. The most striking feature of acetylcholine (Ach) action at moderate concentrations or with moderate stimulation of the vagi is a marked hastening of the termination of the spike and hence a shortening of the plateau in auricular and pacemaker fibers; there is little effect on the spike amplitude when E'_m is not increased (29*I*, 231*I*, 245*I*, 255*I*, 507*I*). S and E'_m are usually increased when they are low to begin with (Part I). The slowing of the rising phase of the spike by quinidine has recently been reported to be counteracted by Ach (119), a result of possible relation to atrial fibrillation induced by Ach (26).

b. Epinephrine. When the rate of contraction of rat atrial fibers is kept constant, this amine, at low concentration (5×10^{-7} M), lengthens spike duration about 13% and spike amplitude is reduced slightly—by an amount equal to the 4.5% reduction in E'_m (507*I*). The shortening of spike duration observed by Fingl *et al.* (145*I*) is probably due to the higher rate of beating caused by epinephrine rather than to a direct effect; under these conditions of uncontrolled rate, the maximum membrane potential was not affected by the amine or by sympathetic stimulation (145*I*, 245*I*).

12. Refractory period. It has long been known that during most of the spike another spike cannot be evoked (absolutely refractory period) and that for a period thereafter (relatively refractory period) a shock greater than required for the first spike is necessary to initiate the second spike. Here we shall be concerned with the size and configuration of the spike during the refractory periods rather than with the shock strengths required. The latter are dealt with later in relation to the excitation process.

a. Absolutely refractory period. The failure of a second nodal spike to be initiated during the repolarization phase of a previously induced spike is not due to the exhaustion of the mechanisms concerned with spike generation. This was clearly shown by Tasaki (214) and Lüttgau (326*I*) when they succeeded in obliterating the repolarization phase of a spike, before it was normally completed, with a brief, strong anodal shock and then in immediately inducing a second spike with the usual cathodal pulse. An additional important observation was that, the longer the first spike was allowed to last, the smaller and shorter was the second spike, its magnitude approximating that at which the first spike was terminated. Hence, during the first spike, the mechanism of spike generation becomes progressively weaker so that by the time the spike terminates the node cannot produce a second spike for a brief period. The last might be considered a true absolutely refractory period.

Stimuli applied at increasing intervals following the end of the first spike reveal gradual recovery of the spike-generating mechanism, reflected in the progressively larger and longer spikes obtainable (214).

Similar studies on the possibility of restimulating following premature termination of the spike with an anodal pulse have not been carried out in detail in other fibers; but in view of evidence that the spike of the squid axon (151) and the plateau of Purkinje fibers (226) can be terminated as in nodose fibers, similar observations may be anticipated in these systems. We may note that the development of inexcitability during the spike appears to be identical with the corresponding changes during a depolarization and described in Section 2 above.

In general, there is a clear correlation between spike duration and the length of the absolutely refractory period (nodes: 477*I*; heart fibers: 21). Recently, exceptions have been reported. Thus, when the spike is prolonged at nodes by Ringer solution made hypertonic with NaCl (326*I*) or in cardiac fibers by veratrine (figure 67 in 21), the absolutely refractory period appears to terminate before the plateau of the spike. Tasaki and Hagiwara (218) suggest that in such

cases the second spike actually arises in another region of the fiber where repolarization has already occurred.

In studies on the heart it is not uncommon to use the maximum frequency of stimulation as an index of spike duration and of the absolutely refractory period (see 112 for references). In such measurements the decline of threshold to a point at which propagation can occur, rather than the duration of the spike, may be the controlling factor (477*I*).

b. Relatively refractory period. We have noted that during this interval, which follows the normal spike, the spike-generating mechanism gradually recovers. Such recovery is observed at the node of Ranvier (393*I*) and in Purkinje fibers (227) upon removal of a previously sustained electrical depolarization, which apparently is equivalent to the spike in causing inactivation; this recovery, as well as that following the spike, have been pointed out to have the same characteristics as the disappearance of "inactivation" in squid axons under "voltage-clamp" conditions (227, 326*I*, 393*I*) (Section IV A 1).

Urethane slows the rate of recovery of the spike height at the node of Ranvier (221, 223). Prolongation of subnormality is particularly marked in giant axons exposed to yohimbine (405*I*), which does not alter the resting potential when applied (Part I). Successive spikes, separated by intervals as long as several hundred msec, develop more slowly, attain lower amplitudes, and, in general, increasingly take on the appearance of spikes from fibers in low $[Na]_o$. A period of rest leads to recovery, and the sequence can be repeated many times. The small positive after-potentials (less than ½% of the injury potential) associated with these marked changes in spike amplitude (405*I*) cannot possibly be regarded as the cause but rather as an electrical sign of the mechanisms involved.

Similar observations on the effect of yohimbine on the prolonged refractory period have been reported for whole toad nerve and single muscle fibers (147, 435*I*). It is pointed out that the similarity of the alkaloid to low extracellular sodium is evident (a) from their lowering of spike amplitude and in the slowing of the rate of repolarization, (b) from the progressive depression of spikes with repetitive stimulation that occurs in both, (c) from the recovery of the spike in both as a result of anodal current, and (d) from the synergism between them. Quinidine has also been observed to cause prolonged subnormality in frog nerve (202).

In both squid axons and muscle fibers yohimbine differs from low $[Na]_o$ in that its effect is reversed but slowly. Shaw *et al.* (435*I*) assert that the rising phase in muscle spikes is unaffected by yohimbine, but this appears not to be the case in squid fibers (405*I*).

13. Recapitulation. a. Rate of rise and amplitude of the spike. In all but crab muscle fibers, the initial part of the spike has been found to be dependent on a high $[Na]_o$ and on a low $[Na]_i$. In some cases other cations may be able to replace Na_o^+. The rising phase is affected by stabilizers and by depolarization as though $[Na]_o$ had been lowered. In keeping with this (a) hyperpolarization or an increase in $[Na]_o$ may counteract stabilizers and (b) the effectiveness of

stabilizers is accentuated by a decrease in $[Na]_o$ and, conversely, the effect of low $[Na]_o$ is accentuated by low concentrations of stabilizers. During the relatively refractory period excitable fibers behave as though stabilized or treated with low $[Na]_o$; in keeping with this, stabilizers delay recovery during the relatively refractory period. These observations are in keeping with evidence that the spike normally arises by virtue of a rapid entry of sodium ions (Section IV).

Calcium differs from other stabilizers in having its blocking action profoundly accentuated by hyperpolarization and relieved by depolarization; moreover, the action of calcium resembles hyperpolarization and differs from that of other stabilizers in that, although the stimulus strength required to initiate the spike is increased, once the impulse is initiated it may develop more rapidly and to a larger amplitude. These results indicate that the role of calcium in spike generation is a more labile one than in the case of other stabilizers; moreover, this ion is of critical importance for spike generation in all systems that have been studied, including crab muscle fibers.

Available evidence is consistent with the view that the development of the spike is depressed by metabolic inhibition only insofar as (a) E'_m is decreased or (b) $[Na]_i$ is increased. Analytical data remain to be obtained simultaneously with spikes to verify the latter. Thus, the role of metabolism in spike production appears to be an indirect one, *viz.*, that of active transport, whereby the characteristic ionic gradients of the cells and the associated resting membrane potential are maintained (Part I).

b. Duration of the spike. Conditions which reduce the amplitude of the spike in general prolong its duration when a distinct plateau is not normally evident (giant axon, muscle fiber)—a result of a slower rate of repolarization. When a distinct plateau is evident as part of the normal spike, it undergoes changes which may be quite independent of the initial part of the spike. Thus, despite no change in spike amplitude, plateau duration may be prolonged at the node of Ranvier by repetitive activity, high hydrostatic pressure, low temperature, or hypertonic solutions, all of which slow the repolarization, so that the time at which the membrane potential rises to a critical value, at which it suddenly returns to the normal E'_m, is delayed. The shortening of the nodal spike by stabilizers and during the relatively refractory period is due not to a change in repolarization rate, which is little altered, but merely to the smaller spike amplitude from which recovery occurs.

The shortening of the plateau of spikes of cardiac fibers by potassium and low $[Na]_o$ is completely out of proportion to effects on spike amplitude. The difference between the initial and later parts of the spike is shown by the fact that restoration of E'_m electrically in the presence of elevated $[K]_o$ restores the rate of rise and peak amplitude of the spike whereas the small residual plateau is further shortened. This dissociation of the initial and later parts of the spike in cardiac fibers was also accomplished by a variety of agents or conditions that may affect only the plateau: Ach, digitalis, calcium, rapid heart rate, and metabolic inhibition, which shorten it, and epinephrine and low temperature, which lengthen it. No single factor can be pointed to as underlying the actions of these different conditions (see Section IV E).

Stabilizers, as a group, do not have a marked effect on the recovery phase of the spike. At the node of Ranvier the shortening in urethane is due primarily to the smaller spike amplitude rather than to a change in rate of recovery; in Purkinje fibers the plateaus were depressed, in other cardiac fibers they were slightly lengthened by a variety of local anesthetics. However, one special class of stabilizers—derivatives of quaternary ammonium such as TEA—exhibit a special propensity for prolonging the spike in all fibers where its action has been studied. The concomitant increase in spike amplitude which may occur cannot be divorced with certainty from the slowed repolarization, for analogy with the effects of temperature indicates that in such cases the faster repolarization prior to treatment limited the height attained by the spike. The possible relation of these results to P_K, which is depressed by TEA (Part I), will be discussed in Section IV E.

C. Factors modifying the positive potential, PP

1. Resting potential. The presence of a positive potential, PP, depends on the level of membrane potential. Thus, prior electrical depolarization of single skeletal muscle fibers, which normally do not exhibit PP, causes it to appear (16); conversely, in squid axons, which normally have a substantially lower E'_m and produce a positive potential, PP disappears when E'_m is elevated electrically to a critical level (160*I*). Similarly, nodose fibers with low membrane potentials by virtue of puncture with microelectrodes exhibit PP, which is suppressed with electrical hyperpolarization (55*I*). It will be recalled that E'_m is normally low compared to E_K in nodose fibers, but apparently not sufficiently to give rise to PP. The rate of subsidence of the positive potential also is augmented by prior depolarization, but this is due to oscillatory waves which follow (see "oscillations" below).

In rhythmic fibers of the heart (pacemaker, sinus, auricular, and Purkinje cells) the spike is followed by a sequence of electrical changes resembling a positive potential, that is, E_m rises immediately to a maximum and then declines gradually; the latter is referred to as the diastolic depolarization (21, 57*I*, 245*I*, 512*I*).

Records showing moderate depolarization of Purkinje fibers, *e.g.*, after cocaine (511*I*) or quinidine (35), reveal that PP is increased. This is by virtue of E'_m being lower, rather than the absolute transmembrane potential at the peak of PP, PP_m, being larger (see Fig. 1), as is the case in other excitable cells when E'_m is reduced electrically. Stronger depolarization by K^+ eliminates PP; Weidmann (511*I*) suggests that this is due to the shortened or absent plateau, for when the membrane is kept depolarized for a longer period by passage of current, sudden cessation of this depolarization then gives rise to a transitory hyperpolarization resembling PP. This effect is comparable to the transitory hyperpolarizations seen following cathodal depolarization in vertebrate nerve trunks (*e.g.*, 177) and in muscle fibers (30*I*) and which it will be recalled are attributable to the delay in the subsidence of P_K elevated during the depolarization (Part I). Hence, it appears that a sufficiently sustained depolarization in heart fibers also increases P_K.

Hyperpolarization of cardiac fibers by lowering of $[Na]_o$ reduces PP, but this is because E'_m is raised with little further increase in PP_m (96*I*), again as in the other excitable cells.

2. Extracellular potassium. Unlike the initial part of the spike, but like the plateau of cardiac fibers, PP is affected by $[K]_o$ in a manner different from that to be expected from the change in E'_m it produces. Thus, a decrease in $[K]_o$ produces only an increase in PP of the squid axon, despite the increase in E'_m, and an increase in $[K]_o$ substantially reduces PP although E'_m is reduced. These changes in positive potential are proportionately greater than in spike or resting potential (158*I*, 221*I*, 405*I*).

We may note that the specific effect of potassium is understandable as a consequence of the dependence of PP not on the absolute value of E'_m but on how much smaller E'_m is than E_K (the equilibrium potential for potassium, *i.e.*, that value of potential which is governed solely by the potassium ions, as described in Part I). Thus, the increase in E'_m electrically brings it closer to E_K, thereby reducing PP; the increase in $[K]_o$ reduces E_K more than E'_m, hence PP is decreased although E'_m is smaller. In Purkinje fibers excess $[K]_o$ also depresses PP (figure 29 in 512*I*), but systematic observations are not available.

3. Sodium. In squid axons, where lowering $[Na]_o$ does not change E'_m appreciably, PP is little changed (221*I*). It was noted in Section 1 above that in Purkinje fibers PP is reduced in low $[Na]_o$, but that this is due to E'_m being larger and hence closer to the peak value of absolute membrane potential attained during PP. This follows from closer proximity of E'_m to E_K.

4. Stabilizers. Increase of $[Ca]_o$ increases PP in the giant axon, and a decrease has an opposite effect (405*I*). This result is to be expected if an increase in P_K during the spike is greater when the spike is larger (Section IV A). In Purkinje fibers an increase in $[Ca]_o$ has no effect either on spike amplitude or on PP; as pointed out in Section 1 above, other stabilizers (quinidine, cocaine) affect PP only insofar as E'_m is lowered (511*I*).

5. Cardiac transmitters. The most prominent effects of acetylcholine and epinephrine are, respectively, slowing and acceleration of the development of the diastolic depolarization potential (230*I*), which underlies their effect in slowing and accelerating heart rate (21, 512*I*). In frog sinus fibers, in which it will be recalled E'_m normally is lower than in the auricles or ventricles (Part I), vagal stimulation during the plateau of the spike causes repolarization to be not only more rapid but to a higher level. Thus, an increase in P_K (by Ach) and an increase in G_K (by elevation of $[K]_o$) both terminate the spike more rapidly, the former carrying the membrane potential to a higher level, the latter to a lower level, in keeping with the value of E_K in the two cases.

D. Oscillations (and after-discharge)

The oscillation in membrane potential seen following the spike or the positive potential does not actually require the spike for its initiation. Negative, and under certain conditions positive, pulses of current, of intensities insufficient to induce a spike, can produce such oscillations (*e.g.*, figure 1 in 405*I*). The positive po-

tential itself appears to be a reaction to the spike in a manner similar to the positive wave following a cathodal pulse (405*I*); the probable identity of the two positive swings of potential is further indicated by the similar fashion in which they vary under a variety of conditions (*cf.* the cathodal break response and the "R_1" deflection as described by Lorente de Nó for frog nerve trunks in 310*I* and 311*I*).

Two conditions have long been known to favor oscillation of membrane potential, *viz.*, sustained cathodal depolarization (*e.g.*, in giant axons: 7, 9, 41*I*) and low $[Ca]_o$ (in giant axons: 6, 21*I*, 405*I*; lobster nerve fibers: 1). Both conditions have been pointed out to increase P_K (and probably P_{Na}) (Part I); their similarity of action suggests that cathodal depolarization may accomplish the same effect, *viz.*, the reduction of surface calcification, which normally "damps" or minimizes fluctuations in permeability (see Part I and Sections IV A, F, and G). Excitability fluctuations indicating a basic oscillatory behavior in other systems such as vertebrate nerve and skeletal muscle are discussed later. Here we may note that the spontaneous, rhythmic succession of compound spikes, decreasing in amplitude, (the "after-discharge"), which follows a single, brief, adequate stimulus under similar conditions also is indicative of an oscillatory process in vertebrate nerve trunks (138, 310*I*).

A reduction of $[K]_o$ increases the oscillations but may also reduce them (405*I*). The two opposing effects may be due to the hyperpolarization in low $[K]_o$, on the one hand, since electrical hyperpolarization suppresses oscillations (*e.g.*, 7), and to an increase in E_K, on the other, which, as in the case of the positive potential, would augment potential oscillations if changes in P_K are involved (Section IV A 2 d).

Veratrine, in keeping with its classification as a labilizer, also accentuates the oscillations in giant axons (405*I*). An interesting feature of the oscillations induced with this alkaloid mixture is their rapid increase in amplitude and decrease in damping (*i.e.*, decrease in the difference in amplitude of successive waves) when the fiber is driven so that it produces 9 to 50 impulses a second; such oscillations can grow to the point where they become adequate as stimuli and initiate spikes (405*I*). A similar phenomenon is reflected in the initial growth in the spontaneous repetitive response, *i.e.*, the after-discharge, during repetitive stimulation of veratrinized frog nerve (413*I*). This, as well as similar observations with phosphate, serve to indicate that after-discharge is an indication of oscillatory behavior. However, other factors may play a part in repetitive activity; they are considered later (Section VI A).

With yohimbine (405*I*) and cocaine (424*I*) the oscillatory response of squid axons to successive subthreshold shocks decreases. Although low $[Na]_o$ reduces the oscillations, it does not interfere with their augmentation with repeated shocks (424*I*).

The oscillatory characteristics of excitable cells, whether seen directly or reflected in spontaneous activity, are not necessarily a consequence of a depolarized state of the membranes. In low calcium, for example, depolarization may not occur or may be very small before or following stimulation (Part I, 19*I*).

The same has been described for DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane) in crab nerve (406*I*). Feng (60) noted the lack of a simple direct correspondence between after-discharge and the negative after-potential produced in veratrine-treated frog nerve.

A wide variety of compounds, including DDT, DDT analogs, pyrethrins, paradichlorbenzene, naphthalene, nicotine, and even quinoline and physostigmine, which at higher concentrations exhibit stabilizer properties in vertebrate nerve (Part I), give rise to after-discharge in insect (cockroach) and especially cray fish and crab leg nerve (513*I*); these studies give no information concerning E_m in the various drugs. As with veratrine and DDT (*e.g.*, 60, 406*I*, 407*I*, 413*I*), but unlike low $[Ca]_o$, these compounds do not *initiate* spontaneous discharges at lower concentrations but rather require at least one stimulus to set off a train of impulses, as might be expected of oscillations of membrane potential. However, the possibility must also be explored that the action may be like that of TEA, which induces repetitive activity by delaying the repolarization phase of the spike (Section VI A).

Depression of oscillations in squid axon is achieved by anodal hyperpolarization (7), by elevated $[Ca]_o$ (6, 405*I*), and by procaine and cocaine (405*I*, 424*I*), that is, by stabilizers or stabilizing conditions. Low $[Na]_o$, previously noted to act like a stabilizer (Part I), also depresses them (424*I*). After-discharges are reduced or prevented in vertebrate nerve by the same conditions, *viz.*, hyperpolarization (21*I*), increased $[Ca]_o$ (21*I*, 60, 413*I*), and anesthetics (413*I*). Calcium was also found to be effective against the many compounds active in arthropod leg nerve (179*I*). It is noteworthy that veratrine-induced after-discharges (and associated contraction) in curarized muscle are suppressed by low $[Na]_o$, physostigmine, DFP, and procaine (127), and quinine and quinidine (281*I*), all previously shown to have stabilizer properties by virtue of other effects (Part I); veratramine, which acts similarly (281*I*), may be expected to exhibit other characteristics of a stabilizer. In all these studies low concentrations—far lower than needed to block spike production or conduction—sufficed to depress or prevent after-discharge.

It is tempting to propose, as suggested by Gordon and Welsh (179*I*), that the after-discharges and oscillations are due to displacement of calcium from the sites where it exerts its stabilizing influence. The effectiveness of many other stabilizers in the absence of Ca_o^{++} indicates that the action of calcium in these phenomena either does not involve a highly specific interaction with the cell membrane, or that the same end effect can be accomplished by less specific interactions. The hyperpolarization may be of this non-specific nature or it may enhance calcification (Part I). The wide variety of agents causing after-discharge in arthropods, and the rapid reversibility of many of them, are interpreted as indicative of a non-specific, physical interaction with the membrane (513*I*). That physostigmine may cause after-discharge at low concentration and block (stabilize) at high concentration is reminiscent of the two-fold action of acetylcholine at junctions, *viz.*, depolarization and stabilization (Part I). This is discussed in detail later (Sections IV F and G).

E. Negative after-potential (NAP)

1. In the absence of veratrine or its alkaloids. The negative after-potential, which received considerable qualitative study in nerve trunks earlier (*e.g.*,

13, 59, 77, 78, 82, 310*I*, 311*I*), has more recently been shown to decline as a simple exponential in the squid giant axon (158*I*, 405*I*, 415*I*), in frog nerve (415*I*), and in muscle (69a). In nerve it clearly does not have a rising phase of its own; its apparent rise is due to the presence of the preceding positive potential (415*I*), a conclusion also reached by Lorente de Nó (310*I*, 311*I*) for frog nerve on the basis of his observations of what he designates the "R_1 deflection" (the P_1 of Gasser and Erlanger), which appears to correspond to the positive potential (see also 79).

The presence of NAP usually depends on E'_m being above a critical value, and its magnitude is governed by the amount E'_m exceeds this. Thus, in frog muscle fibers, which normally have a high value of E'_m (Part I) and a substantial negative after-potential (*e.g.*, 92*I*, 266*I*, 344*I*, 488*I*), prior depolarization of the membrane by cathodal current to a level equal to or less than that at which the repolarization wave suddenly slows down to produce NAP—about 75 mV (92*I*)—eliminates the negative after-potential (16, 69a); similarly, increase in E'_m by lowering $[K]_o$, leaves the potential at which NAP appears unchanged, with the result that NAP is increased by the amount E'_m is raised (92*I*, 69a). Single nodose fibers, with low values of E'_m by virtue of penetration with microelectrodes, lack a negative after-potential, and electrotonic hyperpolarization causes NAP to appear (55*I*). In squid axons hyperpolarization likewise causes the appearance of NAP at a critical value of E'_m somewhat higher than the maximum potential attained at the peak of PP when the resting potential is normal. In C fibers the hyperpolarization following repetitive stimulation also reveals that NAP appears at a particular value of E'_m, and continues to arise at this level as E'_m increases, so that NAP attains the magnitude by which E'_m exceeds the critical value (174). Similar relationships have been observed with vertebrate nerve trunks when E'_m is increased in numerous ways, *e.g.*, by anelectrotonus, CO_2, and post-anoxic repolarization (310*I*, 311*I*). Earlier observations on nerve trunks are also in keeping with these effects of polarization (*e.g.*, 79). A negative after-potential is not seen following a single spike in crab nerve (406*I*).

The negative after-potential is additive, *i.e.*, a rapid succession of spikes is accompanied by a progressive depolarization which attains a steady level governed by the rate of stimulation (*e.g.*, 158*I*, 310*I*). It must be recognized that the cumulative NAP is of at least two types: That due to accumulation of K around the fibers because of its release during each spike (*e.g.*, in crab nerve: 406*I*, 412*I*; in squid axon: 158*I*, 405*I*) and that due to another process, apparently involving changes in membrane permeability (Section IV C).

That a negative after-potential can result from potassium accumulation was first suggested for crab nerve and squid axon on the basis of the following: (a) the increment in $[K]_o$ in the interstitial space, calculated from the depolarization produced by repetitive stimulation, agreed with the actual amount of potassium liberated or anticipated; (b) agents expected to reduce the liberation of potassium during the spike, such as procaine and cocaine, reduced the amplitude of NAP; (c) recovery from this negative after-potential following cessation of stimulation, normally obtained under moist chamber conditions, could be accelerated by washing the nerve; and (d) recovery from repetitive stimulations under moist chamber conditions could be prevented by oxygen lack, presumably by preventing active potassium reabsorption (405*I*, 406*I*, 412*I*). The temporary rise in

$[K]_o$ immediately around squid axons as the basis of NAP in these fibers has since been confirmed as follows: (a) Low temperature, which increases the potassium loss per spike, increases NAP correspondingly; (b) the concomitant changes in E_m, in the amplitude of the spike, and in PP all correspond to the same change in $[K]_o$; and (c) the cumulative changes of E_m, S, and PP with repetitive stimulation, over a range of different potassium concentrations in the medium, are essentially the same and matched by the effects expected from an increase in $[K]_o$ surrounding the fibers (158*I*). The last study concluded that the estimated rise in $[K]_o$ is that to be expected in a "space" about 300Å wide; the kinetics of decline of this excess $[K]_o$ suggests that this space is enclosed by the unselective membrane with the low resistance of 4 ohm cm^2 indicated by other types of measurements (158*I*). On the basis of the alteration of NAP with repetitive stimulation, especially as modified by changes in $[K]_o$, C fibers are also considered to produce an accumulation of potassium at their surfaces (366*I*).

These studies focus attention on a transitory rise in $[K]_o$, either in a restricted region immediately around the fiber as in the giant axon, or in the more remote regions of the interstitial space as in crab nerve. In the former, the disappearance of the excess $[K]_o$ (and NAP) is relatively rapid, for the ion can escape into the more remote part of the extracellular space or into the surrounding solution; in the latter, the decline in NAP will be slower, being only as fast as active reabsorption or diffusion from the interstitial space. No quick recovery from NAP was seen in crab nerve corresponding to that in squid axons (406*I*), hence the membrane that has been suggested to surround giant axons seems lacking in crab fibers. One is inclined to wonder whether the relatively inelastic sheath, characteristic of the giant axon (Part I), may constitute the postulated barrier, rather than the axoplasm-Schwann-cell junction, proposed by Frankenhaeuser and Hodgkin, which is also present in crustacean leg nerves (168*I*).

In any case, it is apparent that rapidly disappearing negative after-potentials may be a consequence of changes in $[K]_o$ near the active membrane surface and that these must be distinguished from membrane permeability changes *per se*.

In the squid axon (158*I*, 405*I*) and in frog nerve trunks (78, 310*I*) the amplitude of the negative after-potential is much more sensitive to extracellular potassium (and rubidium) than that of the spike or E'_m, but the time constant of decline is unaffected (405*I*). This is also true in muscle (69a). In view of the accumulation of potassium in the interstitial spaces which is now known to have occurred in earlier experiments involving metabolic inhibition (Part I), the greater sensitivity of NAP than the spike to inhibition (59, 310*I*) is more probably due to the rise in $[K]_o$ than to a special relation between metabolism and the after-potential. Halving or doubling $[Na]_o$ does not affect NAP in muscle (69a) but increase of $[Ca]_o$ raises both E'_m and NAP (118, 250*I*). Increase of $[Ca]_o$ reduces the height of the after-potential without altering the time course in squid axons (405*I*); the opposite effect on amplitude, and a lengthening, has been reported for sheathed nerve trunks (78, 80, 138). In nerve trunks NAP is much more sensitive to urethane than the compound spike (77). The depolarization (*i.e.*, potassium release) with repetitive stimulation in crab nerve was completely unaffected by a 3-fold increase in $[Ca]_o$, but repolarization (*i.e.*, the rate of potassium reabsorption) was hastened (412*I*).

A recent finding, described so far only for muscle, is the augmentation of NAP by replacement of $[Cl]_o$ with other anions, especially thiocyanate (17, 59b, 144); the sequence of effectiveness of the anions is same as their effectiveness in increasing membrane resistance and in decreasing the penetration of potassium

into muscle (Part I). The falling phase of the spike may also be affected (144), and the after-potentials may take on a plateau-like appearance or develop an initial rise phase (17, 59b) such as is also observed in some fresh muscle fibers (69a). Probably related to the increase in muscle NAP by replacement of Cl_o^- with more poorly penetrating anions, is the observation that long soaking in Ringer which increases $[Cl]_i$ (138*I*), also increases NAP (69a).

The possibility that movement artifacts may have contributed to the variable appearance of these negative after-potentials requires examination. This now can be done easily, for contraction can be prevented by hypertonic solutions without affecting spike production (98a).

2. After treatment with veratrine or its alkaloids. The qualitative aspects of the action of veratrine alkaloids on nerve and skeletal muscle in increasing and prolonging the negative after-potential have long been known (130)—an observation since extended to cardiac fibers (21). More recently it has been demonstrated that, although cevadine and veratridine each produces an increase and prolongation of the negative after-potential, which declines as a simple exponential, the time constant of repolarization (*i.e.*, the time required for the amplitude to decline to 37% of the maximum) is five to eight times longer with veratridine—an effect characteristically the same in squid giant axons and in crab and frog nerve (415*I*). The basic identity of the behavior of their membranes is strikingly demonstrated by this quantitative similarity. Moreover, when the two alkaloids are both present in about equal concentrations, as in veratrine, each continues to exert its characteristic effect on the time course, with the result that the decline is then composed of two exponentials, each with the same time constant obtained with the pure alkaloids.

These observations on the time course appear to rule out any single factor, such as a transitory increase in $[K]_o$, as the cause of the veratrine-induced negative after-potential. That fluctuations in $[K]_o$ do not directly underlie these after-potentials is further indicated by (a) the appearance of a fast-disappearing component of NAP on early cessation of repetitive stimulation of veratrinized crab nerve, although such a rapid component is not apparent in untreated preparations (406*I*) and (b) an increase in NAP far in excess of that to be expected from the increment in potassium loss with the alkaloids (412*I*, 415*I*).

It is noteworthy that after only a brief exposure of nerve fibers to these alkaloids the increased negative after-potentials persist for a long time. This indicates a relatively rapid uptake followed by a very slow loss of the alkaloids (*e.g.*, 415*I*).

In most respects the veratrine-augmented NAP responds to experimental conditions as before: Increase with hyperpolarization, as in vertebrate nerve trunks (79, 310*I*); marked decrease with elevated $[K]_o$, as in giant axons, crab nerve, and vertebrate nerve (310*I*, 311*I*, 405*I*, 406*I*); and reduction in amplitude, with little change in time course, with low concentrations of anesthetics, as in giant axons (405*I*). Reduction of $[Na]_o$ markedly reduces the NAP, with little effect on the spikes in crab nerve (203, 425*I*). In the cephalopod axons the

effect of $[Ca]_o$ on the negative after-potential is reversed, *i.e.*, it now causes an *increase* rather than a decrease in amplitude (405*I*).

The difference in the response to calcium of the normally occurring NAP and veratrine-induced NAP in squid axons may reflect a difference in the mechanism of negative after-potential production. Reasons were given above for considering NAP in untreated squid axons to be due to a transitory change in $[K]_o$. Increase in $[Ca]_o$ probably does not decrease K^+ liberation during the spike (Section 1 above), but it will reduce the depolarizing effectiveness of $[K]_o$ (Part I); the reduction of NAP in this agent, therefore, is attributable to the latter. On the other hand, if permeability changes are affected by veratrine alkaloids, say by interfering with the return of calcium to the membrane at the end of the spike (405*I*; see Section IV C, F, and G), then more of the initial membrane calcium, expected to be greater in higher $[Ca]_o$, would be subject to displacement by the alkaloids.

Regardless of specific mechanisms, it does appear that calcium has a different effect when surface $[K]_o$ is responsible for the NAP than when another process, probably involving permeability, is affected. The augmentation of the naturally occurring NAP by calcium in untreated frog nerve, reported by Graham (Section 1 immediately above), therefore suggests a process more related to that enhanced by veratrine than that ordinarily seen in giant axons.

In crab nerve, the growth of depolarization with repetitive stimulation is hastened and the final magnitude is larger with veratrine, the former in keeping with the NAP which appears with individual spikes and the latter in accord with the 60% greater release of potassium (406*I*, 412*I*). Here, again, one notes that the large NAP after individual spikes is so out of proportion to the increase in the potassium liberated that the electrical phenomenon following single spikes is not attributable to transient changes in $[K]_o$; the slow build-up in the residual depolarization with repetitive activity, however, is dependent on $[K]_o$. Increase in $[Ca]_o$ accelerates the depolarization of veratrinized nerve with repetitive stimulation, as expected from the larger NAP in its presence, but the final level attained is unchanged, indicating that the actual liberation of K^+ during the spike has been unaltered (412*I*). The repolarization of previously stimulated nerves is slower but larger in veratrinized nerve; only the effect on rate is counteracted by elevated $[Ca]_o$.

Because veratrine-induced negative after-potentials are additive with repetitive activity, the after-discharge frequently accompanying a single stimulus may produce an NAP which appears to have a rising phase (*cf.* 13 and 406*I*). However, when after-discharge is absent, a rising phase may not be apparent, as in crab nerve (406*I*). Frog nerve, on the other hand, shows a distinct, slow rising phase, occasionally a plateau, in the absence of after-discharge—as may be accomplished by treatment of veratrinized trunks with local anesthetics or calcium (413*I*) or by anodal polarization (79). Whether this may be an artifact due to the summation of after-potentials with different time courses in a mixed nerve (320*I*) cannot be answered at present. Its absence from more homogeneous fibers makes it suspect.

If we consider the absence of a distinct rising phase to be a basic characteristic feature of the negative after-potential, the implication is that it reflects a residual condition which develops during the spike. This is supported by the simple

exponential nature of the decline of NAP in untreated fibers and in fibers in the pure veratrine alkaloids.

F. The positive after-potential (PAP)

This slow, small hyperpolarization frequently follows the negative after-potential, especially in B and C fibers, and is augmented by repetitive activity and by treatment with alkaloids such as yohimbine and quinine. Earlier studies on vertebrate nerve are summarized in (59, 82, 310*I*, 311*I*).

In crab nerve the development of a very late positive after-potential after repetitive stimulation was shown to be related to the depletion of potassium from the extracellular space. This was done by demonstrating (a) that when the potassium released during stimulation is allowed to accumulate in the interstitial spaces—by the nerve being mounted in a moist chamber—a small positive after-potential appears (406*I*), which corresponds to a small excess of potassium absorbed from the medium when the nerve is returned to a solution containing K^+ following these conditions (412*I*); and (b) that when potassium escapes during stimulation into a large volume of solution, upon cessation of stimulation a much larger amount of potassium is taken up from the medium (412*I*); this is correlated with a substantially larger positive after-potential under the same conditions (406*I*).

The concept of depletion of $[K]_o$ has recently been applied to less direct experiments on the hyperpolarization that succeeds activity in C fibers (366*I*). Thus, it was found that appearance of the after-potential is prevented by (a) interference with active transport by low temperature, dinitrophenol, cyanide, iodoacetate, azide, or ouabain, (b) complete removal of extracellular potassium, thereby preventing hyperpolarization since there is no $[K]_o$ to be reduced, and (c) replacement of extracellular sodium with lithium, since lithium taken up during activity cannot be extruded in exchange for extracellular potassium; also, the prolongation of the after-potential in low $[Na]_o$ solutions was attributed to slower operation of active transport as a result of less increase in $[Na]_i$ during previous activity.

It should be noted that the experiments on C fibers are susceptible to another interpretation based on active transport. It will be recalled from Part I that in the sciatic nerve of the toad there is evidence that active transport effects an elevated potential by "sodium exclusion", *i.e.*, by preventing some of the sodium, which leaks into the membrane from the outside, from passing through to exchange with intracellular potassium; instead, this sodium is ejected in exchange with extracellular potassium, thereby elevating the influx of potassium and reducing that of sodium. The results described by Ritchie and Straub (366*I*) would still follow and for the same reasons, except that the action of low $[K]_o$ would be attributable to interference with sodium exclusion, which depends on $[K]_o$. The rapidity with which the after-potential develops in C fibers, in contrast to crab nerve, would seem to make this alternative view the one of choice, unless it can be shown that an appreciable barrier to diffusion exists around C fibers as in squid axons.

The decrease in $[K]_o$ or of membrane sodium appears to be a satisfactory explanation for the positive after-potential in small axons which, like crab and C fibers, have high rates of recovery metabolism (126*I*, 134) and of potassium reabsorption (412*I*, 419*I*). This high rate of recovery metabolism has been pointed out to be a necessity in such small nerve fibers which, by virtue of their high surface:volume ratio, must lose proportionately more potassium and gain more sodium per impulse than large or heavily myelinated fibers (421*I*). This view is subscribed to by Ritchie and Straub (366*I*), with the additional suggestion that the greater increment in $[Na]_i$ in C fibers contributes to their higher recovery metabolism—a proposal which many consider an important basis for the regulation of active transport under other conditions as well (see Part I).

As in the case of the negative after-potential, therefore, it is unlikely that all positive after-potentials reflect a transient change in $[K]_o$. This may be especially true for the small positive after-potential following single spikes that can be induced with yohimbine, as in the squid giant axon (405*I*), which has an exceedingly low recovery metabolism (47*I*), and in crab nerve before it has lost appreciable potassium (406*I*). The reduced effectiveness of the spike generating mechanism during these after-potentials (Section B 12 b above) indicates the likelihood of a more specific membrane process rather than simply an increase in E_m by virtue of a change in $[K]_o$—perhaps the depletion of membrane sodium suggested above. This will be discussed later.

In conclusion, we may note that membrane hyperpolarization, by electrical means, augments PAP in nerve trunks (79), as does low $[K]_o$ in the case of yohimbine-induced PAP in crab nerve. After-potentials in yohimbinized squid axons and crab nerves are reduced by an increase in $[Ca]_o$ and augmented by a decrease in the alkaline earth (405*I*).

IV. HYPOTHETICAL CONSIDERATIONS

A. General principles

A limiting factor in the analysis of events responsible for the action potential has been the complexity of the situation during propagation of the all-or-none impulse. The observer is unable to control (a) the explosive change in membrane potential designated as the all-or-none spike and (b) the local currents arising between the region occupied by the spike and that ahead and inactive and that behind and partly recovered (Fig. 2) (*e.g.*, 28).

An important step in the analysis of events during the spike was that of Marmont (150). He applied electronic feed-back to segments of squid giant axon on either side of the central, excited region such that their transmembrane potential varied in exactly the same way as in the central region. These segments therefore functioned as "guard rings", which prevented the longitudinal flow of current and the associated membrane currents, such as normally occur in propagation, from contributing to the potential changes. This arrangement is spoken of as a "space-clamp". In effect, then, propagation is eliminated, as might also have been accomplished if the entire fiber were activated at once and were completely uniform in its response.

The next contribution was an additional feedback system—the "voltage-clamp"—to fix the membrane potential difference rigidly at values set by the

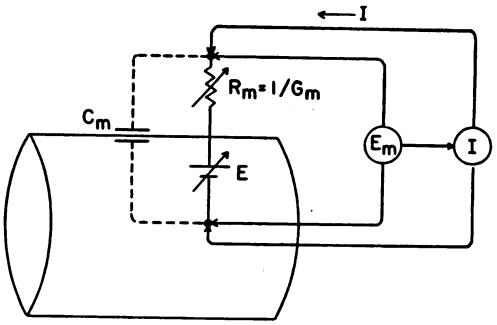

FIG. 3. Simplified representation of the membrane variables—resistance, R_m, and intrinsic e.m.f., E—that govern the flow of current, I, when membrane potential is "clamped" at arbitrary values of E_m. Membrane capacitance, C_m, has a negligible effect on results under these conditions.

observer. This has the important effect of eliminating the contribution of membrane capacitance to the electrical phenomena—a matter discussed later. The first descriptions of results with this method on the squid axon were reported by Cole (41*I*) and Hodgkin *et al.* (107). The latter proceeded to examine the events taking place during clamping with remarkable quantitative precision and completeness (102–105, 108). Their findings have so many implications for other excitable systems and phenomena that they will serve continually as a point of reference in the rest of this review. Hence, the important details and inferences from their work must now be described.

1. Voltage-clamp. Fig. 3 shows very schematically the basic elements of the voltage-clamped, central segment of the squid axon. E_m is the actual potential difference across the membrane and is set and measured by a circuit which also controls the current, I, flowing from or to the membrane so that any tendency for E_m to change from its clamp value is compensated for by an increase or decrease in the current I; this constitutes the feed-back circuit. Thus, R_m and E_m are the resting resistance and potential of the axon membrane, and E equals E_m when no current is flowing through R_m. E may also be called the "intrinsic electromotive force" (e.m.f.) of the membrane. If, now, I flows through R_m, E_m is changed by an amount equal to R_mI, being increased when I is an *inward* (hyperpolarizing) current, as shown, and decreased when I is an *outward* (depolarizing) current. As long as R_m and E do not change, Ohm's Law is obeyed and E_m will alter by an amount directly proportional to I, R_mI, and to the same extent but in the opposite direction when the direction of I is reversed. If, when E_m is set to a value less than E by an initial *outward* I, R_m proceeds to fall, the feedback circuit will maintain E_m at the originally set value by increasing I so that R_mI remains constant. Thus, changes in I will vary inversely with R_m when E is constant. Now, suppose only E changes when E_m is set at a low value, and that it begins to decrease; again, I must change, this time to *decrease* R_mI to compensate for the approach of E to E_m, an effect obtainable by decreasing the outward current initially used to set E_m less than E. It is important to notice that if E declines below E_m, I must become an *inward* current. If both R_m and E decline, a larger inward current will develop.

Results and inferences. Cole (41*I*) and, with far more detail, Hodgkin *et al.* (107) found that a sudden, sustained change in E_m of small amplitude, whether

an increase or decrease, had little effect except for a minor increase or decrease in R_m, respectively; as the *changes*, $V(= E_m - E_m')$, in E_m were made larger, R_m increased somewhat more with hyperpolarization, but radically different results were obtained during depolarization, namely, a rapid brief surge of inward current followed by a better sustained, large outward current—indicative of a maintained decrease in R_m as well as a transitory decrease in E. As the step-like decreases in E_m (or increases in V) were made larger (E_m itself actually smaller), the amplitude of the inward current reached a maximum (when E_m was about zero, or V equal to the original resting potential), and a further decrease in membrane potential (E_m now actually reversed in polarity) caused it to decline, so that when E_m was about equal to the reversal potential of the spike of the unclamped axon (and hence V = S; see Fig. 1) the inward current was zero; and, finally, a further stepwise reduction in membrane potential (the reversed potential difference $-E_m$, still larger) caused an initial *outward* transient of current corresponding to the inward surge seen with smaller depolarizations. The late outward current, obtained at all depolarizations, was progressively larger with greater depolarizations.

The following conclusions were drawn (102–105, 108): (a) The transient inward current is due to the entry of sodium ions resulting from a transient increase in P_{Na} that is measurable as an increase in sodium conductance (G_{Na}). This is based on several observations: The inward current is decreased by reduction of $[Na]_o$; this current disappears at the higher depolarizations at a value of E_m (Fig. 3) that corresponds approximately to E_{Na}, *i.e.*, the sodium equilibrium potential given by $58\log [Na]_o/[Na]_i$, at which sodium influx should equal sodium outflux and hence no net current (sodium) flow should occur (Part I); when $[Na]_o$ is lowered, the transitory current flow changes from inward to outward at a value of E_m corresponding to the new value of E_{Na}.

(b) The sustained outward current is due to an increase in P_K, actually expressed as potassium conductance (G_K), which develops very slowly at first when the step depolarization is applied, and then rises in sigmoid fashion to its maximum. The time course of the increased G_K is given by the outward current when the inward current transient is minimized, as when $E_m = E_{Na}$ or when $[Na]_o = [Na]_i$. The linearity of the maximum outward current as a function of larger values of V demonstrates that Ohm's Law is obeyed and hence that P_K (or potassium conductance, G_K) is constant beyond a certain magnitude of depolarization. The evidence that the outward current is due to an increase in P_K is as follows: A sudden return of E_m to the original resting potential when the outward current has become large reveals a transitory outward current which is eliminated when $E_m = E_K$; this follows from P_K having increased during depolarization so that the other terms of the Goldman equation (Part I) are negligible, hence E is equal to E_K at the time E_m is restored to normal levels, for P_K is slow in returning to its rest level. Measurements of radiopotassium flux during membrane depolarization show that potassium carries practically all the current (106).

(c) The cessation of inward current (and hence of the increase in P_{Na}), in

contrast to the sustained outward current (and hence of increased P_K), during continued depolarization is due to a distinct phenomenon designated as "inactivation". It always develops more slowly than the initial increase in P_{Na}. Its rate of development increases with the extent of the depolarization. Similarly, removal of depolarization causes subsidence of inactivation—"reactivation"—at a rate governed by the extent of the repolarization. The giant axon, at its "normal" resting polarization in sea water, is in a partial state of inactivation, *i.e.*, can produce a peak inward current or P_{Na} increase of only about 0.6 of the maximum possible. The maximum inward current (increase in P_{Na}) is obtained when the initial value of E_m, E'_m, is at a level of 100 mV before the depolarization clamps are applied; it declines along a sigmoid curve as a function of the E'_m until it is almost zero when E'_m is about ⅔ to ½ of the normal resting potential (figure 5 in 104).

The fact that generation of an increase in P_{Na} can be prevented by a decrease in E'_m to ⅔ to ½ the normal resting potential should be recognized as providing the basis (a) for the block of nerve conduction or spike generation by depolarization (*e.g.*, by excess extracellular potassium, metabolic inhibition, veratrine) and (b) for restoration of conduction and spike generation under depolarizing conditions by electrical repolarization. The other features of inactivation are in keeping with (a) the time course of decline of spike production with electrical depolarization and the time course of recovery with electrical hyperpolarization, (b) the limited increase in spike amplitude with hyperpolarization, (c) the decline of the spike-generating mechanism during the period of the nodal spike, as revealed when spike generation is tested after sudden termination of the plateau, and (d) the recovery of the spike mechanism during the relatively refractory period or after the termination of the spike. All of these features were described for a variety of excitable cells in Section III B.

(d) The rapid rise in G_{Na}, and the less rapid fall in G_{Na} and rise in G_K, as well as their maximum amplitudes, are continuous functions of the membrane potential and are independent of membrane current. Both the peak G_{Na} and G_K increase exponentially with the magnitude of depolarization for smaller displacements of E_m, a 2.7-fold increase occurring in G_{Na} with each 4 mV depolarization and in G_K with each 5 to 6 mV depolarization; beyond 20 to 30 mV of depolarization, the peak values of G_K and G_{Na} do not increase much with V and approach a maximum asymptotic value (figures 9 and 10 in 102).

In Part I other evidence for the inverse dependence of membrane conductance in squid axons—largely due to G_K (or P_K)—on E_m was pointed out; additional evidence is now available from the decrease in G_m during the positive potential (4, 203) (see Section 2 g below).

(e) The current changes during voltage clamps, and the associated changes in R_m and E, can be explained almost completely in terms of the temporal characteristics of G_{Na} and G_K in series with their respective, oppositely oriented equilibrium potentials, E_{Na} and E_K, which do not change appreciably during the clamp, as in Fig. 4.

In the resting fiber G_K is much larger than G_{Na} ($P_K \gg P_{Na}$ in the Goldman equation), hence simple application of Ohm's Law shows that E_m is governed largely by E_K. This is

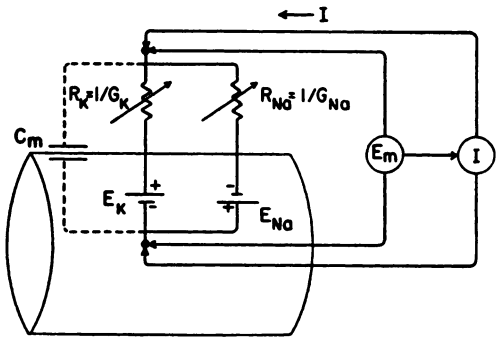

FIG. 4. Simplified representation of membrane variables—resistance to potassium ions, R_K, and resistance to sodium ions, R_{Na}—in series with respective constant equilibrium potentials of potassium (E_K) and sodium (E_{Na}), which account for the changes in both R_m and E in Fig. 3. The minor contribution of other ions (*e.g.*, Cl^-) is neglected.

easily seen by expressing the intrinsic e.m.f., E, in terms of the resistances and the absolute values of E_{Na} and E_K:

$$E = E_K - \frac{R_K}{R_K + R_{Na}} (E_{Na} + E_K) \qquad (I)$$

R_K is normally much smaller than R_{Na}, hence the second term reduces E below E_K by only a small amount governed by R_{Na} relative to R_K, much as when P_{Na} is not zero relative to P_K in the Goldman equation (Part I). Since E_{Na} nearly equals E_K, E becomes zero when R_{Na} equals $R_K(P_K = P_{Na})$; as R_{Na} decreases further, so that it is negligible compared to R_K, ($P_{Na} \gg P_K$), $E = -E_{Na}$, the potential reversing because E_{Na} is directed inward (inside positive)—a consequence of $[Na]_o$ being greater than $[Na]_i$ (Part I).

An additional parallel circuit representing leakage resistance and potential due to chloride, *etc.*, is not shown; it is regarded as constant and does not affect these considerations appreciably.

R_m, of course, is merely R_K and R_{Na} in parallel, or $R_K R_{Na}/(R_K + R_{Na})$.

Thus, the initial rapid decline in R_{Na} (increase in G_{Na}) changes the intrinsic e.m.f. E (Fig. 3) from a value originally close to E_K to one approaching E_{Na}. The decline in G_{Na}, resulting from the more gradual inactivation, alone would restore *E* to its original resting value approaching E_K. But the gradual increase in G_K, which occurs in addition, hastens the return to the resting level and causes E to approach closer to E_K than it was originally; this is because the membrane potential is now less sensitive to the sodium leak at rest (in other words, P_{Na}/P_K is smaller in the Goldman equation).

From Fig. 4 it follows that the sodium current, I_{Na}, (*i.e.*, the rate at which sodium is transferred across the membrane) and the corresponding potassium current, I_K, are given by

$$I_{Na} = G_{Na}(E_m - E_{Na}) = (E_m - E_{Na})/R_{Na} \qquad (II)$$

$$I_K = G_K(E_m - E_K) = (E_m - E_K)/R_K \qquad (III)$$

2. Applications. a. Rising phase of the spike. To describe the relation of the above observations to, say, the space-clamped axon now requires taking into account the membrane capacitance shown in Fig. 3 and 4. This has the effect of delaying changes in E_m due to current flowing through the membrane.

For example, in the resting axon the capacitance C_m in Fig. 3 will have the same potential difference across it, E_m, as the intrinsic e.m.f., E, because of the charge Q on it given by

$$Q = C_m E_m = C_m E. \tag{IV}$$

If the intrinsic e.m.f. suddenly falls, say to zero, Q cannot change instantaneously but leaks off only gradually through R_m. As a result, the potential difference across the membrane, E_m, declines much more slowly than E. It declines at a rate proportional to its own magnitude and inversely as the time constant ($\tau_m = R_m C_m$) of the membrane:

$$\frac{dE_m}{dt} = \dot{E}_m = E_m/R_m C_m, \tag{V}$$

which may also be written

$$\dot{E}_m = I/C_m \tag{VI}$$

since E_m/R_m is the current flowing through the membrane.

Obviously, a decrease in R_m alone will do nothing to the membrane potential in the resting fiber since E and E_m are equal, but combined with a decline in E, the change in E_m will occur more rapidly.

The concept of membrane time constant, τ_m, is an extremely important one for transient electrical phenomena, for it sets a limit to the speed with which the membrane potential will respond not only to changes in intrinsic e.m.f.'s, but to extrinsically applied potentials or currents. For a final maximum decrease in potential, V_M, the time course is given by the exponential

$$V = V_M e^{-t/\tau_m}, \tag{VII}$$

so that V falls to 1/e or 37% of its full decrease, V_M, in the time τ_m (or to ½ of its full decrease in the "half-time", $t_{1/2}$, equal to 0.69 τ_m). Conversely, the rise in V to a final total increment, V_M, is given by

$$V = V_M(1 - e^{-t/\tau_m}) \tag{VIII}$$

Thus, if the phenomena observed during the voltage-clamp apply to a space-clamped fiber, the rate of rise of the spike should reflect I_{Na} (equation VI), the maximum rate of depolarization giving the maximum I_{Na} and hence the maximum G_{Na} or P_{Na}. Moreover, the maximum reversal of membrane potential (S_m in Fig. 1) should approach but not exceed E_{Na}, given by 58log ($[Na]_i/[Na]_o$). And since propagated action potentials differ but slightly from unpropagated ones (Section III A), the same conclusions should apply to them as well.

It will be recalled that the rising phase and the amplitude of the spike of cephalopod axons and of other fibers, except those of crab muscle, exhibited characteristics commensurate with this view: (a) Reversal of membrane potential occurred at the peak of the spike; (b) the magnitude of the reversal varied as log $[Na]_o$; (c) the magnitude of the reversal was reduced by an increase in $[Na]_i$; and (d) the rate of spike rise varied with $[Na]_o$.

Studies of the magnitude of the reversal potential, S_m, have usually not been carried out on the same cells as analyses for Na^+. Average values available for cephalopod axons show E_{Na} and S_m to be close (*e.g.*, *cf.* 221*I*, 509*I* with 273*I*; see also table 3 in 219*I*).

b. Falling phase of the spike (in the absence of a plateau). According to the voltage-clamp studies in the squid axon, depolarization initiates two processes that contribute to repolarization—(a) inactivation, which shuts off the increase in $P_{Na}(G_{Na})$ and hence eventually stops the inward, depolarizing sodium current, thereby driving E_m back to the original resting level, and (b) an increase in $P_K(G_K)$, which hastens the process by keeping the time constant of the membrane short and which provides additional charge (K^+ moving outward) to bring E_m towards E_K. Both inactivation and the increase in potassium permeability develop more slowly than the increase in P_{Na}. Hence not until near the peak of the spike does G_{Na} begin to shut off and does G_K, which shows a substantial delay in turning on, begin to become appreciable.

Hodgkin and Huxley (105), on the basis of empirical equations set up to describe the experimentally observed amplitudes and time courses of G_{Na}, G_K, and inactivation as functions of membrane potential in voltage-clamped axons, have been able to reconstruct space-clamped and propagating spikes and positive potentials which conform remarkably well to those actually observed. Fig. 5 is an example of their computations for a propagated spike; it gives the changes in G_K and $G_{,Na}$ and their sum, G_m, as well as V (*i.e.*, the deviation from E_m') as functions of time. The general configuration of the spike conforms well to that actually observed, and their computations showing that the increase in conductance during the spike outlasts the potential change are consistent with the earlier findings to this effect (43*I*). More recent observations of the changes of membrane conductance accompanying the spike reveal two distinct stages in the decline of conductance (4) consistent with the two processes involving G_{Na} and G_K as proposed by Hodgkin and Huxley.

We may also note that since the amplitude and rate of development of the increase in G_K depend on the degree of depolarization, spikes of reduced amplitude will tend to develop a smaller increase in G_K. This, combined with a smaller difference between E_m and E_K, will make $I_K[=G_K(E_m - E_K)]$ smaller and hence repolarization will be slower, as seen in giant axons and muscle.

Cole *et al.* (30) note that the computed spike shows a delay in repolarization which is not seen in axonal spikes. However, this probably is due to a deviation of the empirical curve, used by Hodgkin and Huxley, from the actual $G_K - V$ relationship (see figure 5 in 105), as discussed in Section g below.

The remarkable success that has been achieved in quantitatively reconstructing the normal spike (as well as the other phenomena to be discussed) from observations made under voltage-clamp conditions represents an outstanding demonstration that in the squid axon the three variables G_{Na}, G_K, and inactivation are governed solely by membrane potential and completely represent the processes underlying the spike. But, as emphasized by Hodgkin and Huxley (105), the formulations relating these variables to V do not necessarily have an intrinsic molecular significance; they were selected for convenience as reasonably good fits of the data to verify the computability of several components of the action potential, of associated conductance changes, and of ionic movement.

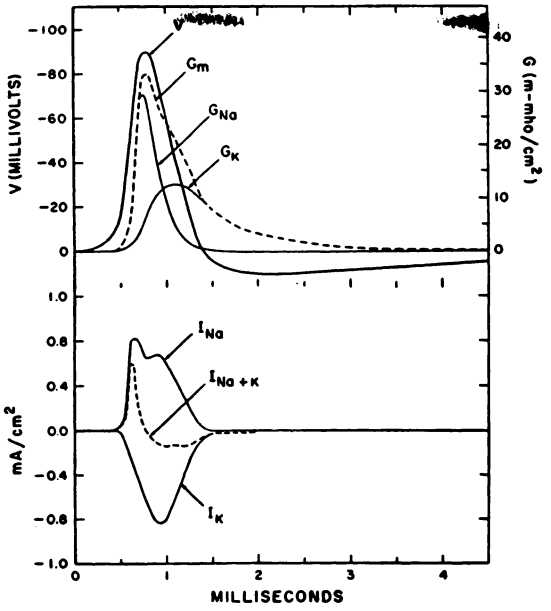

FIG. 5. Time courses of the propagated spike and positive potential (V) and underlying conductances (G) and currents (I) as computed by Hodgkin and Huxley. Modified from (105).

V begins to rise before G_{Na} because of electrotonic spread from the arriving spike and because of the time required for G_{Na} to increase. As G_{Na} rises, the inward current, I_{Na}, increases, but starts to fall again as V approaches E_{Na} since I_{Na} depends not only on G_{Na} but also on the *difference* between E_m and E_{Na} (equation II). G_{Na} starts to fall because of inactivation at about the time G_K begins to increase appreciably. The increase in outward current, I_K, accompanying G_K causes the *net* current, I_{Na+K}, which is the algebraic sum of I_{Na} and I_K, to fall and reverse even though I_{Na} is rising again; the secondary rise in I_{Na} occurs despite falling G_{Na} because the dropping V, resulting from outward I_{Na+K}, temporarily increases ($E_m - E_{Na}$) proportionately more. The initial part of membrane conductance, G_m, is predominantly G_{Na}, while the late part is chiefly G_K.

The calculated behavior of G_m at late times is obtained experimentally at lower temperatures and when $[K]_o$ is twice that of sea water, whereas in sea water, especially at room temperatures, G_m generally falls temporarily below that at rest (4); this minor discrepancy is probably due to PP and a steeper dependence of G_K on E_m than assumed by Hodgkin and Huxley (Section IV A 2 g).

c. The positive potential (and delayed rectification). This potential is a consequence of the prolonged increase in P_K. It will be recalled from the Goldman equation in Part I that the resting potential of axons in sea water is well below E_K, apparently because P_{Na} is appreciable compared to P_K, and that the resting potential can be made to approach E_K by either a decrease in P_{Na} or an increase in P_K. The latter appears to be the situation during PP in the axon.

This provides an explanation for the inverse relation between PP and E_m' when the latter is changed electrically, and the direct dependence on E_m' when the latter is varied by altering $[K]_o$ (Sections III C 1 and 2). When E_m' is increased electrically, it is brought closer to E_K as determined by $58\log([K]_i/[K]_o)$. Hence, the larger P_K during the spike,

which cannot raise the membrane potential above E_K, will not increase the potential as far from the higher initial E'_m. Of course, when E'_m is set equal to E_K by electrical means, there will be no PP, and when E'_m is made still larger, the spike will terminate at E_K, and then E_m will rise gradually to the set value at a rate determined by the membrane time constant. On the other hand, when E'_m is increased by lowering $[K]_o$, E_K is simultaneously raised and by more than E'_m because the latter is limited by membrane leakiness to sodium (Goldman equation, Part I); in this case, therefore, PP is augmented.

We emerge with the principle that when E_m changes by virtue of alterations in P_K, prior electrical change in E'_m will affect the potential fluctuation due to P_K oppositely from a modification in E'_m with K^+. On the other hand, if permeability changes other than in P_K are involved, then the effect of previously altering E'_m with either K^+ or electrical current will be the same, and the latter can be used to counteract the action of K^+. The equivalence of electrical or potassium polarization changes on spike amplitude or rise (see Section III B 2) therefore is an example of this principle, as might be expected since the early part of the action potential is not dependent on P_K but rather on P_{Na}. This principle will prove useful when the plateau is discussed later.

Another feature of these considerations is that they provide an explanation for the similarity of the transitory hyperpolarization following cathodal depolarization to PP. The delayed rise in P_K during flow of a constant outward (depolarizing) current across the membrane of squid axon is evident as a sudden cessation of the fall in E_m (which otherwise would continue to rise as with a constant inward current) followed by a rise so that only a small depolarization is maintained as long as current flows outward; that is, the rise in P_K raises E back towards E_K and, by making R_m smaller, also makes R_mI, or V, smaller (41*I*, 105). The sudden transition in the depolarization towards repolarization is termed "delayed rectification" (219*I*). When delayed rectification has occurred, sudden cessation of outward (depolarizing) current not only results in collapse in R_mI but, since P_K declines slowly, E_m will swing towards E_K, resulting in a transitory hyperpolarization, which then subsides as P_K declines to normal. The converse may be expected with anodal currents, especially when E'_m is low compared to E_K, as may be achieved electrically or by increasing P_{Na} (as in veratridine); hyperpolarization will now cause a delayed decrease in P_K, hence a secondary partial depolarization will occur during flow of current; furthermore, when the current flow ceases, E_m will fall below the original level and recover as P_K rises again. Such transitory changes during and following cathodal and anodal current flow have been described in the giant axon (*e.g.*, 6, 7, 9), in spinal roots (372*I*), in desheathed frog sciatic nerve (395*I*), in "slow" muscle fibers (30*I*), and in "fast" muscle fibers, although in the last rectification may become reversed (266*I*, Part I).

d. Oscillations. The delayed increase (or decrease) in P_K (G_K) with depolarization (or hyperpolarization) and the delayed potential changes resulting from membrane capacitance provide the chief ingredients necessary for the oscillations, as shown by actual calculation (105). Thus, a brief depolarization causes G_{Na} to begin to rise, leading to an increase in inward current and a further depolarization; this in turn, begins to subside, partly because of inactivation but largely because outward (repolarizing) I_K develops as G_K starts to increase; when E_m is back to normal G_K is still elevated although beginning to decline, consequently E_m continues to rise (because of I_K); as E_m increases further, G_K continues to fall and attains a value below normal; by then E_m is falling again (because of

decreased outward I_K), and as it declines I_{Na} increases with the increase in G_{Na}.

This sequence of events is a consequence of the presence of membrane capacitance, which retards E_m relative to the membrane currents, and of the lag between the G_K and the G_{Na} changes that keeps the opposing I_K and I_{Na} out of phase.

Detailed analysis of the effect of alteration of membrane parameters by calcium and other stabilizers on oscillations has not been attempted. But qualitatively we may note that when $[Ca]_o$ is lowered, both resting P_{Na} and P_K are increased (Part I); thus, an increase (or decrease) in E_m can cause larger absolute (but still alternating) changes in I_{Na} and I_K and hence in membrane potential, as actually found (Section III D). The very recent studies of Frankenhaeuser and Hodgkin (157*I*) showed that lowering $[Ca]_o$ was equivalent to lowering E_m' insofar as it increased the rest magnitudes of P_K and P_{Na} and the response of these permeabilities to sudden changes in E_m, although the magnitude of the resting potential was actually practically unaltered. Conversely, agents which limit the increase in P_{Na} and P_K and the associated currents should suppress the oscillations—as has been observed with stabilizers (Section III D). A condition which reduces the ionic currents without affecting the permeabilities, namely, low $[Na]_o$ (since I_{Na} or G_{Na} is dependent on both P_{Na} and [Na], as pointed out in Part I and indicated by equations II and III above), should reduce I_{Na} and the associated depolarization and therefore suppress oscillations, as found (Section III D). The change in oscillations with $[K]_o$ would depend on whether G_K (which in Part I was shown to be roughly proportional to $[K]_o$) is changed more than $(E_m' - E_K)$ (see equation III above). Hence, the variable results noted with potassium (Section III D) and the "inductive" (oscillatory) behavior of giant axons observed with moderate increase in $[K]_o$ (28) are consistent with the Hodgkin-Huxley approach.

e. Subthreshold behavior and excitation. The oscillations in potential were pointed out to be obtainable with brief shocks too weak to induce the spike, *viz.*, subthreshold shocks. When these oscillations are particularly "damped", *i.e.*, successive waves decline very rapidly or do not appear at all—the more usual situations—the electric shock can elicit two obviously different subthreshold potentials. One of these is a "passive" subthreshold response—that to be expected from the cable network (*e.g.*, 228*I*)—which is essentially the same whether an anodal or cathodal shock is applied and declines exponentially with time. The other is the "active" subthreshold response; it becomes apparent as an excessive depolarization under the cathode as the shock strength attains about ½ or more of the threshold, and the return to E_m' is increasingly delayed or may even be replaced with an additional transitory, abortive depolarization.

These subthreshold, non-linear potentials were once designated "local" responses in the belief that they represented activity of a localized region that had not become sufficiently extensive to be propagated (*e.g.*, 181). But the same phenomena occur in space-clamped axons (150) and at single nodes of Ranvier (123, 196, 208, 210, 214) as well as in invertebrate fibers generally (96, 171), in skeletal muscle (*e.g.*, 249*I*, 265*I*), and in cardiac fibers (37, 42), and therefore reflect a general process inherent in the membrane itself. Stämpfli's suggestion that they be called "subthreshold" or "subliminal" potentials (455*I*) is therefore followed.

Hodgkin and Huxley (105) have shown that their equations predict active subthreshold potentials comparable to those obtained experimentally. These potentials reflect the rise in G_{Na} followed by inactivation and a rise in G_K as

in oscillations, but the latter variables are more delayed and their levels are lower so that the subsidence of potential is asymptotic or damped rather than oscillatory.

That the subthreshold potentials, like the oscillations, are related to the changes in G_{Na} and G_K underlying the spike itself is shown by their depression by the lowering of $[Na]_o$ (235), by elevated $[Ca]_o$ (6), and by the presence of a refractory state (inactivation) for these responses following a single active subliminal response (171). Conductance increases can also be seen to result from subthreshold shocks (31, 264*I*).

When the increase in the active subthreshold response lowers E_m to a critical value, E_T, I_{Na} begins to outstrip I_K and inactivation; E_m continues to fall, which further increases P_{Na} and therefore I_{Na}, which still further decreases E_m, *etc.* Hence, an explosive decline in E_m occurs—the all-or-none response—by virtue of the regenerative "chain-reaction" of subsequent events. The minimum amount of membrane depolarization which initiates the spike constitutes the "threshold of excitation", V_T. This Cole finds to be truly critical in the Hodgkin-Huxley equations, for a difference of only 1 part in 60,000 in a current pulse of 10 μsec duration determines whether the calculations predict a regenerative spike or only a subthreshold response (29a, 30). In the axon, of course, spontaneous fluctuations in the excitability, such as occur in other cells, would prevent so critical a threshold (Section i below).

Cole (29a) finds that the Hodgkin-Huxley equations predict a very narrow range of V_T (varying less than 1 part in 50,000) within which the spike amplitude theoretically may assume intermediate amplitudes. The spontaneous fluctuations of the membrane normally would prevent the demonstration of such intermediate states. However, this provides a basis for the observation that under certain conditions, *e.g.*, stabilization, completely graded action potentials are demonstrable (*e.g.*, 220).

f. Refractory period. Hodgkin and Huxley (105) point out that in the squid axons two factors contribute to the ability of the fiber to respond during and immediately following the spike—the growth and later subsidence of both P_K and inactivation. While the former is large, any attempt to depolarize the membrane causes a large outward current (I_K) which will largely neutralize any inward current (I_{Na}) that may arise; and while the latter is large, little I_{Na} can develop to generate the spike. The computations of recovery of the spike generating mechanism as a function of time after a previous spike agree well with observation (105).

g. Conductance changes. It has already been pointed out that, in keeping with the Hodgkin-Huxley equations, in squid axon the initial large increase in conductance during the spike outlasts the latter and declines in two distinct stages (Section b above). The initial increase in conductance is further identified as G_{Na} by virtue of its decrease with $[Na]_o$, while its decrease with elevated $[K]_o$ has been shown to be in keeping with the inactivation to be expected from the effect of $[K]_o$ on E_m' (83).

A careful study of the later conductance changes following the spike has revealed that the final decline occurs far more rapidly than expected and, es-

pecially at higher temperatures, falls well below the resting level—substantially more than expected from the equations for the hyperpolarization during PP—prior to returning to the original value at rest (4, 203). This is not a serious discrepancy, for it probably merely indicates that one of the empirical equations does not relate G_K to E_m properly; this can be seen to some extent in the depolarization range of figure 5 in (105), for G_K actually decreases more rapidly with increase in E_m than the empirical curve, a difference that might be even more marked in the hyperpolarization range, for which, unfortunately, no experimental data are provided. The effect of this late decrease in G_m (probably G_K) would be to slow the subsidence of PP and to reduce the tendency of the fiber to oscillate.

Oscillations in a veratrine-treated giant axon are accompanied by fluctuations in membrane conductance; an examination was not attempted of the phase relations of the potential and conductance changes, although from the records it appears that the first wave of increased conductance stops more abruptly than would be expected from the Hodgkin-Huxley equations (203). Veratrine also tends to eliminate the decline of G_m below the resting value (203), but this may be due to the accumulation of extracellular potassium, which also depresses the phase of increased resistance (4). Subthreshold fluctuations in conductance during the application of weak cathodal currents to squid axons are also apparent in early records (31).

h. Ionic movement. The increase in P_{Na} and in P_K and the temporary decline and reversal of membrane potential during the spike would be expected to lead to a gain in sodium and a loss in potassium with each impulse. Equation IV may be used to predict the *minimum* charge that sodium must provide by diffusion to change the potential difference across 1 cm^2 of squid fiber capacitance from the resting potential to the reversal potential; this also gives the minimum amount to be supplied by potassium to recharge the membrane. With C_m equal to 10^{-6} farad, and the total change of potential 100 mV, Q is 10^{-7} coulomb; dividing this by the Faraday (ca. 10^5 coulomb/equiv.) gives 10^{-12} moles of sodium and of potassium as the least that can produce the spike. In squid and *Sepia* the actual shifts per impulse are reported to lie between 3 and 4 times this figure, the larger one probably being correct (268*I*, 273*I*, 419*I*); so more than enough ions are transferred for spike production. (See also 82 and 374*I*.)

The Hodgkin and Huxley analysis permits a more exact determination of ion movement—unidirectional as well as net (table 5 in 105). It predicts net shifts of about 4×10^{-12} mole/impulse in squid axons at room temperature, in excellent agreement with available data. Moreover, the voltage-clamp studies had shown that low temperature prolonged the duration of the permeability changes without appreciably interfering with the magnitude of the permeability increases (in keeping with general prolongation of spikes by low temperature), on the basis of which a large increase in net ionic movement was predicted at low temperatures. The expected increase—slightly smaller but in keeping with the lower temperature coefficient exhibited by the observed action potentials—was subse-

quently found (419*I*). Similar increases in potassium loss during activity at lower temperature have also been described for crab fibers (419*I*).

All the predicted unidirectional fluxes, when compared with those of *Sepia* (268*I*), are not in as good agreement. Thus, the expected increment in potassium outflux is within 25% of that found (at a slightly lower temperature), but the predicted small increment in influxes is more than four times too high. The single-file movement of potassium, which has since been proposed to explain the reduction in flux in one direction by an increase in flux in the opposite direction, is now believed to account at least in part for the much smaller increment in influx actually observed (226*I*; see Part I). Sodium influx in *Sepia* increases about twice as much as predicted for squid, and outflux over six times more than expected; in view of the evidence for sodium transfer in other than ionic form in the unexcited membrane (Part I), these discrepancies may merely indicate enhanced movement of sodium by exchange diffusion or possibly as part of an ion pair (Section IV G 2).

i. Recapitulation. The technical approach designated the "voltage-clamp" has proved a potent tool for analyzing the concomitant changes in membrane resistance, R_m (or conductance, G_m), and the intrinsic e.m.f., E, in squid axons. R_m changes are attributable to fluctuations in P_{Na} and P_K, which also determine the changes in E, since they govern whether E_{Na} or E_K dominates the membrane potential. The initial part of the spike is due predominantly to an increase in P_{Na}, which leads to an inrush of sodium ions (I_{Na}) that discharges and reverses the charge on the membrane capacitance; this constitutes the rising phase of the spike. The falling or repolarization phase results from the decrease in P_{Na}, brought about by an unidentified process designated "inactivation."

Although outward flow of potassium ions (I_K) would still occur and restore E_m as P_{Na} comes back to normal, we actually find that I_K, and hence repolarization, is accentuated in the giant axon by an increase in P_K as well. Because of its delay in arising, the increased P_K is present chiefly during the falling phase of the spike and during the initial part of the positive potential. The positive potential is a consequence of the elevated P_K and of the fact that the resting potential is less than E_K.

This viewpoint is impressive because of the variety of phenomena accounted for quantitatively and qualitatively in the squid giant axon: Magnitude of the reversal of the membrane potential at the peak of the spike and its dependence on $[Na]_o$, $[Na]_i$, $[K]_o$, and E'_m; the positive potential and its dependence on $[K]_o$ and E'_m; oscillations and their alteration with stabilizers, $[K]_o$, and low $[Na]_o$; the active subthreshold response and the presence of a threshold; the absolutely and relatively refractory periods; the conductance changes accompanying the spike (and the oscillations and subthreshold response?) and their dependence on $[Na]_o$ and $[K]_o$; and the magnitudes of sodium and potassium interchange with activity. Some indication has already been given of the generality of these features in other systems. This will now be examined in greater detail. Then, the possible relation of these and other general principles to the after-potentials, the plateau, subthreshold potentials, and excitation phenomena, especially as affected by drugs and ions, will be considered.

It should be noted that oscillatory fluctuations of membrane current have been observed under voltage-clamp conditions in the squid giant axon (157*I*, 216, 432*I*) and more

recently at nodes of Ranvier (48, 217) when clamp voltages are in the vicinity of the normal threshold. At least two explanations can be offered: (a) That it is a technical artifact due to incomplete clamping by virtue of excessive resistance between different parts of the internal current electrode and the protoplasm (157*I*) or between the external electrodes and the active membrane (73), or (b) that it is a consequence of different patches of the membrane being in slightly different states, perhaps by virtue of random fluctuations in physical state (48). Frankenhaeuser asserts that such oscillations result when feedback is inadequate, and finds that they disappear with adequate clamping (73). It would be interesting to determine whether changes in tonicity of solutions, which appear to change diffusion resistance to potassium at the nodes of Ranvier (Part I) may augment or depress such oscillations. On the other hand, the long-known random fluctuations of excitability (*e.g.*, 58, 166, 193) and more recently observed "quantal" fluctuations in subthreshold response at single nodes of Ranvier (51) are in keeping with the second possibility. Available data are insufficient to reject or accept one of the alternatives completely. In either case, however, the general principles elucidated by the studies of Hodgkin, Huxley, and Katz rest on so broad a foundation that serious revision of the concepts as applied to the giant axon appears unlikely on the basis of such findings. It is more likely that refinements of the details will be the outcome of these and future studies.

B. Applicability to other excitable systems

1. Node of Ranvier. a. Voltage-clamp. This is now being applied to single nodes (50, 215). Fresh nodes give evidence of both inward and outward currents, whereas those which have been subjected to repetitive activity, which prolongs the spike without appreciably affecting the amplitude (206), exhibit only an inward current that suddenly shuts off (50). As pointed out in Section IV A 2 i, only an increase in P_{Na} well above P_K is required to produce the rising phase of the spike, and only the decline of P_{Na} to normal (through inactivation) is needed to restore the resting potential; this apparently can be the case at the node. The fact that the increase in P_K may or may not be present indicates that it may not play an important part and that inactivation is not necessarily linked with it. The parallelism between the conductance and potential changes of the spike have been indicated to be inconsistent with a secondary increase in P_K (480*I*, 481*I*). The inward current during voltage clamp is depressed by low $[Na]_o$ (215).

These observations raise several important questions: Do fibers with short time constants (in Table 1 of Part I this is seen to be only 40 μsec at the node) dispense with the increase in P_K? Does the increase in P_K have a threshold, as also suggested by the long delay in its appearance in giant axons; if so, what determines its initiation? The sudden inactivation observed by del Castillo *et al.* (50) also is suggestive of a threshold for this process, an observation of special importance in view of the sudden secondary decline in excitability observed in nerve trunks during the flow of constant cathodal currents (56).

b. Rising phase of the spike. This was seen in Section III B to exhibit all the properties to be expected if an increase in P_{Na} is the responsible factor: Reversal of membrane potential under normal conditions and decrease in rate and amplitude in low $[Na]_o$ when E'_m is low (electrically or with excess $[K]_o$) and during the refractory period. To this may be added its association with an inward current under voltage clamp conditions (Section a above) and with a large decrease in membrane conductance (393*I*, 480*I*, 481*I*).

In multifiber systems the uncertainties of determining $[Na]_i$ because of the large error introduced by having to correct for a large $[Na]_o$ in the extracellular

space, as well as the possibility of inhomogeneity in the fiber population (Part I), render strict comparison of gross tissue analyses with single fiber potentials of questionable value. However, we may note that in toad fibers, for which $[Na]_i$ has been estimated with a number of precautions, $[Na]_i/[Na]_o$ is about $\frac{1}{3}$ (428*I*). Hence, $E_{Na} = -28$mV. The spike of single fibers averages 101 to 102 mV (479*I*). If the resting potential is 70 mV as in frog fibers (246*I*), the reversal potential is 31 to 32 mV, which is closer than one would expect from the possible sources of error.

The findings by Lüttgau (146a) with guanidine hydrochloride (Gua^+), described in Section III B 3, are most easily interpreted in terms of its penetration at the same sites as Na^+ during excitation, but more slowly. Since the number of sites is limited, as shown by the maximum G_{Na} obtainable with hyperpolarization, the presence of Gua^+ when $[Na]_o$ is high would interfere with the magnitude of inward current, as observed, for it would compete with Na^+ for these sites; on the other hand, when $[Na]_o$ is low, I_{Gua} will be additive to I_{Na} since adequate sites are now available for both Gua^+ and Na^+, also as observed.

The possibility that guanidine may exert a mild stabilizing action cannot be ruled out; its decrease of E'_m, associated with an increase in R_m, suggests a reduction of P_K in the resting cell. However, since in guanidine when $[Na]_o$ is low the inward current (rising phase of the spike) is greater, a careful comparison of I_{Na} and I_{Gua} individually (*i.e.*, in the absence of each other) and together is needed to establish whether the increase in P_{Na} is interfered with by Gua^+.

Data on the conductance changes during the spike and the fluxes of Na^+, K^+, and Gua^+, at rest and during activity, should clarify the action of guanidine hydrochloride.

c. Falling phase of the spike. This frequently occurs in two stages—a slow fall followed by a sudden rapid one. Thus, one is led to suspect that somewhat other details are involved in its production than in the falling phase of the spike in giant axons. This has been verified by conductance studies showing a close parallelism between the decline in potential and conductance (480*I*, 481*I*); in such preparations potassium conductance changes may be lacking or poorly developed, as observed with the voltage-clamp, so that the decline is slower and is governed solely by inactivation of G_{Na}, as suggested by Lüttgau (326*I*) from the time course of development of inactivation during the plateau (see Sections III B 12 a and IV A 2 f).

In large single fibers, the node and adjacent internode (1 mm) have a combined capacitance of 3 $\mu\mu$F and a resistance of 36 megohm (478*I*), or τ_m of 100 μsec. Therefore, in contrast to the situation in giant axons or skeletal and heart muscle fibers, C_m may not limit the rate of potential change; hence, especially in the falling phase, E_m at any moment can be governed directly by the relative magnitudes of G_{Na} and G_K.

d. The positive potential. The appearance of this phenomenon in sufficiently depolarized single fibers (Section III C 1) is indicative of the increase in P_K under these conditions. The usual absence of PP at ordinary resting levels, although E'_m is below E_K, again suggests P_K may not alter under these conditions.

e. Delayed rectification. It was pointed out in Section IV A 2 c that this process, reflected by a delayed partial repolarization during a cathodal depolarization and by a transitory hyperpolarization at the end of the depolarization, is charac-

teristic of a delayed increase in P_K during the depolarization. Both the partial repolarization and decrease in resistance have been observed in desheathed nerve, but with depolarizations close to stimulating intensities and larger (395*I*). The observations by Frankenhaeuser (72) on single nodes confirm these findings and show, further, that at low $[Ca]_o$ rectification occurs with weaker currents. These electrical changes have also been seen in spinal roots of mammals with make and break of cathodal current (372*I*).

f. Ionic transfer. The failure of early efforts to demonstrate ionic movement during vertebrate nerve activity (*e.g.*, 61), and the delay in the appearance of potassium during repetitive stimulation (5), were undoubtedly due at least in part to the low penetrability of the sheath (epineurium) surrounding the nerve trunk (Part I). A similar delay in the appearance of potassium in the medium during metabolic inhibition has been shown to be quantitatively accounted for by the low permeability of the sheath (414*I*). Removal of the epineurium from bullfrog nerve increases the amount of potassium appearing per impulse by almost fifteen-fold to 20 $\mu\mu$mole/g, and this figure is obtained for low (20 stimuli/sec) as well as high (100 stimuli/sec) rates of stimulation (411*I*).

Hodgkin (219*I*) estimates the effective capacitance of whole nerve to be 8 μF/g. For a spike of 100 mV (479*I*), this represents $8 \times 10^{-6} \times 0.1/10^5$ or 8 $\mu\mu$mole/g minimum charge required to generate a spike. It will be recalled that in cephalopod axons the actual amount of sodium or potassium transferred was four times the minimum. We see that vertebrate nerve is similar in actually transferring almost three times more of the ions than required. In both cases the additional interchange may be attributed to the heightened permeability lasting longer than required to simply charge and discharge C_m.

It should be noted that under the conditions employed for study of nodal spikes, 1 mm of internode is included with the node, and this has a total capacitance of 3$\mu\mu$F, which for a 100 mV spike represents 3×10^{-13} coulomb. The charge drawn from a node (ca. ½ peak action current × time) at room temperature averages about 10^{-12} coulomb (206, 207, 478*I*), and hence capacitative charging is 30% of the total charge delivered by a spike, in good agreement with the ratio of estimated capacitative ionic transfer to the transfer actually obtained above.

A recent preliminary note reports that in frog nerve potassium outflux is increased by stimulation but influx is not significantly changed (54). These results are comparable to the findings with cephalopod axons (Section A 2 h above). A rise in P_{Na} alone would increase O_K but would also reduce I_K (see Section V 2 a in Part I). The lack of a change in I_K therefore suggests some increase in P_K.

With respect to the properties discussed, therefore, nodose fibers conform to the pattern observed in squid axons. The increase in P_K may or may not occur, and when it does, it usually requires a greater degree of depolarization.

2. Cardiac fibers. a. Rising phase of the spike. Weidmann's studies with Purkinje fibers provide important support for the similar role of increased P_{Na} in the production of this part of the spike. The pertinent data are (a) the dependence of the initial part of the spike on E'_m, (b) the similarity of the time course of

recovery of spike generation, upon termination of a sustained depolarization or following a spike, to subsidence of inactivation, and (c) the dependence of the rising phase on $[Na]_o$ (Section III B). His additional demonstration of a large decrease (to about 1%) in membrane conductance during this period (figure 7 in 229; figure 16 in 512*I*), is additional confirmation. The general dependence of spike production on membrane potential in other cardiac fibers under a variety of depolarizing or hyperpolarizing conditions has been pointed out (Section III B 2; 512*I*); it has also been noted that the decline of the spike during metabolic inhibition, when E'_m is not affected, could be due to an increase in $[Na]_i$ (Section III B 8), but studies of the sodium content of the fibers under these conditions remain to be carried out.

All cardiac fibers reverse the membrane potential at the peak of the spike, and the magnitudes of S_m are consistent with estimates of $[Na]_i$ and $[Na]_o$ (42, 512*I*).

b. The positive potential. The sudden transition from the end of the plateau to a maximum E_m, which then subsides, has been shown to duplicate the behavior of PP in squid axons in that its magnitude depends on E'_m being below E_K at the start of the spike; thus, PP is reduced in low $[Na]_o$, which raises E'_m towards E_K, and by elevated $[K]_o$, which lowers E_K towards E'_m (Section III C). The finding that membrane conductance undergoes more than a three-fold increase during PP development, followed by a slow decline (229), further emphasizes the homology to the squid PP. It will be recalled, too, that stimulation of the vagus, which increases P_K (Part I), augments the hyperpolarization (Section III C 5).

The considerable delay in the development of an increased G_m—presumably G_K—is not altogether a new effect. Attention was called in Section IV A 1 to the delay in the squid axon in the turning on of G_K. The absence of an increase in G_K in repetitively stimulated nodes of Ranvier may reflect a similar delay. Membrane calcium may be an important factor, for high $[Ca]_o$, or elevated E'_m, prolongs the delay in squid axons and at nodes of Ranvier (72, 157*I*). In keeping with these observations is Weidmann's suggestion that one factor underlying an increase in G_K and PP is a minimum period of adequate depolarization; in fibers which do not develop a plateau, PP is lacking, but when the membrane is kept depolarized electrically from the beginning of such spikes for a time corresponding to the duration of a plateau, upon removal of the depolarizing current an overshoot similar to PP is obtained (511*I*). Weidmann (512*I*) also calls attention to his failure to obtain early delayed rectification as further indication of the relative inertness of P_K in Purkinje fibers.

c. The diastolic depolarization. The slow decline in membrane potential is that to be expected from the fall in G_K suggested by the gradual decrease in G_m during this period. The fact that low $[Na]_o$ reduces the diastolic depolarization has been suggested to indicate the involvement of sodium (512*I*). But we should recognize that this effect is also easily explained in terms of G_K. Thus, the effectiveness of a decrease in G_K in causing depolarization depends on the presence of a small leak to sodium, for if P_{Na} is zero, or E'_m is close to E_K by virtue of

no extracellular sodium, then a change in P_K can have no effect on membrane potential (see the Goldman equation in Part I).

Coraboeuf *et al.* (34, 37) report that in aged Purkinje fibers, in which E_m' is low, the rise in E_m with sustained, inwardly directed (hyperpolarizing) current is followed by a very weak secondary depolarization during the continuous flow of current, giving the effect of a weak overshoot or hyperpolarization. Further maintenance of the membrane in a hyperpolarized state, followed by a depolarization and repolarization, reveals an improved overshoot, and this can be repeated with still further improvement in the distinctness of the overshoot; in fact, the secondary depolarization following the overshoot may become large enough to initiate a spike. The improved secondary depolarization may be related to a substantial lowering of G_K and G_{Na} by the prior hyperpolarization (see Section IV F 1), which thereby restores the effectiveness of the fall in G_K during diastolic depolarization. Conductance data are needed for a more complete evaluation of the situation.

d. Ionic transfer. Wilde *et al.* (232, 233) have attempted to correlate the pulsatile release of K^{42} from "hot" turtle ventricle with specific parts of the action potential. Since collections were by way of the venous system of the heart, an uncertain time delay was involved. A "slug" of K^{42} injected into the coronary arteries provided a correction for delay and dispersal in the blood vessels; on the basis of this, the major release of potassium appears to occur at the time of development of the positive potential, when P_K is increased. However, the possibility of another delay to be corrected for—that due to the diffusion time from the cells to the capillaries—could place the potassium loss earlier in the cycle. Weidmann points out that, even if there were no change in P_K during the spike, the sustained depolarization during the plateau could account for a large part of the increased K^{42} liberation with activity (512*I*).

If we take the resting potential as 58 mV, and the plateau potential as zero, then from the former the resting potassium outflux is given by equation III in Part I:

$$O_K = [K]_i(P_K E_m/25)(e^{E/25} - 1)^{-1} = 0.25[K]_i P_K$$

whereas when $E_m = 0$,

$$O_K = [K]_i P_K.$$

Hence, the outflux during the plateau would be four times that during diastole if the intervals are equally long. Since Wilde (quoted by 42) estimates the actual factor to be about 8, this would leave additional potassium loss to be accounted for during the early part of the spike and at the termination of the plateau.

Weidmann (512*I*) estimates a factor of ten times by assuming a larger resting potential and a reversed potential during the plateau. However, the *in vitro* preparations of Wilde *et al.* are more likely to have had lower values of E_m' and of plateau potential [see the turtle auricle figures in table 1 of (42)].

Flux measurements carried out at low temperature, which would lower the heart rate, prolong the spike, and increase the ionic interchange of activity, might more clearly indicate the time and sequence of augmented potassium release.

The net potassium loss per contraction in frog heart, under conditions that prevent active potassium reabsorption, has been estimated by Hajdu (192*I*) to be 20 $\mu\mu$mole/cm^2. This is five-fold larger than in squid axon and twice that

in skeletal muscle, in keeping with the relative capacitances of the fibers (see Table I in Part I and Section 3c below).

3. Skeletal muscle fibers. a. Rising phase of the spike. Again with respect to its dependence on $[Na]_o$, $[Na]_i$, E'_m, and its delay in responding to prior depolarization (Sections III B 2 and 3), the initial part of the spike exhibits the familiar characteristics. The associated decrease in membrane conductance has also been observed (120*I*, 191*I*, 264*I*).

In fresh frog muscle, the reversal potential was found to be 33 compared to a calculated E_{Na} of 46 mV; after conditions causing a gain in sodium, S_m was reduced to 13 mV compared to the corresponding E_{Na} of 11 mV (92*I*).

Shaw *et al.* (438*I*) report that, as $[Na]_o$ is increased, $[Na]_i$ increases in proportion; osmotic changes may have contributed to this. In any case, it is in keeping with the constancy of S_m. Although the same dependence of $[Na]_i$ on $[Na]_o$ appeared to hold when $[Na]_o$ was reduced by replacement with sucrose, the spike was found to be lowered. Whether this is due to a discrepancy in the theory, as Shaw *et al.* maintain or to other factors, *e.g.*, uncertainties in the estimate of $[Na]_i$ (see Part I), or insufficient time for $[Na]_i$ to change prior to electrical measurements, remains to be seen. We may note that they also report a lowering of E'_m in ½ and ⅓ normal $[Na]_o$; this is contrary to the findings of others and suggests that the leakage of potassium which occurs in low sodium raised $[K]_o$ around the fibers (see Part I).

b. Falling phase of the spike. Transverse impedance measurements on whole muscle indicate a substantially longer period of augmented conductance than the duration of the spike (264*I*); a second distinct increase in conductance following that associated with the rise phase has been described for single fibers (120*I*, 191*I*). The absence of a positive potential has been pointed out to be the consequence of the proximity of E'_m to E_K; the fact that PP becomes evident when E'_m is reduced (Section III C 1) indicates the presence of an increase in G_K under these conditions.

c. Ionic transfer. Many studies are available showing the release of potassium and uptake of sodium with muscle activity (*e.g.*, 149*I*). In general, these do not lend themselves to a quantitative comparison with the electrical changes. However, data provided by Fenn and Cobb (63) on rat muscle are particularly complete and permit a preliminary calculation. Thus, stimulation at a rate of 400 shocks/min for 30 min gave contractions sustained at ⅓ to ½ the maximum tension; hence, approximately 3,000 to 4,000 stimuli were effective. About 17 μmole potassium/g wet weight was released. If each muscle fiber is taken as 100 μ in diameter, and the fibers represent that fraction of the total muscle given by the ratio of fiber water to total water (60/75), then the total surface/g wet weight is 310 cm^2. Hence, roughly 17/(310 × 3000) or 17/(310 × 4000) μmoles or 14 to 18 $\mu\mu$ mole/cm^2 is the potassium released per impulse. The sodium gain was slightly larger (17 to 21 $\mu\mu$mole/cm^2), and chloride gain was about equal to their difference.

In mammalian muscle the spike is 120 mV (500*I*), consequently with C_m about 5 $\mu F/cm^2$ (see Part I, Table 1, p. 71), the minimum sodium or potassium required to discharge or recharge C_m is 6 $\mu\mu$mole/cm^2. Thus, as with squid axon and vertebrate nerve, the actual amount of ionic exchange is several-fold greater than the minimum required to discharge and recharge the membrane.

We should note that Fenn and his associates find a gain of Na^+ in excess of K^+ lost, the difference being made up by Cl^- uptake; Ca^{++}, Mg^{++}, and total phosphate were unchanged (63, 64).

After this review was completed, Hodgkin (98) provided figures on unidirectional fluxes obtained during an impulse with single frog muscle fibers: $I_{Na} = 18.8$, $O_{Na} = 3.9$, hence net sodium gain of 14.9 $\mu\mu$mole/cm^2; $I_K = 2.0$, $O_K = 11.3$, hence net potassium loss of 9.3 $\mu\mu$mole/cm^2. The net figures are surprisingly close to the rough estimates based on the Fenn and Cobb data.

C. Negative after-potential

It has already been pointed out that one distinct type of negative after-potential is that due to elevation in $[K]_o$ because of the accumulation of potassium released with each impulse—as seen in the squid giant axon and in repetitively stimulated crab nerve (Section III E). NAP brought about by the alkaloids cevadine and veratridine involves another mechanism because, although the potassium released is increased, the amount is smaller in crab nerve (412*I*, 415*I*) and in frog nerve (411*I*, 415*I*) than can account for the augmented after-potentials seen following individual spikes; also, the marked difference in the time course of NAP with the two alkaloids cannot be accounted for by a single mechanism such as potassium accumulation (Section III E).

1. The veratrine-induced negative after-potential. Impedance studies on the squid axon reveal that the after-potentials induced by the alkaloids are paralleled exactly by a small elevated membrane conductance, and that the distinctive effects of cevadine and veratridine on the time constant of decline are exerted in each other's presence, as in the case of the after-potentials (203). It was pointed out that a conductance increase to potassium alone could not be involved, since this would lead to a hyperpolarization (see Section III D 3 in Part I); an increase in G_{Na} alone could account for the direction of the change but could not have been as large as indicated by G_m. An increase in G_{Cl} would provide the proper magnitude and direction if assumptions of a high resting P_{Cl} (221*I*) were correct and because $[Cl]_i$ is greater than expected from E'_m (Part I). But there is reason to suspect that P_{Cl} is not as great as believed (Part I), and it was noted that NAP is reduced by low $[Na]_o$ (203).

In view of the evidence for an increase in P_K and especially in P_{Na} by higher concentrations of veratrine and veratridine (Part I), it is tentatively proposed that at the lower concentrations these alkaloids produce a similar effect by occupying sites made available during the spike, and that they are gradually displaced, each pure alkaloid with a time constant dependent on its own characteristic affinity (association constant) for these sites, as indicated by the time constant of after-potential and of conductance decline.

The primary event, as at high veratrine concentrations, can be looked upon as a maintained, higher P_{Na} at the end of the spike which underlies the depolarization. This in itself is too small to affect G_m, but we may inquire what is the effect of the lower E_m at the end of the spike on the level of G_K, for it will be recalled that G_K is an inverse function of E_m (Section IV A 1 and Part I). Veratridine produces a 1 mV NAP under moist chamber conditions that give S equal to 36 mV (405*I*, 415*I*). Therefore, this is equivalent to a trans-

membrane potential of about 3 mV. Since a 5 mV decrease in E_m will increase G_K 2.7-fold, if G_K is about that of G_m (with R_m about 1500 ohm cm², G_m is 0.7 mmho/cm²)[3] the increment in this case will be about 1.2 mmho/cm². For 3 mV the increment will be about 1 mmho/cm². Hodgkin and Huxley (105) point out that the peak conductance during the spike, determined experimentally and by calculation, lies between 31 and 53 mmho/cm², and hence the maximum elevated conductance associated with NAP under veratridine would lie between 2 and 3% of the conductance increase during the spike. Shanes *et al.* (203) found the figure to average 2.6%. Such agreement suggests, then, that the conductance changes during the veratrine alkaloid-induced NAP may be a *consequence* of the delayed repolarization, resulting from a slightly raised P_{Na}, rather than the *cause* of NAP. This viewpoint is also consistent with the requirement for extracellular sodium in the production of NAP and with the augmented release of potassium with activity in the presence of the veratrine alkaloids.

The available data do not rule out the possibility that P_{Cl} may also be increased to some extent, a possibility which may be examined by replacing chloride with less permeating anions, as described below.

An interesting problem is the basis of the increase in NAP amplitude by calcium that occurs without a change in the time constant of repolarization. At present, the simplest explanation appears to be that Ca^{++} normally occupies the sites to which the alkaloids become attached after a spike and thereby prevents the alkaloids, at low concentration, from occupying them until the spike occurs; this is consistent with (a) the increased oscillatory behavior with successive spikes, such as would occur with progressive lowering of $[Ca]_o$ (Section III D), and (b) the lack of spontaneous firing unless one or more spikes are first produced (Section III D). From this standpoint, the higher $[Ca]_o$, the more sites will be occupied and protected by Ca^{++}, and the more sites will be released during the spike, when calcium is temporarily depleted from the surface (see Sections IV F 1 and G 2, 3). The unchanged time constant of repolarization in elevated $[Ca]_o$ is consistent with this view.

That a competition may exist between veratrine alkaloids and Ca^{++} is suggested by the depolarization of resting fibers produced by higher veratrine concentrations, and the counteraction of such depolarization by increase in $[Ca]_o$ (Part I). It must be remembered that other stabilizers, such as local anesthetics, also act in this way. However, unlike Ca^{++}, they *depress* the veratrine alkaloid-induced NAP. This difference between Ca^{++} and local anesthetics with respect to NAP amplitude focuses attention on the fact that their mechanism of action in reducing P_{Na} is not identical, although the final effect is essentially the same insofar as they reduce slow electrochemical changes (Part I). A similar conclusion may be drawn from the difference in their actions on the spike. This will be discussed further in Sections IV F and G.

The slowness with which the veratrine-induced NAP disappears after removal of the alkaloid mixture from the medium may reflect good initial penetration of the alkaloids into the fibers, perhaps with substantial adsorption on protoplasmic components, which then provides a reservoir of alkaloids for repeated NAP's. In any case, clarification of the mechanisms involved may be anticipated from studies of the kinetics of penetration and emergence of these alkaloids in resting and active fibers—preferably in a preparation such as the giant axon where the contribution of axoplasm can be distinguished from other structures.

2. The naturally occurring negative after-potential. It was pointed out that, in vertebrate nerve, which is composed largely of A fibers, in C fibers, and in skeletal muscle, the NAP appears to arise at a critical level of membrane po-

[3] Frankenhaeuser and Hodgkin (158*l*) note that previous neglect of the fact that the potassium concentration at the surface of the fibers does not decline at low $[K]_o$ because of leakage led to an underestimate of P_K from E_m-$[K]_o$ curves.

tential; the magnitude of the slow repolarization that proceeds from this potential depends on the extent to which E'_m has been raised above this critical potential.

One possible interpretation is that, as with veratrine-induced NAP, a slow stage of P_{Na} decline occurs above a certain value of E'_m. The similarity of the action of raised $[Ca]_o$ in increasing NAP in veratrine-treated and in untreated sciatic nerve may be indicative of the same mechanism. Information on the effect of $[Na]_o$ is desirable. An additional possibility, and not necessarily exclusive of the preceding one, is that P_{Cl} is raised during the spike. If, as may be the case in squid axon (273*I*), sciatic nerve (427*I*, 428*I*), and muscle (18*I*, 138*I*), $[Cl]_i$ is larger than expected from the resting potential, an increase in P_{Cl} will cause a depolarization towards the chloride equilibrium potential, E_{Cl}, which is less than E'_m. It was pointed out in Part I that the high $[Cl]_i$ might be the consequence of entry of Cl^- partly in undissociated form, as appears to be the case with Na^+, so that its passage through the resting membrane is not completely governed by E'_m. Thus, if P_{Cl} is appreciably elevated at the end of the spike, the repolarization will return to a value approximating E_{Cl}, and then, as P_{Cl} falls, E_m will rise gradually towards E_K.

If an increase in P_{Cl} is indeed involved, replacement of extracellular chloride with a less penetrating anion should augment the amplitude of the negative after-potential. It will be recalled from Part I that conductance and penetration experiments with potassium salts indicate that muscle permeability to anions decreases in the sequence $Cl^- > Br^- > NO^-_3 > I^- > SCN^-$. An increase in anion permeability, with a slowly penetrating anion like SCN^- outside while Cl^- is inside, should therefore cause a greater depolarization and therefore a larger negative after-potential because anion influx is smaller than outflux; extracellular I^- and NO^-_3 could be expected to be less effective in augmenting NAP since their influx would be closer to that of chloride when anion permeability is raised. These considerations therefore provide a possible basis for the relative effectiveness of SCN^- and other anions in increasing the negative after-potential in muscle. The increased NAP in muscle fibers soaked in Ringer (69a) would follow from the gain in $[Cl]_i$ under such conditions (138*I*). Moreover, the insensitivity of muscle NAP to changes in $[Na]_o$ (69a) suggests that P_{Cl} is more important than P_{Na} in this case.

It remains to be seen how sensitive the negative after-potential in other fibers is to $[Na]_o$ and to the anions of the milieu. Also, it is important to determine whether the rising phase, noted earlier to be a variable phenomenon, is a genuine feature of NAP or merely an artifact due to muscle contraction. A true rising phase is not readily accounted for in terms of the principles discussed. As already mentioned, contraction can now be prevented by hypertonic solutions, which leave the action potential undisturbed.

In conclusion, the available data suggest that the negative after-potential may reflect 2 processes:

(a) A decline in the transitory increase in $[K]_o$ because of the liberation of potassium during the impulse.

(b) A delayed decrease in the augmented permeability to ions—at least to Na^+ and to Cl^-.

D. Positive after-potential

The information on this phenomenon is most meager, but it has been pointed out for crab nerve that considerable evidence supports the view that it may arise by depletion of potassium from around the fibers by active reabsorption and by the effect of this depletion on E_m. In C fibers the same process has been suggested to operate largely because of the dependence on metabolism. Since the rate of repolarization in C fibers is so rapid, the proposal was made in Section III F that the results are better understood from the standpoint of active sodium exclusion, which also causes hyperpolarization. Studies of potassium movement in C fibers may be expected to be definitive in determining the mechanism involved.

The well-known dependence of PAP in vertebrate nerve trunks on metabolism (see p. 301 in 311*I* for references) was more recently described by Lorente de Nó (his R_3 deflection, pp. 300 ff. in 311*I*). He points out its much greater sensitivity than NAP to anoxia, a difference understandable from the standpoint that NAP is not affected until $[K]_o$ rises as a result of leakage of potassium, whereas PAP requires active transport to be operative. It is significant that during post-anoxic recovery, when respiration (and active transport) is enhanced, PAP likewise is greater (311*I*). It should be emphasized that respiratory processes need not be expected to be the only source of energy; anaerobic glycolysis may also function for transport (Part I), which may explain Lorente de Nó's observation that a nerve in which conduction block (depolarization?) was slow in developing continued to produce a good PAP in the absence of oxygen.

If the above considerations are correct, the fact that yohimbine accentuates PAP, or causes one to appear where one was not apparent, suggests that the operation of augmented active transport following activity becomes more evident in the form of elevated membrane potential.

The ability of cocaine to counteract the leakage of K^+ and gain of Na^+ by veratrine-treated nerve provides a possible model for this action (Part I). Thus, fibers which have been losing K^+ under veratrine not only cease this loss but begin to absorb K^+ from the medium when cocaine is added, and undoubtedly repolarize as shown by the restoration of conduction in veratrine-blocked nerve (Part I). Evidently, the transport processes which were inadequate in the very leaky veratrinized fibers become more than adequate, without necessarily undergoing any change, when leakiness is reduced. It was also pointed out in Part I that cocaine affects predominantly the passive rather than the active transfer of ions. Hence, a stabilizer, by reducing general leakiness (especially to Na^+) will render the same transport processes more effective for the transfer of ions.

Whether an actual change in the intracellular ion content of the cells has occurred sufficient to produce even the small augmentation in after-potential in the invertebrate fibers is doubtful. More likely, especially in the light of the marked concomitant effect on spike production (Section III B 12 b), is an alteration of the ion distribution within the membrane itself. For example, it will be recalled from Part I that in vertebrate nerve, where membrane potential depends in part on "sodium exclusion", cocaine has no marked effect on active transport but can prevent failure of sodium exclusion from causing depolarization and increased Na_o^+-K_i^+ exchange; these effects would be explained if cocaine acted *below*, *i.e.*, further within the membrane, than at a more superficial site of sodium rejection. If, now, before a stabilizer is applied, sodium rejection is stimulated (as by activity), this will tend

to lower the effective sodium concentration—let us call this $[Na]_m$—at the outer layer of the membrane, but diffusion outward of sodium from the axoplasm limits the extent of this fall in $[Na]_m$. When a stabilizer (yohimbine) is present, it will slow the outward diffusion of sodium; hence $[Na]_m$ will fall further, and presumably E_m will rise higher, especially if it was originally well below E_K.

No doubt other possibilities may be proposed. But one additional feature they must share is a basis for the depressed entry of sodium ions during PAP, which, as the behavior of the rising phase of the spike indicates, occurs at the same time. The above proposal would require that the depletion of $[Na]_m$ is related to a limited availability of sodium ions for spike production.

On the basis of the suggestion which has been made, *viz.*, that the reduced inward leak of sodium, by virtue of augmented active sodium exclusion, is responsible for the positive after-potential, it follows that increase of $[K]_o$—beyond the range in which the exclusion process itself is dependent on it—will depress PAP. This should be particularly marked when E'_m is ordinarily substantially below E_K by virtue of an appreciably high P_{Na}, as in amphibian nerve, for the effect of active exclusion is comparable to a decrease in P_{Na} and thereby will elevate E_m towards the high E_K; if, now, $[K]_o$ is raised, E_K is brought closer to E_m, and hence, the same decrement in P_{Na} will cause a smaller increment in E_m. This effect by $[K]_o$ has been described (311*I*).

More extensive studies, including the role of ions in the milieu as well as ionic movements, especially in the presence of yohimbine, may be expected to provide important information for delineating more clearly the mechanisms involved.

E. The plateau

1. Cardiac fibers. Considerable information is at hand concerning the effect of ions and other factors on the characteristic plateau of heart fibers (Section III B). The effects, and possible interpretations in the light of the principles which have been elaborated, may be summarized as follows:

(a) Low $[Na]_o$ shortens it—inward leak of Na^+ normally contributes to the sustained depolarization.

(b) Elevated $[K]_o$ shortens it independent of its effect on E'_m—P_K normally is decreased during the plateau and thereby contributes to the depolarization.

(c) Metabolic inhibition shortens it—the rise in $[Na]_i$ it produces reduces inward leak of Na^+ and hence limits the depolarization.

(d) Cardic glycosides shorten it—the reduction of P_{Na} changes, shown by Dudel and Trautwein (55a) on the basis of the rapidly reduced rate of rise of the spike, decreases inward leak of Na^+; the less direct effect of suppressed active transport, resulting in a rise in $[Na]_i$ and a fall in $[K]_i$, would supplement the more direct membrane effect at later times.

(e) A longer interval between spikes lengthens it—the extent of the gradual fall in G_K during the previous diastolic period (Section IV B 2 c) determines its level at the time immediately after the rise phase of the spike and therefore the amplitude and duration of the plateau as in (b) above; this implies that G_K does not rise during the spike but rather continues to fall (see below). The finding of Trautwein and Dudel (493*I*) that an increase in the interval *instantane-*

ously and *fully* restores a larger plateau serves to eliminate a number of other possibilities; the same observers also reported that the processes concerned with the refractory period are not involved since spike amplitude and membrane potential are fully restored even at the shorter interval.

(f) Acetylcholine shortens the plateau—the increase in G_K it produces (Part I) augments I_K and causes repolarization.

To these must be added Weidmann's additional important observations on Purkinje fibers that (a) delayed rectification (and hence the secondary rise in G_K) does not occur except after cessation of long depolarizations in heart fibers and (b) that membrane conductance (G_K?) falls during the plateau as well as during the diastolic depolarization (229, 512*l*). These observations indicate that G_K continues to fall throughout the period from diastole to the end of the plateau, being uninfluenced by E_m, although this is not evident during the initial part of the spike because the increase in G_{Na}, which is in parallel, obscures the situation with respect to G_K during this period. This proposal is susceptible to more direct test by terminating the plateau and the initial part of the spike at different times with brief anodal pulses to determine G_m.

Assuming tentatively, then, that G_K falls continuously, we may predict that, as G_{Na} declines from its very high value during the initial part of the spike, a point will be reached when it nearly equals G_K; if E_K is about equal to E_{Na}, E_m is zero (Fig. 4). At this point inwardly directed I_{Na}, which tends to depolarize, is balanced by outwardly directed I_K, which tends to repolarize. Now if G_K and G_{Na} continue to fall together, the fiber remains depolarized while the membrane resistance rises, as observed during the plateau. This can continue to operate only as long as $I_{Na} = I_K$. If I_{Na} alone, or chiefly, is reduced (by decreasing $[Na]_o$, increasing $[Na]_i$, or by lowering P_{Na}), then the plateau will be shortened; if I_K alone, or chiefly, is increased (by raising G_K, as with elevated $[K]_o$, with acetylcholine, or with short intervals between spikes), then the plateau also will be shortened. On the basis of both G_K and G_{Na} remaining about equal and decreasing, then, the observations (a) to (f) above as well as the available conductance data appear to be satisfactorily correlated.

The effect on plateau duration of drugs, ions, or other experimental conditions will depend on which permeability is affected more. It will be recalled that while P_{Na} and P_K are generally altered in the same direction, one is often affected more than the other. If differences in membrane structure, such as may exist in different parts of the heart or in hearts from different species, can alter the relative effect of agents on P_{Na} and P_K, it follows that the effects of these agents on plateaus will differ depending on the preparation, as actually found (Section III and 42). Thus, when the plateau is lengthened the decline in P_{Na} may have been slowed or P_K may have been decreased; the reverse action on the permeabilities is to be expected when the plateau is shortened. A systematic examination of specific permeability and plateau effects by the many agents that have been studied in the past should determine the validity of this postulate. Of course, in such studies care would have to be exercised that other effects (*e.g.*, on active transport and therefore on $[Na]_i$ or $[K]_i$) are not present. Rapidity of action or evidence of effects on membrane conductance, as in the case of k-strophanthin (55a), are important criteria in this respect.

In the meantime, it is of interest that veratrine, which increases chiefly P_{Na} in other fibers, greatly prolongs the plateau; Ba^{++}, which seems particularly potent in decreasing P_K (Section VI A 2), also prolongs the plateau (Section III B). One may venture to predict a marked lengthening of the plateau by TEA if it depresses P_K more than P_{Na}, as in other

fibers. The lengthening of the plateau by low $[Ca]_o$ would be accounted for if, as in squid axons, the shut-off of increased G_{Na} is delayed (157*I*; see Section IV F 1).

The significance of an initial rising phase to the plateau, as has been described occasionally, remains to be determined.

The dependence of the plateau on experimental factors can therefore be accounted for qualitatively in terms of the special characteristic of a continuing fall in G_K throughout the systolic and diastolic periods except at the very end of the plateau and a comparable slow decline in G_{Na} during the plateau, with the result that I_{Na} is of the same order of magnitude as I_K. It would be desirable to test this viewpoint further, for example by observing whether the conductance changes during the plateau conform with those to be anticipated under the various experimental conditions that have been tried, and by examining the pulsatile release of K^{42} under the same conditions.

2. Tetraethylammonium-treated squid axons. The detailed observations by Tasaki and Hagiwara (218) on the marked prolongation of the spike by injected TEA provide another important test of the general applicability of the principles which have been suggested to underlie the plateau. Their data will be shown to provide a possible basis for the termination of the plateau.

The situation is unlike that in heart fibers in that the conductance remains, slightly above normal throughout the plateau and declines to, or slightly below normal during the termination of the plateau. Their figure 6 suggests a later increase in G_m corresponding to a positive potential which appears after the plateau. Their figures 10 and 12 are illuminating in showing that, at clamp depolarizations under 60 mV, the usual (but smaller) transient inward current is followed, not by an outward current, but rather by a small, very slowly declining *inward* current. This suggests that G_{Na} increases almost as before but that subsequently it does not decline completely to zero during the plateau; also, that an increase in G_K may not occur during the plateau. The conductance changes shown are in keeping with this. Thus, the sequence of events appears to be (a) an increase in G_{Na} well above resting G_K to give E_{Na} (the spike peak); (b) incomplete inactivation, so that when G_{Na} approaches G_K it then declines very slowly and E_m is about zero because E_K nearly equals E_{Na}; and (c) as E_m rises to a critical value (threshold of a secondary delayed inactivation?) G_{Na} returns quickly to normal while G_K temporarily increases. The dependence of the plateau on a high $[Na]_o$ as in cardiac fibers is also in keeping with the proposed sequence of events.

An additional striking feature is observed upon lowering $[Na]_o$ surrounding TEA-treated axons. The late currents observed during voltage-clamp are greatly increased and outward, a phenomenon also seen with TEA-treated axons in ordinary sea water only when the clamp depolarization approaches E_{Na}. These observations indicate that the increase in G_K is delayed by the flow of sodium ions inward; for when I_{Na} is reduced by lowering $[Na]_o$ or by adjusting the clamp potential close to E_{Na}, large outward currents (I_K) are obtained. In keeping with this, veratrine, which keeps P_{Na} elevated following a spike (Section IV C) can produce large, indefinitely long plateaus in TEA-treated muscle (191*I*).

The termination of the plateau by anodal pulses in cardiac fibers, nodes of Ranvier, TEA-treated muscle fibers, as well as in TEA-treated squid axons, shows that another factor is operative in lowering I_{Na}, for anodal pulses themselves, everything else being equal, would increase I_{Na}. A possible clue to the action of the anodal pulses is the increase in membrane resistance it produces in active cells (*e.g.*, 102, 214). This could be the consequence of restoration of calcium to the fiber surface (Sections IV F and G). Although the possibility that raising $[Ca]_o$ will shut off the plateau sooner in TEA-treated axons has not been examined, it will be recalled that calcium has this effect in cardiac fibers. The PP and elevated conductance that may follow the termination of the plateau suggest that the turning-off of raised P_{Na} precedes that of P_K—the same sequence characteristic of the turning-on process in spike generation.

That outflow of potassium cannot occur during inflow of sodium is not unprecedented, for it will be recalled from Part I that outflow of potassium is reduced by increased inflow of potassium when E_m is maintained constant (226*I*). This was interpreted as the consequence of single-file passage through membrane pores. In the presence of TEA, then, it appears that the increase in G_{Na} subsides quickly only in part (incomplete inactivation?), and while I_{Na} is still appreciable I_K for repolarization is negligible (G_K unchanged); when I_{Na} has fallen to a certain level (G_{Na} further reduced), I_K takes over (G_K rises).

The TEA-treated fiber therefore suggests a new principle—the interaction of sodium and potassium fluxes when G_{Na} and G_K are of the same order of magnitude. It is tempting to propose that this also underlies the sudden cessation of the plateau associated with an increase in G_m in Purkinje fibers, for it will be recalled that the conditions that reduce I_{Na} (*e.g.*, low $[Na]_o$, high $[Na]_i$) or enhance I_K (*e.g.*, Ach), shorten the plateau.

The conclusions which have been drawn are different from those reached by Tasaki and Hagiwara. Their voltage-clamp curves have been found to indicate that in TEA the changes in G_K are no longer independent of G_{Na}; in fact, that increased outflow of potassium cannot occur while sodium is entering at an appreciable rate.

The finding by Tasaki and Hagiwara that calculations based on the independence of sodium and potassium currents lead to discordant results is therefore not surprising. Their rejection of a sustained increase in G_{Na} as the basis of the plateau also depends on the assumption that the G_K values in low $[Na]_o$ apply in sea water, an assumption which is valid only if I_K and I_{Na} are independent. Other reasons for favoring the view that a maintained, elevated G_{Na} is an important feature of the plateau have already been given. A more thorough quantitative analysis of voltage-clamped, TEA-treated axons and measurements of ionic fluxes as a function of $[Na]_o$ should be helpful in establishing the correct relationships.

How TEA produces its effects presents an interesting problem. It will be recalled that it exhibits stabilizer effects, reflected by a decrease in P_K in spinal roots and interference with changes in the permeability of junctional membranes (Part I). However, effects on R_m are reported to be inconsistent (193*I*, 218). The results with squid axons indicate interference with the increase in P_{Na} and especially in P_K and perhaps with the development of inactivation as well. Whether other stabilizers will act like TEA remains to be seen. Cocaine and procaine do not appear to produce the same phenomena, but their reduction of the increase in P_{Na} may be too similar to that in P_K; it should be remembered,

however, that, with respect to their action on crab muscle action potentials, quaternary ammonium compounds and procaine behave similarly.

3. Other fibers. The effects of TEA in skeletal muscle (191*I*) are much less striking. Thus, the lengthening of the spike appears to be limited to the time constant of the resting membrane. This suggests that TEA prevents a rise in P_K which normally hastens the end of the spike. In TEA the conductance increase during the muscle spike is smaller and more prolonged. This could be a consequence of a decrease in the P_{Na} and especially the P_K increments during the spike, as well as of the prolongation of spike repolarization. The former permeability appears to be less affected, for spike amplitude is somewhat better when TEA substitutes for sodium than when choline does; this would follow from elimination of an increase in P_K that arises early enough to limit the extent to which I_{Na} discharges the membrane capacitance—a possible alternative to the proposal (191*I*) that TEA contributes directly to the spike.

In crab nerve the limitation of the effect of the TEA (and tetrabutylammonium, TBA) to a slowing of the falling phase (25) again suggests interference with the increase in P_K. The prolongation to "several milliseconds" suggests that here, too, the time constant of the membrane may be the limiting factor in the termination of the spike (Part I, Table 1).

Crab muscle reacts by a lengthening and increase in the spike to a wide variety of compounds in addition to TEA and other quaternary ammonium compounds (123*I*). The compounds employed in these studies read like the reagents on the shelf of the electrochemist concerned with salts soluble in solvents of low dielectric constant, but also capable of large phase boundary potentials (74a*I*). Although phase boundary potentials may play a part, they can be considered seriously only when the electrical changes can be shown to be substantially greater than expected from observed increases in conductances and in ionic movement.

4. Plateau versus negative after-potential. While the negative after-potential is generally small compared to the plateau, in the heart or in TEA-treated muscle veratrine eventually produces either an after-potential of great amplitude or a plateau of great duration (Section III B 7). If the definitions are restricted to the amplitudes of potential, a certain measure of arbitrariness is involved. The above considerations suggest one basic important difference: NAP of the type that results from membrane permeability changes is due to delay in the decline of elevated P_{Na} or P_{Cl}, or both, probably accompanied secondarily by *elevated* G_K, whereas the plateau is related to a fall or a delay in the rise in G_K associated with a delay in the decline of P_{Na}. Hence, from the standpoint of the behavior of G_K, the late effect of veratrine is a prolongation of the plateau.

5. Conclusions. These considerations therefore lead to the view that plateaus reflect a delayed increase in P_K and incomplete decline in P_{Na}, as though complete inactivation were at least partly linked to an increase in P_K. This appears to be related at least in part to competition between leaving potassium ions and entering sodium ions. From this standpoint, the effect of many experimental conditions on the duration of the plateau can be understood, for if entry of

sodium, I_{Na}, is reduced (by increase in $[Na]_i$, or decrease in P_{Na}) or if exit of potassium, I_K, is increased (by increase of P_K) the plateau terminates sooner, and opposite changes in I_{Na} and I_K prolong the plateau.

F. The action of stabilizers

In many respects calcium and other stabilizers such as local anesthetics were seen in Part I to produce similar effects, indicative of a reduction in both P_K and P_{Na} at rest, yet two differences have already been mentioned, namely, the increase in certain preparations of the spike and of NAP by calcium although they are reduced by the local anesthetics (Sections III B 4, III E, and IV C). The difference was suggested some time ago (405*I*) to be the result of the reduction of calcium in the fiber surface during the spike, whereas local anesthetics continue to exert their effect throughout the spike. Recent studies support this point of view and add important details.

1. Calcium. Weidmann (511*I*) first pointed out in Purkinje fibers that, although a stronger shock is needed to initiate the spike in raised $[Ca]_o$, the rate of rise of the spike is substantially elevated, showing that the entry of sodium is facilitated (*i.e.*, inactivation reduced). By comparing the rate of spike rise at normal and elevated $[Ca]_o$ as a function of E_m' (modified by anodal or cathodal polarization), he found that increasing $[Ca]_o$ does not affect the maximum obtainable rate of sodium entry but partly counteracts the reduced entry of sodium when E_m' is low. In other words, inactivation is reduced.

This was also found for squid giant axon with voltage-clamp (157*I*). This study found the converse also true, *viz.*, that low $[Ca]_o$ leaves the fiber more inactivated (G_{Na} change or I_{Na} increment reduced), although E_m' was little altered; electrical elevation of membrane potential counteracted this effect of low $[Ca]_o$. Additional important observations were made when the precaution was taken of working with E_m set at an initial hyperpolarized level to assure negligible inactivation at all $[Ca]_o$'s.

These may be summarized as follows (157*I*):

(a) For smaller clamp depolarizations, the maximum inward current (peak G_{Na}) was larger for a given depolarization the smaller $[Ca]_o$. An analysis of the curves relating peak I_{Na} to V (the *change* in E_m) revealed that the lower $[Ca]_o$, the greater was the resting G_{Na}, and the greater the increments in G_{Na} with small depolarization, but the maximum increase in G_{Na} obtainable, although reached with small depolarization, was not different. The general effect was that a decrease in $[Ca]_o$ from 22 to 4.4 mM was equivalent to working with a preparation requiring 15 mV less depolarization for a given effect; conversely, an increase from 22 to 112 mM was equivalent to requiring 15 mV more depolarization. This applied, too, to the rate of development of the increase in G_{Na}.

(b) The ability of elevated G_{Na} to shut off, when a depolarizing clamp voltage is suddenly removed before inactivation has set in, is markedly interfered with by low $[Ca]_o$ and enhanced in elevated $[Ca]_o$. The effect is appreciably greater than expected for the 15 mV equivalent polarization changes noted above. This effect is not due to the inactivation process since the latter is not given time to develop. When inactivation is present, however, the delay in the return of G_{Na} to low values in low $[Ca]_o$ is much less striking. Thus, as inactivation develops at 22 mM $[Ca]_o$, the rate of return increases—an effect not very marked at 112 mM $[Ca]_o$. Also, if G_{Na} is not given time to develop fully (by using too short a depolarization) low $[Ca]_o$ has less of an effect on the shutting-off of increased G_{Na}.

(c) The effect of $[Ca]_o$ on I_K (and G_K) was essentially similar to that on the sodium parameters. Thus, in lower $[Ca]_o$ (4.4 mM), G_K at rest was higher. Hence, with either an anodal or cathodal clamp (under conditions in which I_{Na} was negligible) inward or outward current was larger to begin with; under anodal clamp this inward (K^+?) current fell gradually (G_K declined), while under the cathodal clamp it increased further (G_K increased) towards the same maximum which could be obtained, at higher $[Ca]_o$, with stronger depolarizations. Another important observation was that increased $[Ca]_o$ prolonged the delay in the development of an increase in G_K during a depolarizing clamp, and that this effect could be duplicated at lower $[Ca]_o$ by previously raising E_m before clamping to the same level of depolarization.

These studies therefore reveal that in most respects $[Ca]_o$ (without an associated change in E_m') and E_m' are comparable in their effects on G_{Na} and G_K in the fiber at rest and during activity. Low $[Ca]_o$ or E_m' increases G_K and G_{Na} (therefore P_K and P_{Na}; see Part I) and hastens and increases their increment in response to a sudden depolarization; particularly striking is a shortening of the delay in the rise in G_K.

These observations provide further support for the proposed basis of oscillatory behavior described in Section IV A 2 d. Thus, not only are G_{Na} and G_K initially higher with low $[Ca]_o$ or E_m', so that a given depolarization induces larger increments in these parameters, but they develop more rapidly, especially in the case of G_K, which ordinarily exhibits an appreciable delay in responding. The reviewer is inclined to attach special importance to the reduction in this delay for oscillatory behavior. At nodes of Ranvier, too, the earlier onset of increased G_K is shown by earlier rectification in lower $[Ca]_o$ during passage of linearly increasing current (72). The observations also provide a basis for the better developed, less damped oscillations obtained with a cathodal, sustained pulse of current than with a corresponding anodal current (Section III D). The development of the positive potential (G_K increased) with sustained depolarization of Purkinje fibers (Section IV B 2 b) appears related to these findings.

The improved spike amplitude of giant axons and the faster rise of the spike in Purkinje fibers in elevated $[Ca]_o$ discussed earlier are related to the reduction of the inactivation in the resting state. It will be recalled from Section IV A 1 that the giant axon, and from Section III B 2 that Purkinje fibers, are partially inactivated in the resting state. Frog nodes, on the other hand, show little improvement in spike amplitude with elevated $[Ca]_o$; in fact, extremely great precautions must be taken in order to be able to lower $[Ca]_o$ sufficiently to reduce spike amplitude appreciably (72). This probably is only a qualitative difference from squid, one that reflects a much greater affinity (association constant?) for calcium in frog nerve, a system that operates at calcium concentrations 10-fold lower [or 20- to 30-fold lower if allowance is made for the larger amounts needed for squid axons when magnesium is lacking, as seen by Shanes (405*I*) and very recently by Frankenhaeuser and Hodgkin (157*I*)]. It is of interest, therefore, that rheobase-$[Ca]_o$ curves plotted by Brink (figure 7 in 19*I*) are indicative of a 20-fold greater association constant for calcium in frog fibers.

Consequently, the fact that higher concentrations of calcium depress the spike

at the node of Ranvier may merely reflect that the sites concerned with sodium transfer are fully occupied at relatively low $[Ca]_o$, and that higher $[Ca]_o$ concentrations function like less specific stabilizers such as local anesthetics, now to be discussed.

2. Local anesthetics and cardiac glycosides. Weidmann (511*I*) showed that the rising phase of the spike is slowed by the local anesthetics, and that this could be counteracted, but not completely, by raising E_m'. From their effect in slowing the rise and amplitude of the spike (Section III B 5) and from the standpoint of the many observations discussed in Part I, one would predict that they reduce the increase in P_{Na} and probably P_K during excitation. Voltage-clamp studies on squid axons carried out by the reviewer in collaboration with Grundfest, Freygang and Amatnike in the summers of 1956 and 1957 (432*I*) confirm the reduction of both inward and outward currents by procaine and cocaine, and the inability of increased E_m' to counteract this effect completely. E_{Na}, and probably E_K, were little altered, so that the effect on the currents is attributable to the predicted effect on the permeability changes. Taylor (224) recently described similar experiments and results with procaine that confirm our findings. The reduction of the impedance change at active nodes of Ranvier by cocaine (221) is consistent with these results.

Thus, the interference with the excitation process appears to be related predominantly to a depression of the increase in I_{Na}, as can occur with prolonged depolarization; the difference, of course, is that depolarization does not take place, and the underlying mechanism is probably not the same (Section G below).

Moreover, if the limitation of spike production is merely an insufficient increase in P_{Na}, so that at a particular $[Na]_o$ I_{Na} is small [since I_{Na} is dependent on P_{Na} and $([Na]_o - [Na]_i)$], elevation of $[Na]_o$ should be able to correct the situation, as we have seen in Section III B 9.

That the inactivation by local anesthetics is partly reversed by hyperpolarization as well as by elevated $[Na]_o$ (Section III B 9) requires further analysis. If it could be shown that elevated $[Ca]_o$ acts similarly—which does not seem likely—then the possibility of displacement of calcium from the membrane by local anesthetics, *e.g.*, by "lateral pressure", would be indicated as the factor reversed by raising E_m'. Many other possibilities can be suggested depending on the assumption made to account for inactivation (see 105 and 157*I* and the following section).

Cardiac glycosides have been included under "stabilizers" because their effects on membrane permeability and its changes, as shown by studies on the rate of rise of the spike, on the plateau, and on membrane conductance (55a, 472*I*) are the same as those of local anesthetics. This conclusion is consistent with Glynn's observations on red cells which showed that the passive fluxes of both sodium and potassium, as well as the active fluxes, are depressed by cardiac glycosides and related compounds (76a).

Whether cardiac glycosides are "soluble" in the membrane, as postulated for the other stabilizers, and thereby exert their effects on passive fluxes in a manner

distinct from that on active transport, or a more specific interaction is involved which is related to that interfering with active transport, cannot be answered with certainty. There is reason to suspect that the cardiac glycosides may raise the calcium content of the membrane, perhaps by complex formation such as may occur with more polarizable anions (Section VI B). On this basis, the similarity between the cardiac glycosides and, say, SCN^-, in raising membrane resistance (356a*I*) and in interfering with passive and active ion transfer (108a*I*) may not be coincidental but the reflection of the same basic membrane interactions.

One might expect efflux to be reduced by the lower P_K suggested by the resistance measurements. Rayner and Weatherall (172a) recently confirmed earlier reports to the contrary. However, the electrical measurements also showed a depolarization and increased conductance at later times, which could obscure the initial effect; moreover, these measurements were carried out in fibers made more leaky by low $[Ca]_o$, which would have accentuated the electrical effect. It may be recalled, too, that only a transitory decrease in O_K was obtained with cocaine on vertebrate nerve (Part I and 200a), a result attributed to cocaine action limited to the outer layer of the membrane. Whether such factors may have obscured an effect by the cardiac glycosides on efflux under the conditions employed for measuring the efflux remains to be determined.

G. Conclusions

1. General aspects. With respect to the spike, positive potential, oscillations, and the subthreshold potentials, then, the underlying phenomena appear to be changes in P_{Na} and P_K, as also indicated for slower changes in resting potential under a variety of experimental conditions (Part I). This conclusion is based on the same type of electrochemical evidence (*viz.*, the interrelated changes of potential, conductance, and of ion movement) that was summarized in Part I for the resting cell.

As in the case of the unexcited cells, P_{Na} and P_K are susceptible to change independently or to different degrees. An additional difference apparent from the study of the transitory electrical phenomena is in the speed of their response, P_{Na} changing more rapidly than P_K. Indeed, the latter exhibits a delay which can be lengthened to the point where it may not arise at all.

The initial event in the impulse of all but one of the excitable cells that have been considered, and probably for many more not discussed, appears to be the same. From the electrochemical criteria employed, it consists of an inrush of sodium ions due to an increase in P_{Na}. This is invariably followed by inactivation —a progressive decrease in the ability to produce another increase in P_{Na} as long as the system is appreciably depolarized. The increase in P_{Na} and inactivation are two common denominators which could account for the extensive similarities among the different fibers in the dependence of their spike-generating mechanism on several factors, *e.g.*, on E'_m, which when too low causes inactivation; on $[Na]_o$, which when too low limits I_{Na} to the point where inactivation (or increased G_K) can prevent it from rising to the level of regenerative depolarization; on $[Na]_i$, which when too high also limits I_{Na}; on $[Ca]_o$, which when very low causes inactivation; and on local anesthetics, which limit the increase

in P_{Na} so that I_{Na} is inadequate. The basic factor, then, is the ability of sodium to enter and cause depolarization. Repeated references to the similarity of low $[Na]_o$ to local anesthetics (*e.g.*, 57) are thus seen to have a basis from the theoretical standpoint which has been presented.

Unlike the initial process, the later events of the spike can differ appreciably in various excitable cells. A major factor appears to be the presence or absence of a change in P_K, or the delay with which it arises. Further study is required to determine the extent to which the inactivation process may differ in other excitable cells from that in the squid axon. Tetraethylammonium (or tetrabutylammonium) appears to exert a more specific effect in interfering with the P_K changes. Local anesthetics, on the other hand, although they affect both permeabilities, most obviously depress the P_{Na} change. Veratrine may alter P_{Na} chiefly, but the possibility of P_{Cl} involvement cannot be ruled out as yet. A further clarification of the events involved may be expected from a systematic study of all ion movements (*viz.*, Na^+, K^+, Cl^-, and Ca^{++}) under these various conditions.

2. Possible nature of the changes in P_{Na}, P_K, and inactivation. a. General. From the considerations discussed in Part I, it seems not unlikely that the early entry of sodium (or of lithium, since it can function like sodium; see 221*I*, 247*I*, and 198) reflects the smaller radius of this ion when it is unhydrated. This could be the consequence of an adsorption (binding) process that favors sodium over potassium at certain sites on and/or in the membrane, much as has been found for muscle protein (62, 185) and for condensed phosphates (153).

The dimensions of unhydrated calcium are practically identical with those of Na^+, but it is well-known to be much more strongly bound by complexing agents. Apparently, its greater charge and polarizability and the strength of the coordination complexes it forms more than suffice to counteract its higher hydration energy (153), and hence greatly favor its binding over sodium. Niedergerke and associates (346*I*, 347*I*) provide data based on the contractility or contracture of heart muscle and on Ca^{45} uptake that suggest a competition between Na^+ (and K^+) and Ca^{++} for the fiber surface. This provides a basis for the increase of G_{Na} (and G_K) as $[Ca]_o$ is lowered. The transitory increase in G_{Na} during a depolarizing clamp might therefore also follow from removal of Ca^{++} from these sites, especially in view of the fact that when $[Ca]_o$ is raised or lowered a larger or smaller depolarization is needed to achieve a given increase in I_{Na}.

The analysis by Hodgkin and Huxley (Section IV A) established that the rises in I_{Na} and I_K, or in P_K and P_{Na}, are due to the decrease in transmembrane potential, not to current flow. This might be accounted for by two assumptions: That the decrease in the electric field in the membrane allows calcium to form ion pairs, say with an anion, either an organic one or chloride, present in the membrane, and that this association depletes Ca^{++} from the sites on which sodium entry depends. Such ion pairs would proceed to diffuse through the membrane towards the interior of the cell, where the thermodynamic activity of Ca^{++} is low. As pointed out in Part I, the strong electric field in the membrane of at least 100,000 V/cm, the result of a resting potential of 0.1 V across a membrane 100 Å (10^{-6} cm) thick, might well be important in keeping oppositely charged ions from associating. The possibility of a quantitative analysis based on this proposal must await detailed evidence in its

favor, but we may note that Onsager has provided a theoretical basis for ion pair association in electric fields (in 86).

Several predictions can be made from this hypothesis:

(a) Ca^{++} influx (I_{Ca}) will increase during the impulse and with depolarization. This has just been reported for giant axons (109).

(b) As Cl^- is replaced with more polarizable anions (*e.g.*, NO_3^-, I^-, and especially SCN^-), the tendency for anion pair formation will be improved, as described in Part I, and I_{Ca} should increase. Direct evidence for this is not yet available, but the effects of anions on muscle contraction are consistent with this view if muscle shortening is taken as an index of Ca^{++} entry (Section VI B).

(c) Excitable cells permeable to anions should be more sensitive to a lowering of $[Ca]_o$ and to depolarization when Cl^- has been replaced by a more polarizable anion, for this will favor ion pair formation (Part I). Chao (27) found that excitation of muscle by potassium in calcium-free media containing different anions increased in the following sequence: $Cl^- < Br^- < NO_3^- < I^- < SCN^-$. This will be recognized as the sequence of increasing polarizability (Part I). Replacement of Cl^- with NO_3^- also increases the excitability of less excitable fibers in normal Ringer (121).

(d) The ability of sodium influx to shut off will depend on the rate of displacement of Na^+ at the sites by Ca^{++}, which should be a function of $[Ca]_o$, as reported by Frankenhaeuser and Hodgkin (Section IV F 1). It may be added that there is reason to suspect from studies of muscle contraction (Section VI B) that more polarizable anions also favor the presence of more calcium in the membrane. The faster decline in NO_3^- of the hyperirritability of muscle following brief subthreshold shocks (121) would therefore be in keeping with a faster shut-off of elevated G_{Na}; it cannot be attributed to a reduced r_m, for R_m is increased by replacement of Cl_o^- by NO_3^- (Part I).

Why is the increase in P_K delayed but sustained whereas that in P_{Na} is rapid but subsides (*i.e.*, is inactivated)? Only conjecture is possible at this point. With respect to the former, presumably Ca^{++} leaves potassium sites more slowly. If the suggestion of the role of anions in the removal of Ca^{++} from its sites is correct, a depletion of anions from the membrane would achieve inactivation; how this might be accomplished does not merit speculation unless it can be shown that replacement of $[Cl]_o$ with impermeant anions (glutamate?) hastens the development of inactivation.

Such studies must be carried out in systems such as muscle where extracellular anions penetrate readily; whether giant axons are in this category is uncertain (Part I). Another possibility is that Ca^{++}, present in the membrane at a higher level near the inner surface (and on K^+ sites) by virtue of the electric field—*i.e.*, greater than that at the outer surface by a factor approaching $e^{2E_mF/RT} = e^{E_m/12.5}$ (*cf.* 157*I*)—declines and diffuses toward Na^+ sites in the membrane when E_m is lowered (as well as into the cell with Cl^-) and thereby increases G_K while reducing sodium entry.

The second possibility is appealing in view of the similarity of the time courses of inactivation and P_K in giant axons. Also, it will be recalled that when inactivation is allowed to develop while $[Ca]_o$ is low (22 mM), the shut-off of G_{Na} becomes much more rapid—proportionately much more than when $[Ca]_o$ is high (112 mM) (157*I*). The second proposal would contribute to recalcification of Na^+ sites, which also receive Ca^{++} from the medium, and hence would be more apparent in decreasing G_{Na} when $[Ca]_o$ is low. Granted that such recalcification occurs, there would nevertheless still have to be a depletion of membrane anions or the involvement of other Na^+ sites if reactivation is not to occur.

In fibers like TEA-treated axons or in the heart, where at least part of the decrease in P_{Na} appears to occur without an increase in P_K, a modification of these suggestions or another approach may be necessary.

Other possibilities for the mechanisms that may be involved in the changes in permeability and inactivation have been considered by Hodgkin and his associates but rejected for one reason or another (105, 157*I*). For example, the possibility of a negatively charged carrier, which accumulates at the outer surface at normal values of E'_m, appears to be inconsistent with the conductance changes at low temperature, with the lack of a large initial outward current, or with the appreciable time required for G_{Na} to increase during depolarization. Nevertheless, the idea of carriers continues to be employed (*e.g.*, 157*I*, 512*I*). More complicated possibilities mentioned by Hodgkin and his associates are not susceptible to experimental test. It was noted, too, that simple displacement of calcium by the electrical field cannot of itself account for the sensitivity of membrane permeability to E_m.

The proposal that ion pair formation underlies removal of Ca_m^{++} is susceptible to experimental test. Thus, it can be predicted that, if Cl^- is the anion involved, its replacement in the medium with a sufficiently large anion should slow the rate of rise of G_{Na}, while its replacement with a more polarizable anion (that can penetrate the membrane) may increase the rate of rise of G_{Na}. These effects would follow from slower or faster release of the sites on the membrane along which unhydrated (or weakly hydrated) Na^+ passes. Moreover, it can be predicted not only that entry of Ca^{++} into the cell will be augmented by activity, evidence for which has already been mentioned, but that the presence of more polarizable anions may accelerate it further. The necessary measurements remain to be carried out.

Evidence that Cl^- enters during activity has been available for some time [*e.g.*, for the giant axon (273*I*) and for skeletal muscle (63, 64)], although this is related largely to entry with Na^+, perhaps also as ion pairs. It will be recalled (Section IV A 2 h) that the unidirectional fluxes of Na^+ increase more than expected from the conductance data obtained from giant axons. Data on Na^+, Ca^{++}, and Cl^- fluxes, as functions of membrane potential or during spikes prolonged by TEA, should be helpful in verifying the validity of ion pair formation as a basis of movement of these ions.

Are all increases in permeability due to release of sites occupied by divalent ions? Are new channels produced or are critical points in preexisting channels enlarged by removal of Ca^{++} from the membrane? Such questions cannot be answered with great certainty, but there is some indication of some of the events from the differences among ions and drugs in their effects on the resting permeability and on *changes* in permeability, to be discussed in Section 3 below.

b. Inactivation. This term, as employed in the literature and in this review, probably represents more than one process. It refers to an end-effect, *i.e.*, the reduced ability of sodium movement to increase during a sudden depolarization. At least two different conditions of increased inactivation can be recognized: (a) That accompanied by a decrease in P_K (as with local anesthetics) and (b) that associated with an increase in both P_{Na} and P_K (as with low $[Ca]_0$ and depolarization).

The last may be due to the limited number of sites (indicated by the maximum G_{Na} obtainable) being already partly occupied by Na^+; hence further increase in the number of sites is restricted. Type (b) may be identical with (a) with respect to sodium, but its coupling to an increase in P_K is suggestive of a transfer of a common component, perhaps Ca^{++}, from the K^+ to the Na^+ channels.

Both types of inactivation are reduced by raised $[Na]_0$, presumably because, although the increase in P_{Na} is still limited as before, I_{Na} depends on *both* P_{Na} and $[Na]_0$, consequently I_{Na} is increased with $[Na]_0$. Type (b) is counteracted by increased $[Ca]_0$, but type (a) may not be so counteracted as long as E'_m is unchanged; experimental data are unavailable to establish this point. All types of inactivation are reduced by hyperpolarization, but type (a) prevents all of the previously available sites from appearing regardless of the strength of hyperpolarization; that type (a) is counteracted at all may merely reflect removal of E'_m-sensitive inactivation that was present before application of the agent, rather than an effect on the inactivating mechanism subsequently produced. A clarification of this point is desirable. The effect of E'_m on type (b) suggests that the effectiveness of Ca^{++} in occupying the Na^+ sites is augmented. Stämpfli's finding that action potentials are restored in small vertebrate nerve bundles by hyperpolarization in the absence of Ca^{++} (Part I) cannot be regarded yet as indicating a more complicated mechanism. It will be recalled that Frankenhaeuser found that special precautions are necessary to lower $[Ca]_0$ sufficiently to interfere appreciably with spike production at single nodes.

3. The pore-solvent hypothesis and ion and drug action. Attention was called in Part I to the necessity for considering limitation of ion penetration in terms of (a) the difficulty of removing water from the ions (hydration energy) and (b) the crystal ionic radii. It was also pointed out that the action of drugs and ions in altering membrane permeability was twofold, namely, on the permeability directly, on the ability of the permeability to undergo change, or both.

The drug and ion effects on the action potential that have just been described and that will be related shortly to the excitation process, further emphasize these factors and call attention to additional possible details.

The entry of stabilizers into the membrane in the interpore region, proposed for the local anesthetics to account for their limited effect on resting permeability but marked effect on *increases* in permeability (Part I), may well be less random than simple "solution". This is evident from the studies with quaternary ammonium compounds, such as TEA, which affect P_K more than P_{Na}; it is also suggested by the different effects of stabilizers on plateau duration. Local anesthetics did not exhibit a striking difference in effect on P_{Na} and P_K changes, although their action on spike generation calls attention to the effect on the first event, *viz.*, the increase in P_{Na}. It should be stressed that even with TEA, P_{Na} is also affected.

Granting that solubility (*i.e.*, entry by displacement of interpore molecules) in the interpore region occurs, we must also consider as likely that configuration of the agent, as in inclusion compounds of urea and thiourea, coordination bonds, as in inclusion compounds of deoxycholic acid and fatty acid (*cf.* 41), may favor the presence of some drugs and ions closer to the channels of potassium than of sodium and *vice versa*. Specificity cannot be very great, however, since both ion species are generally affected.

The inclusion compounds discussed by Cramer (41) are highly suggestive of the means whereby channels are maintained in the membrane and the manner in which even large molecules could enter or pass through a semi-rigid membrane such as has been postulated. Straight chains of fatty acids, for example, can function as nuclei around which assemble other (membrane) molecules as a result of coordination linkages, van der Waals' forces,

etc. to form a long channel around the individual "guest molecules". The spaces between the enclosed guest molecule and the surrounding molecules are of the dimensions of the unhydrated ions; moreover, it seems a possibility that the different sites for Na^+ and K^+ entry could reflect different positions within the circumference of such channels. This offers a possible basis for the interactions that appear to be present between Na^+ and K^+ movement during the plateau and for the similarity of the time course of P_K and inactivation changes found in giant axons.

Entry of agents into the region between channels is considered as the simplest mechanism to account for interference with increase in permeability with little change in the permeability at rest. The increase in lateral pressure will affect channel diameter at rest only to the limited extent permitted by short range, intermolecular repulsive forces and by various intermolecular bonds.

However, when the resting permeability is appreciably affected, it is most simply regarded as the consequence of occupation of sites normally available to the monovalent ions. The effects of Ba^{++} on excitability and membrane conductance (Section VI A 2) are most easily interpreted as a reduction of P_{Na} and P_K, but somewhat more in the case of the latter—hence the final depolarization and raised excitability. Mg^{++} has been pointed out to be weaker but acts on G_{Na} and G_K like Ca^{++}, which affects both conductances about equally (157*I*). This is of interest because in a number of chelating agents, *e.g.*, Versene (ethylenediaminetetraacetic acid), the stability (association) constant of Ca^{++} exceeds that of Mg^{++} (and Ba^{++}) (table 5.4 in 153).

Observations have been described which require that the site or mechanism of action of Ca^{++} be somewhat different from that of other stabilizers, although in many respects, particularly in unexcited cells, its action is quite similar. The proposals that Ca^{++} acts directly on the sites where ion passage takes place and that it is displaced during depolarization are in keeping with the improved spikes during the action potential in certain fibers (more sites made available on depolarization) and with the raised threshold (more Ca^{++} to be removed to expose the number of sites required for an adequate inward sodium current). From this standpoint, other stabilizers depress both threshold and spike production because they are not displaced during the spike.

In the case of the cardiac glycosides it cannot be stated whether their action as stabilizers is non-specific, as in the case of local anesthetics, or involves the sites of monovalent ion entry, perhaps by forming a less labile complex with Ca^{++} at such sites. It would be desirable to determine whether, unlike other stabilizers (Part I), the stabilizing action of the cardiac glycosides requires Ca^{++}. It may be significant for the action of these glycosides that digitonin forms complexes with steroids (41). A similar block of sites normally concerned with active sodium and potassium transport could account for the more familiar effect of cardiac glycosides on transport. As pointed out in Part I, an examination of ionic transfer in the presence of cardiac glycosides, during and without metabolic inhibition, and comparison with the action of local anesthetics (422*I*) and calcium under these conditions, should be helpful in delimiting the sites of action.

It is noteworthy that in erythrocytes the passive leak of potassium into low electrolyte solutions is slowed by extracellular calcium but not by 10^{-6} (w/v) strophosid, whereas active recovery of potassium by potassium-depleted cells is blocked by strophosid but unaffected by $[Ca]_o$ (144a).

A systematic study of the action of different ion series, inorganic (monovalent as well as multivalent) and organic (quaternary ammonium, *etc.*), on resting and active permeability changes and inactivation should also prove useful in determining how far information available on complex formation (153) and on inclusion compounds (41) may contribute to an understanding of membrane structure and behavior.

4. Action potentials as diffusion potentials. It should be pointed out that while the viewpoint of diffusion potentials as the basis of the electrical changes induced in excitable cells—whether at rest or during the impulse—is supported by qualitative and quantitative data on the ionic movements which accompany the bioelectrical phenomena, such data either have not been obtained or are not sufficiently quantitative to establish the validity of this viewpoint as the basis for *all* the electrical phenomena which have been described. Nevertheless, less direct observations have been plentiful in providing further support for this view in normal physiological media. In the cases where other cations may substitute for Na^+, radically different concepts still cannot be considered without proof that the conductance changes and the movements of these or other cations in the medium cannot account for the bioelectrical phenomena.

5. The role of metabolism and other energy sources. It has long been known from the heat measurements in A. V. Hill's laboratory (126*I*), from respiratory measurements (*e.g.*, 20), from studies of alterations in metabolic intermediaries (165*I*, 166*I*), and more recently from the aerobic utilization of certain amino acids (159) that nerve activity is accompanied by an increase in energy turnover. Of course, the same is true for muscle, but since much more of the metabolism is associated with the contractile process, the association of metabolism with the bioelectrical phenomena cannot be clearly assessed in this tissue.

In early studies, the gradual failure of nerve and muscle to produce an impulse during metabolic inhibition was assumed to indicate a direct dependence of impulse generation on metabolism. In Section III B 8 it was pointed out that such failure is probably the result of the decrease in E'_m brought about by the accumulation of potassium in the interstitial spaces, for it can be greatly delayed or even prevented by washing nerves continuously. Also, failure of sodium exclusion can lead to a moderate depolarization in vertebrate nerve that will lead to block, or to a rise in $[Na]_i$, which could also interfere with the impulse. In the same Section it was pointed out that the depolarization, rather than reduced metabolism, is directly responsible for functional failure, for repolarization by passage of electrical current restored spikes; this can now be understood from the standpoint of the inactivation which develops when E'_m is lowered and its elimination or reduction on repolarization.

More recently, the demonstration that the elevation of respiration with activ-

ity can be suppressed without impairing spike production (20*I*, 55), and that the ionic interchanges during the impulse as well as the impulse itself are unaltered when active transport is negligible (224*I*, 225*I*), provide further evidence that the immediate energy source for the spike is not metabolism nor does the spike involve active transport directly.

From the principles which have been described and developed, it is apparent that the movement of sodium into the cell to produce the rising phase of the spike, and that of potassium out of the cell to restore the resting potential, are along electrochemical gradients which were established by metabolism much earlier (Part I). Thus, both the resting potential and the deficit of Na_i^+ suffice for the entry of sodium when P_{Na} increases; similarly, the high $[K]_i$ suffices for the later exit of potassium. And in the light of the foregoing, the mechanisms for the permeability changes and the development and subsidence of inactivation, likewise cannot depend on metabolism directly but rather on physical processes linked to E'_m (and perhaps to adsorption forces). In other words, the excitable system stands poised with a large reserve of potential energy in the form of the electrochemical gradients. This is tapped by way of a metastable valve which has a built-in, automatic shut-off mechanism. Whether anything more than removal of the calcium ion can be shown to be involved in the on-off operation of this valve remains to be seen. Certainly, conclusions regarding the involvement of specific metabolites (*e.g.*, acetylcholine), largely on the basis of "specific" inhibitors that are now known to act as nonspecific stabilizers (Part I), cannot be taken seriously until better evidence is available. The inertness of Ach (and carbachol) when injected into cells with anticholinesterases (Part I) raises a serious question of the importance of Ach.

Of course, metabolism plays its part in the restoration of electrochemical gradients so that the systems do not run down from continual activity. It was pointed out that the positive after-potential is the one aspect of the action potential which reflects this role of metabolism. The position of active transport in the cardiac cycle remains to be determined. The recovery of potassium and extrusion of sodium following activity (20*I*, 411*I*, 412*I*, 415*I*), their depression by low temperature (419*I*) and by inhibition of *resting* metabolism by azide (20*I*), and their augmentation by an agent like veratrine that increases the ionic loss per impulse (414*I*, 415*I*) have been demonstrated in nerve. Further evaluation is required of the surprising report that azide, in concentrations which do not alter resting metabolism but suppress the additional metabolism of activity, does not interfere with recovery of electrochemical gradients (20*I*).

The accelerated development of block in inhibited nerve by stimulation (125*I*) is also consistent with the indirect part played by metabolism, for this would follow simply from a hastening of the decline in the electrochemical gradients

Attention was called previously to the special importance of metabolism in small fibers; everything else being equal, their large surface-to-volume ratio will be responsible for a greater relative loss of their electrochemical gradients per impulse.

V. EXCITATION

A. General principles

The events leading to the appearance of the all-or-none response are less accessible to direct measurement, particularly with respect to ion movement, than the action and resting potential. Moreover, matters are complicated by the "passive" electrical characteristics—the "core-conductor" properties—of nerve and muscle, for the techniques usually employed to elaborate the events in excitation are generally not carried out under the simplified conditions of the voltage-clamp or of the space-clamp.

Events involved in excitation have been explored (a) by following the changes in E_m during and following passage of currents of graded intensity and (b) by testing for the "threshold", *i.e.*, the minimum shock required to elicit an all-or-none response as a function of time. Practically all of the "classical" literature was concerned with the latter, as is much recent literature. Since the former provides more direct information, it is discussed first.

1. Subthreshold potentials. It will be recalled that a characteristic generally demonstrable in excitable cells is the non-linear "active subthreshold potential", ASP, which responds to experimental conditions in the same way as the spike and usually appears when cathodal shocks are ½ to ¾ of threshold strength (Section IV A 2 e). When the total change in E_m achieved with ASP equals a critical value, V_T, the spike arises.

V_T represents the *change* in E_m caused by ASP, consequently it is the difference between the values of E_m at rest, E_m', and at the time the spike begins. Inasmuch as E_m' can vary, it is often of interest to know whether the *absolute* transmembrane potential at which the spike arises, the critical potential, E_T, has changed or not; V_T ($= E_m' - E_T$) itself cannot provide this information. Strictly speaking, the larger E_T the more excitable the fiber, *i.e.*, everything else being equal, the less decrease in E_m is required and therefore the smaller V_T and the applied stimulating current.

In the past, and in some recent papers, the excitability of a fiber has been measured in terms of the strength of the stimulus, *i.e.*, the threshold, required to initiate the spike. The effectiveness of the stimulus depends not only on its time course, but on that of the membrane potential, which depends on the "active" processes (G_{Na}, G_K, and inactivation changes) and the general geometry (*e.g.*, size of fiber and electrodes) as well as cable-conductor characteristics of the fiber (R_m, R_i, C_m). Hence, threshold measurements are probably the most complex to interpret, although simplest to make. Consequently, threshold studies will be discussed in this section only when E_m' is known to be unchanged; more complex situations are dealt wih later.

When smaller, longer pulses of constant current are employed, ASP can be expected to be less obvious. G_{Na} increases more gradually, therefore inactivation, and the increase in G_K when present, can keep up better with it and therefore serve to damp its rise; also, when the depolarizing current is kept constant,

the R_mI drop across the membrane tends to fall as G_m ($= 1/R_m$) rises with G_K and G_{Na}, and this will tend to balance the fall in E, the intrinsic e.m.f., as G_{Na} increases relative to G_K. In the case of longer pulses, too, the spike arises when E_m declines to a critical value—about the same or only slightly lower at longer times than that required with short shocks (at the node of Ranvier: 214; in muscle: 249*I*; computed from Hodgkin-Huxley equations for squid axon: 29a, 30).

As rectangular pulses are made smaller, they must act longer to initiate the spike. This is partly because the discharge of membrane capacitance to a given extent is given by the charge delivered, which is the current multiplied by the time. There is a limit, however, to the amplitude of a sustained cathodal pulse that will evoke an all-or-none response; this is the familiar "rheobase", ρ, which is the lower limit of the well-known "strength-duration" or "strength-latency" curve (*e.g.*, 477*I*).

That the process of inactivation or the increase in G_K is setting a limit to the late appearance of the spike with small, prolonged stimuli is indicated in three ways: By the increase in the product of latent period and stimulus strength as stimuli become weaker, by the smaller spikes (muscle: 33, 249*I*; giant axon: 107; calculated for and observed in the axon: 30), and by the fall in the critical firing potential, E_T (249*I*). A depression of the amplitude and rise phase of the spike, and a smaller E_T (or larger V_T), are also usually evident at the node of Ranvier when the latency is lengthened by use of slower, linearly increasing currents as stimuli (53, 71, 194, 222).

Earlier studies, based on the use of exponentially rising stimuli to evaluate "accommodation", the rise in threshold during a subthreshold stimulus, have been found to give misleading estimates of the rate and intensity of accommodation. Thus, the ratio of the maximum charging voltage, V, needed to stimulate for a particular time constant of rise, τ, relative to the rheobase, ρ, was plotted against τ; this gave a straight line increasing with τ except at low values of τ(204). The slope of the linear portion of the curve relating (V/ρ) to τ was predicted to be inversely proportional to the time constant, λ, of the accommodation process by Hill (94; see also 172); on the basis of his hypothesis, which rested on an empirical formulation of accommodation, λ was a time constant that measured the rate of accommodation and its subsidence, which were regarded as exponential processes. However, some time before (*e.g.*, 56), the subsidence of excitability during a sustained stimulus had been shown to be much delayed (which is suggestive of the sudden onset of inactivation described by 50). Another difficulty was that, although the hypothesis predicted an initial more rapid, non-linear increase in (V/ρ) as τ was increased at low values, this frequently was lacking (199, 204).

It has now been shown that the initial curvature in the V/ρ-τ curve that conformed to the Hill hypothesis is actually an artifact arising from the use of multifiber preparations. Single fibers invariably give (V/ρ)-τ curves that are strictly linear (from $\tau = 0$, when $V/\rho = 1$, and up); this has been found for the node of Ranvier (*e.g.*, 188, 190, 194, 210, 211, 222), for crayfish fibers (236), for squid giant axon (137), and for muscle when punctate electrodes are employed (3, 15). In whole nerve, one factor in the curvature at low τ's is the presence of both small and large fibers; the latter are more excitable but have smaller λ's and so drop out early as τ is increased (188, 190). An additional factor appears to be the involvement of more than one node (71). The sheath of whole nerve introduces an additional complication by distorting applied currents, and thereby leads to smaller values of λ (146, 189).

Another important observation was that, although the maximum charging voltages required with more slowly rising currents (τ larger) increased, the actual strength of the potential at which the spike arose (*i.e.*, the threshold intensity) remained essentially constant or rose only 10 to 25% at very late times (194, 210, 222). An analysis of their results led Tasaki and Sakaguchi (212, 222) to conclude that the increase in charging voltage with τ merely reflects the fact that there is a limiting rate ($= V/\tau$) of membrane potential fall below which no response can be obtained. Since the threshold even at the limiting rate was almost the same as the rheobase, it followed that λ is essentially the maximum time that a gradually rising stimulus can act and still produce a response, and this represents a minimal depolarization rate, $u \div \rho/\lambda$.

Thus, λ is a measure of accommodation in the sense that it is that time beyond which inactivation or the delayed rise in G_K develops so rapidly that the increase in G_{Na} cannot exceed them and subsides again. Therefore, λ is related to the utilization time—the maximum (also minimum) time a rheobasic stimulus can act and still produce a response. The late development of rectification seen at the node of Ranvier (Sections IV A 2 c and IV B 1 e), and therefore the increase in G_K, seem to agree in time with the onset of accommodation; but conductance data are needed to ascertain whether inactivation alone also may be involved.

The factors in threshold excitation are discussed further in the section following. At this point it suffices to note that the behavior of excitation processes as well as that of spikes arising with a prolonged latent period are consistent with the development of inactivation and increased G_K. The latter two have not been separated for lack of data on G_m or on the behavior of E_m upon sudden cessation of a cathodal current.

Further indications of the involvement of the three variables, G_{Na}, G_K, and inactivation are seen in "break response" and in oscillatory preparations.

As during flow of continuous outward (cathodal) current, oscillations also can be seen with particularly oscillatory fibers during passage of inward (anodal) current. Very brief cathodal or anodal shocks are also followed by oscillations; in these cases and on sudden termination or "break" of prolonged inward or outward currents, the potential fluctuates with a decrement on either side of the resting potential (6, 7, 9, 405*I*, 424*I*).

The depolarizing overshoot in potential on break of an anodal pulse in squid axon, like the initial depolarization on make of cathodal current, increases linearly at first with the intensity (or duration) of applied current, and then to a greater extent than the applied current, indicating the development of ASP, until it attains an amplitude sufficient to initiate the spike (9,405*I*, 424*I*). When the potential oscillates sinusoidally during passage of anodal current, the amplitude of the break response shows similar fluctuations, but much larger, depending on the time of break. The maximal and minimal responses arise when break occurs while the rates, not the amplitudes, of depolarization and repolarization of the oscillation are at their maxima (9,424*I*). It will be recalled that because of the large membrane capacitance of squid axons (and muscle) (Section IV A 2), the maximum rate of change of membrane potential corresponds to the maximum inward current when depolarization is occurring, and maximum outward current when hyperpolarization is developing; hence, G_{Na} is near its maximum when the largest break response occurs, and G_K and/or inactivation are near their maxima when the smallest break response is obtained.

The same features are revealed by Arvanitaki's studies of the amplitudes of electrical responses obtained with short, subthreshold condenser discharges applied to weakly citrated giant axons that produce spontaneous sinusoidal oscillations at low temperature (8). Thus, the largest response is obtained at the time of the greatest rate of depolarization, the smallest at the time of fastest repolarization.

The studies of Rosenblueth and associates (176, 177, 372*I*) on cat spinal roots are in remarkable agreement with results obtained on invertebrates. Their findings, in the light of the principles that have been developed, are as follows: (a) Increase of the break response with duration of anodal polarization (inactivation decreases during hyperpolarization, so that on collapse of E_m to the resting level the increase in G_{Na} is more marked and I_{Na} greater); (b) smaller effect of relatively refractory period on anodal break response than on cathodal make response (anode hastens decline of inactivation and therefore improves ASP obtained on break); (c) progressive fall of the break response during continuous cathodal depolarization (development of inactivation); (d) cathodal make response falls with time during cathodal depolarization, rises with time during hyperpolarization (development of inactivation in the former, reduction of inactivation in the latter). I have interpreted their results solely in terms of inactivation, although corresponding changes in G_K cannot be ruled out. It will be recalled that in amphibian nerve the onset of an increase in G_K does not occur until close to threshold potentials are employed, and under certain conditions appears not to arise at all. Whether this is the situation in mammalian nerve is unknown at present.

Rosenblueth considered his findings to be different from those in invertebrates; however, his objections, such as the greater cathodal than anodal response with the weakest currents employed, and the frequent development of a spike later than the maximum of the subthreshold response, are referrable to the use of a multifiber preparation and to geometrical factors such as spike arisal beyond the current electrodes (*cf.* 96, 363*I*, 477*I*). Thus, studies with single nodes (*e.g.*, 196) do not reveal the discordant behavior described by Rosenblueth. Frankenhaeuser and Widén (74) have recently summarized the literature and presented additional observations on single nodes of Ranvier that show that anode break excitation is enhanced by conditions (*e.g.*, prior depolarization) which inactivate (and/or increase G_K) (see also 117, 212, 222); thus, prior hyperpolarization counteracts inactivation and increased G_K, and makes G_{Na} changes on break more effective in producing ASP and an all-or-none response.

In summary, the development of the active subthreshold response exhibits behavior under the cathode and anode, and with termination of current flow, that is quite consistent with the behavior of G_{Na} and inactivation, and perhaps of G_K, as elucidated in the studies of the action potential previously discussed.

2. Experimental modification of subthreshold potentials. a. Ions. The effect of ions and drugs on subthreshold potentials has been examined in few cases compared to the many studies on less direct phenomena, such as threshold, to be

discussed shortly. Mention has already been made of the augmented development of ASP in giant axons during weak depolarization and of its similarity to partial removal of divalent ions from the medium. This follows from the augmentation in G_{Na} at rest. The suppression of this increase in ASP by low $[Na]_o$ (235, 424*I*), cocaine (424*I*), or calcium (6, 424*I*) is in keeping with the reduction in inward current all three may be expected to produce. The action of low $[Na]_o$, cocaine, and elevated $[Ca]_o$ in suppressing oscillations and break responses in squid axons (424*I*) are in keeping with the same principles. In all of these conditions the value of E_m at rest, E'_m, is not greatly affected if at all (Part I), and hence it cannot be postulated to be involved in these effects.

In Purkinje fibers, in which increase of $[Ca]_o$ leaves E'_m unchanged, the value to which E_m must be lowered (E_T) to produce an all-or-none response is decreased, *i.e.*, the threshold is raised (571*I*). At nodes of Ranvier, where increase or decrease in $[Ca]_o$ can have little effect on E'_m (Part I), the rheobase provides an inverse measure of E_T. From this standpoint, the results are consistent with the findings in squid axons and heart fibers, *i.e.*, the rheobase is increased when $[Ca]_o$ is raised and decreased when $[Ca]_o$ is lowered (115, 116, 206*I*, 212, 222, 323*I*).

In skeletal muscle, the action of calcium may be complicated by changes in E'_m (Part I) which will affect threshold determinations. Direct determinations of both E_m and E_T therefore are necessary. Fatt and Katz (120*I*) found that E_T is lowered; an increase in V_T, the amount of depolarization required for stimulation, has also been described (118, 249*I*), but since E'_m increases and this was not correlated with V_T, the extent to which the increase in V_T is due to elevated E'_m or lowered E_T cannot be determined.

The utilization time, t, *i.e.*, the maximum (also minimum) time in which a rheobasic stimulus will evoke an all-or-none response, is comparable to λ (see Section 1 above). Therefore, both can serve as an index of the behavior of G_{Na} and inactivation, and perhaps of G_K. An increase in either λ or t connotes a slowing of the last two, a decrease indicates their augmentation, or opposite effects on G_{Na}.

At the node of Ranvier lowering of $[Ca]_o$ increases λ (212) and t (115); λ is also lengthened in lobster leg nerve (1). In muscle, raising $[Ca]_o$ decreases t (249*I*), an effect not attributable to the rise in E'_m since hyperpolarization (in nerve) increases λ (199, 212, 222). The effect of hyperpolarization is understood from the standpoint of reduced inactivation (or reduced G_K when this is pertinent). The action of $[Ca]_o$ requires either that the rate of inactivation be augmented or the rate of increase of G_{Na} delayed. This is consistent with the larger rheobase in elevated $[Ca]_o$. The available data for the squid axon indicate that raising $[Ca]_o$ decreases the rate of development of inactivation and slows the rise in G_K for a given depolarization (157*I*); moreover, at the node of Ranvier elevated $[Ca]_o$ delays the onset of rectification (72). One must conclude, therefore, that in elevated $[Ca]_o$ chiefly the rate of rise of G_{Na} is depressed and this accounts for the shortened λ and t and the increase in rheobase or the lower E_T.

Thus, we have the interesting situation that the increase of G_{Na} during subthreshold depolarization is delayed in elevated $[Ca]_o$, as it is in other stabilizers (see below); yet, once excitation occurs, at least in giant axons and cardiac fibers, the increase of G_{Na} is improved in contrast to the situation with the local anesthetics.

On the basis of the suggestion that Ca^{++} occupies sites on which penetration of Na^+ depends (Sections IV F and G), the effect of elevated $[Ca]_o$ on the subthreshold change in G_{Na} is understood in terms of more Ca^{++} to be removed from the membrane to expose the number of sites required for I_{Na} to exceed I_K; once this has been achieved, the regenerative depolarization (*i.e.*, the rise phase of the spike) releases the additional sites made available by the higher $[Ca]_o$, which has displaced Na^+ from them previously. Skeletal muscle and vertebrate nerve fibers do not show an improvement in the rising phase with elevated $[Ca]_o$ presumably because all sites are already occupied at normal extracellular calcium concentrations. It is possible this phenomenon could be demonstrated in skeletal fibers and at the node of Ranvier if these were previously depolarized or P_{Na} increased by low $[Ca]_o$ in the resting state.

The difference between $[Ca]_o$ and other stabilizers suggested here provides a possible basis for the far greater increase in threshold under the anode when $[Ca]_o$ is raised than when stabilizers are present (148*I*). It will be recalled that hyperpolarization can counteract local anesthetics.

b. Stabilizers. Local anesthetics and other stabilizers depress the development of ASP with little change in E'_m, *e.g.*, cocaine in cat spinal roots (177), urethane at the node of Ranvier (220), and procaine and cocaine in squid giant axons (405*I*, 424*I*). It has long been known that rheobase (and threshold generally) is increased (*e.g.*, 221, 223), which is in keeping with all prior indications of reduced increase in G_{Na}; this is consistent with the greater depolarization required for a given ASP at the node of Ranvier (220) or for initiation of the spike in heart (511*I*) and in muscle (488*I*) fibers. The marked inverse relationship between threshold and spike amplitude at the node of Ranvier under a variety of conditions (*e.g.*, with urethane concentration, at different times during the refractory period, and with membrane polarization) (221, 223) is further indication of the same underlying mechanism, *viz.*, increase in P_{Na}, as the basis of their magnitudes.

3. Threshold studies. a. General aspects. Mention has already been made of some of the factors that may be involved in the determination of "threshold", the minimum shock strength required to elicit a spike. Thus, if the critical membrane potential, E_T, is unchanged, the threshold will show an increase if an experimental variable causes the following:

(a) E'_m to become larger; the *change* in potential required is larger.

(b) R_m to increase; this will reduce the density of membrane current, especially when stimulating electrodes are close together compared to the "characteristic length" [see Section 3 b 2) below], thereby causing reduced excitability (145).

(c) R_m to decrease; since the final potential difference across the membrane is given by R_mI, the stimulating current must be higher.

This situation is complex, since the size and location of electrodes and the duration of the stimulating pulses employed are factors. Core conductor theory is developed to a point where its relation to various special conditions of stimulation have been or can be worked

out (*e.g.*, 228*I*). Only a brief discussion of some of the factors involved, based on more recent studies, is given in the following section. From a functional standpoint, the significant factors in cellular excitability are (a) by how many millivolts E_m must be decreased for it to reach E_T and (b) how readily this is achieved under physiological conditions of stimulation.

We have seen in Part I that resting R_m values frequently are not greatly changed, hence E'_m can be the major factor in addition to E_T. Jenerick's observations on muscle with $[K]_o$ show that the predominant effect of potassium in reducing threshold is attributable largely to its decrease of E'_m down to a certain point, *i.e.*, V_T, the *change* in E_m required to initiate the spike, decreases in proportion to the value of E'_m within a range of the latter lying between 85 and 105 mV; as E'_m is decreased further, the threshold increases again, *i.e.*, E_T now declines faster than E'_m (249*I*). Since the observations were statistical, the exactness of the proportionality is uncertain.

Such results are understandable from foregoing principles as follows: At larger values of E'_m, in that range which does not affect the state of activation or G_K appreciably, E_T changes little with decrease in E'_m, therefore threshold and V_T decrease; with still lower E'_m, E_T falls to a greater extent because of inactivation (and greater G_K) and threshold (or V_T) increases again.

The dual effect of potassium—an increase in excitability at lower potassium concentrations (or when first applied), and a decrease at higher concentrations—has been frequently reported in the past. More recent observations are those on the node of Ranvier (323*I*), on lobster fibers (238), on skeletal muscle fibers (251*I*), and on the squid giant axon (83).

The same principles are applicable to the familiar electrotonus curves, *i.e.*, the increase in rheobase in proportion to hyperpolarization by anodal current (anelectrotonus) and the decrease in rheobase only to a certain point with depolarization by cathodal current (catelectrotonus), and increase in threshold with further depolarization (cathodal depression). A number of recent studies have described this polarization curve for single nodes of Ranvier (*e.g.*, 160, 212, 221, 325*I*). The parallelism between rheobase changes in single myelinated fibers on application and removal of CO_2 (58*I*, 348*I*) with E_m changes in this gas (Part I) further demonstrates the importance of changes in E_m on rheobase changes. Also, lower members of the aliphatic alcohols (methyl to propyl) depolarize and decrease rheobase; those above butyl alcohol hyperpolarize and raise rheobase in frog nerve (167, 292*I*).

These results do not imply that E_T is unchanged, but rather that its changes may be small compared to those of E'_m. Until the changes in E'_m are carefully compared with V_T or E_T, one cannot be certain that changes in E'_m occur in the absence of changes in the latter.

That the processes underlying E_T may indeed be modified as expected is shown by indirect data such as those on the accommodation constant or utilization time. In a system like the node of Ranvier, which has a low capacitance that does not greatly limit the rate of membrane depolarization, simple neutralization of increased E'_m by a greater rheobasic stimulus should not be accompanied by an alteration in λ or t by the conditions that raise E'_m. Here, we find that hyperpolarization by CO_2 or electrotonus does reduce accommoda-

tion (increases λ) at the node (160, 222). In lobster single fibers depolarization with K^+ shortens λ and t (238). The effects of calcium, depolarization, *etc.* were discussed in the preceding two sections. Veratrine acts at the node of Ranvier like lowered $[Ca]_o$ and E'_m in reducing rheobase and λ (190).

Even more striking are the direct observations on both E_m and E_T and on their effects on threshold current shown in 2 records by Weidmann for Purkinje fibers (figure 1 in 511*l*). In these can be seen (a) the repolarization wave of the spike, the positive potential, and the diastolic depolarization obtained with intracellular electrodes, (b) the number of equal, 50-msec increments of depolarizing transmembrane current required to initiate another spike at various times during these membrane potential changes, and (c) E_T at the same times. During the early part of the rapidly falling phase terminating the plateau, when E_m is still less than at the end of diastole, E_T is relatively still lower than in the later stages of diastole presumably because inactivation is present (in keeping with the small, short spike obtainable at this time); however, the interval between E_m and E_T is small and only 4 units of current are required to stimulate. At the peak of PP, E_T is higher than later in diastole [presumably because the hyperpolarization has eliminated inactivation, as shown by the full spike obtainable now (figure 6 in 227)] but twice as much current is needed to drive E_m to E_T because E_m is so much higher still. And during the diastolic depolarization, although E_T falls again [mild inactivation, also shown by the spikes initiated at this time (227)], E_m declines more, so that intermediate intensities of current are required for stimulation. Thus, as pointed out by Brooks *et al.* (21) for auricles and ventricles, the excitability will show a rise followed by a fall followed by another rise (the last designated supernormality), as observed with "stigmatic" cathodes (*i.e.*, localized cathode, diffuse anode); the fact that the threshold for anodal break response can be lower than for cathodal make during the first hyperirritable phase, to which they also call attention (see also 42), is in keeping with the reduction of inactivation that hyperpolarization produces, as noted in Section IV A 1, and as observed in other fibers (*e.g.*, 424*l* on squid axons in low $[Ca]_o$).

The close approach of E_m to E_T during the refractory period in cardiac fibers has important implications for the arisal of arrhythmias and ectopic beating. If the final repolarization should be delayed, as may occur when the increase in P_K or decrease in P_{Na} is slowed [*e.g.*, by certain stabilizers, TEA, low $[Ca]_o$, veratrine *etc.*; see Section IV E 1, (21) and (42)], or if E'_m is lowered with little effect on inactivation (*e.g.*, by a small increase in $[K]_o$; see 87), then it is possible for E_m to attain E_T during repolarization (as occurs in TEA-treated twitch muscle fibers; see Section VI A 1), which will set off ectopic beats or arrhythmias (*cf.* 21). Thus, we see that in cardiac fibers even stabilizers may have potentialities for inducing a phenomenon they would be expected to prevent.

Threshold changes by virtue of changes in E_m relative to E_T appear to be involved in oscillations in excitability that correspond to oscillations in potential to be expected under such conditions. It will be recalled, however (Section V A 1), that in fibers in which C_m is large the level of excitability, as gauged from the ability of ASP to arise, precedes the fluctuations of membrane potential; this is because the excitable state is governed by the relative magnitudes of G_{Na}, inactivation, and G_K, but E_m is delayed in following them by virtue of the membrane charging time as governed by the time constant τ_m. The parallelism may appear stricter between E_m and threshold when capacitance is low, as at nodes of Ranvier, but here, too, the underlying permeability and activation changes are probably more pertinent. The parallelism between augmented excitability and the negative after-potential and between depressed excitability and the positive after-potential (see 82 for references) is much more readily understood from the underlying altered permeabilities (see Sections IV C and D) than from the small absolute changes in E_m.

Parallel studies of E_m and excitability changes, like those carried out by Arvanitaki in the oscillatory squid axon, are needed in systems which have been studied predominantly with threshold measurements. Thus, only oscillations in excitability have been described for the node of Ranvier (324*I*), or for nerve trunks (338*I*). In the latter studies, the hyperirritability immediately following a conditioning brief shock 90% of threshold subsides and is succeeded by a period of low excitability (subnormality); the latter is accentuated by conditions such as low $[Ca]_o$ and mild depolarization (132) that augment oscillatory behavior of membrane potential, and is depressed by hyperpolarization (*e.g.*, with CO_2 or anelectrotonus) (338*I*). Depolarizing aliphatic alcohols (methyl to propyl) augment the subnormality and hyperpolarizing alcohols reduce it (292*I*). The probable relation of the subnormality to inactivation (or increased G_K) is indicated by its increased intensity as the conditioning subthreshold pulses are applied at increasingly later times during continuous cathodal depolarization (32), much as inactivation (or G_K) can be expected to increase progressively during a depolarization. Moreover, Ba^{++}, which causes only depolarization in nerve trunks (314*I*), causes a decrease in R_m at nodes and a secondary increase in excitability superimposed on a decrease in excitability (324*I*). This is consistent with reduced P_{Na}, on which is superimposed a more marked decrease in P_K that is responsible for depolarization. In this case, careful study should reveal a decrease in E'_m greater than the lowering of E_T.

b. Geometrical factors. 1) Excitation. A full consideration of the wealth of experimental conditions described in the literature as modifying the threshold is beyond the scope of this review. The lack of details on the absolute changes in E'_m, activation, and in rectification (G_K) prevents one from determining whether current information on these phenomena suffices to account for the observed behavior. From the more straightforward studies discussed above, it appears that subthreshold excitatory changes, as well as excitation, are governed by the same processes elucidated for the spike. E_m must attain a critical value, E_T, because at this value I_{Na} cannot be overtaken by I_K or inactivation.

A word must be said about complexities arising from the core conductor properties of excitable cells when external electrodes are used for stimulation. Characteristic distributions of potential develop around the electrodes on make and break (228*I*), but their relation to excitatory processes has not been studied in detail. The well-known observation that, when short rectangular shocks are employed, the quantity of electricity (*i.e.*, the charge, given by the product of current and time) needed to stimulate is a constant, has been generally conceded to indicate that the potential difference across the membrane capacitance is lowered to the same value (for V_T is Q/C_m) (*e.g.*, 477*I*). Cole (29) has shown that the Hodgkin-Huxley equations lead to this result for space-clamped axons.

Electrode size was early recognized to modify the shape of strength-duration curves (47, 180). Attention will be directed here only to recent work on this aspect.

Coraboeuf *et al.* (39) have pointed out that, in single crab (*Carcinus*) fibers, excitability changes following a brief subthreshold shock resemble those in nodose fibers only when the stimulating electrode is very fine (*e.g.*, 0.1 mm in diameter), not when it is thick (*e.g.*, 0.8 mm). Thus, with fine electrodes hyperexcitability subsides very quickly and is followed by subnormality, while with

coarse electrodes the hyperexcitability subsides slowly (with a time constant of about 7 msec for 3 mm electrodes) and without a succeeding period of subnormality. In the nodose fibers the results are independent of electrode size.

These findings are readily understood from the properties of core conductors. When a fine cathode is employed, the initial decrease in E_m occurs over a very localized region, especially when the pulse is short so that spread of current to adjacent areas is delayed. On cessation of the stimulus, therefore, the limited amount of charge that has been deposited in the localized region is dissipated in 2 ways: By spread laterally, which charges the adjacent area, and by discharge through the membrane itself through its resistance. On the other hand, when a large area has been depolarized, the lateral spread of charge will be a much smaller fraction of the total dissipation, which may be expected to give a repolarization with a time constant approaching that of the membrane, $R_m C_m = \tau_m$. In keeping with this, τ_m of *Carcinus* fibers is about that obtained for subsidence of hyperexcitability when large electrodes are used, *viz.*, between 4.1 and 8.5 msec (Table 1 in Part I).

The rate with which the potential under a fine electrode rises after cessation of current flow will be slowest when the current flow has lasted long enough for a stationary state to be achieved; a faster repolarization is obtainable with briefer currents because they do not allow sufficient time for adjacent areas to become charged, consequently the dissipation of charge from under the electrode can occur more quickly to the yet uncharged regions when current flow ceases (see figure 2 in 228*I*). It is therefore of interest to find that even after a stationary state is achieved, E_m under a fine electrode will rise 3 times faster than expected from the time constant (228*I*). In the experiments of Coraboeuf *et al.*, an exponential discharge with a time constant about ⅓ that of the *Carcinus* membrane would certainly have led to a still faster recovery of E_m, in keeping with their excitability curves.

Rushton (179) pointed out some time ago that core conductor theory provides a similar qualitative basis for the different strength-duration curves obtained with fine and coarse electrodes in muscle.

Thus, with a fine electrode the membrane potential subsides fast enough to reveal the presence of inactivation (and elevated G_K?), whereas the slow decline in potential with large electrodes enables inactivation (or elevated G_K) to subside with it, and so it is not seen. The comparison by Coraboeuf *et al.* of the situation with fine electrodes and "unmyelinated" fibers to that at the node of Ranvier is quite pertinent; for from the available data on R_m and C_m at an internode and at a node bounded by segments of internode (the latter being the usual conditions of observation) (478*I*) it is easily shown that the time constant at the node is ⅙ of the adjoining myelinated segments. Hence, a short pulse applied to a node of Ranvier, even with a large electrode, will deposit most of the charge at the node, and this is dissipated quickly by spread to the myelin as well as by the low resistance (and time constant) of the node (Table 1 in Part I), so that the presence of inactivation (or elevated G_K if present) is revealed by the residual subnormality as in crab fibers when fine electrodes are employed.

In muscle, too, electrode size is important. Thus, a capillary cathode gives a high rheobase and a low value of λ with a marked temperature coefficient, whereas a large electrode gives a low rheobase and no accommodation ($\lambda = \infty$)

(3, 15). Here two factors must be considered: (a) Under the conditions of observation (volume conductor theory applies), a fine cathode will give an exceedingly high depolarizing current density surrounded by a ring of anodal current; the latter can be sufficiently intense to prevent excitation by limiting the depolarized area, as when a capillary electrode is brought close to the surface membrane (81). Thus, unlike the situation with a large electrode, by which the muscle membrane is relatively homogeneously depolarized, a very steep potential gradient exists around a capillary electrode, so that when it causes excitation the central-most region of the membrane has been exposed to a depolarization well in excess of that required with large electrodes. With such a large depolarization must be associated a greater inactivation (and rise in G_K?). The extent to which a genuine increase in inactivation (or G_K) is involved, as suggested by the temperature coefficient of λ, or merely the time course of development of the anodal surround, requires a mathematical analysis of the latter and further experimental study.

Benoit (15) notes the fast decline of excitability following brief cathodal shocks with a capillary electrode, as might be expected from Coraboeuf's studies with crab fibers. However, in muscle with degenerated nerve, large electrodes give rise to both a rapidly and a slowly declining excitability, the former being comparable in time course to that obtained with a fine electrode, the latter having a time constant to be expected from the time constant of the membrane of denervated preparations (345*I*). The reviewer suspects that the fast component may represent events at the junctions. A simple possibility is that the resistance at the degenerated endplates is much lower than in the muscle membrane proper, and hence, as at nodes of Ranvier, the build-up of potential is greatest there and dissipates quickly because of lateral spread of the charge; the rest of the membrane charges and discharges slowly. This possibility merits examination, for it may bear on the hyperirritability of degenerate myoneural junctions to acetylcholine. It could be tested in the same manner as in studies of the spread of charge from endplates following indirect stimulation (120*I*).

Rushton (178, 179) carefully analyzed the "γ" and "α" excitabilities in freshly dissected muscle many years ago and concluded the fast component (γ) was due to nerve fibers, the slow component (α) to the muscle fibers. If in Benoit's preparation the innervation was completely degenerated, it would be interesting to check whether d-tubocurarine still eliminates the fast component, as observed by Rushton. If it does, one would have evidence of an increased resistance at the endplate by the drug which is not obtainable by more direct means (Part I).

2) Conduction. Propagation of the action potential along excitable fibers is a process dependent in part on (a) the magnitude of the spike relative to the depolarization needed to excite the region ahead (S/V_T), known as the safety factor (also designated as the ratio of normal S to the lowest spike or equivalent shock that can stimulate), and (b) the spread of local currents from the active region, in turn dependent on the core conductor characteristics of the fiber (R_m, C_m, R_i, or more specifically the time constant of the membrane, $\tau_m = R_mC_m$, and the characteristic length, λ_x, the distance in which a maintained electrical change in polarization subsides to 1/e of its value under the electrode). Katz (265*I*) presents the following tentative formulation for the velocity of propagation in a large volume of medium or in a tissue:

$$v = (S/V_T)a/C_m\sqrt{2R'_mR_i} \qquad (IX)$$

in which a is the fiber radius and R'_m is the membrane resistance during the spike. Trautwein *et al.* (497*I*) point out that the effect of low temperature on R_i and R_m (if the latter is used in place of R'_m), the only parameters altered with temperature (*cf.* 59*I*, 211 on rheobase in the heart and node of Ranvier, 482*I* on C_m, 105 on R'_m, and 88*I* on R_m), is too small to account for the conduction change. However, Tasaki (211, 482*I*) finds that although the rheobase is unchanged by low temperature at nodes, the threshold and latency to brief shocks are increased substantially. Measured changes in R_m are too small to account for these effects (88*I*). The delayed development of G_{Na} at low temperature (102, 103) is probably the main factor, for not only are the events too early for inactivation (or G_K) to be involved, but the onset of the latter is also delayed by low temperature, in keeping with the substantial prolongation of λ seen at nodes of Ranvier (211). The observation of Trautwein *et al.*, that the action of low temperature on conduction velocity parallels its effect on the rising phase of the spike, is in keeping with our proposal, for the rising phase, too, depends on rate of increase of G_{Na} under these conditions. This is because the maximum amplitude of G_{Na} is not greatly affected by low temperature (at least in squid axons: 102, 103).

These considerations therefore require that an additional factor be added to equation IX that will take into account changes in the rate of development of G_{Na} (or I_{Na}), such as might occur under conditions that alter the state of activation (*e.g.*, changes in E'_m, stabilizers) or when $[Na]_o$ is changed. A comparison of the effects of such variables on conduction velocities is probably possible from available literature, but will not be attempted here (see 21, 77*I*).

An equation such as IX should also include R_m—that of the as yet unexcited membrane; this would also take into account the ability of the spike to pass a blocked region. Thus, Tasaki (477*I*) has noted that the number of blocked nodes beyond which a spike can be transmitted depends on whether the resistance of the nodes (and myelin) is lowered (as with KCl) or not (as with urethane). In the former case the local currents do not spread as far since they are dissipated by the low resistance. This is one reason why low concentrations of agents that depolarize will affect conduction velocity differently from those that do not depolarize (*cf.* 21).

In the central nervous system changes in membrane resistance may play an important part in the action of inhibitory transmitters (Part I). Thus, the rise in G_m they cause, especially if this occurs on dendrites more central to the soma than the locus of action of excitatory transmitters, could reduce the spread of the electrotonic potentials (or spikes?) that the transmitters produce in the more distal regions of the dendrites.

Thus, the excitation processes and their modification by physiological and pharmacological agents appear to be quite consistent with the behavior of P_{Na}, P_K, and inactivation as elucidated by studies of the spike. However, more direct observations of G_m, E_m, and E_T and further computations based on the Hodgkin-Huxley model under the many conditions that have been described are necessary to establish fully the complete adequacy of the spike processes.

VI. IMPLICATIONS

A. Repetitive activity

The repetitive activity discussed in previous sections can be placed in at least 3 categories: (a) spontaneous, arising with no external stimulation or imposed depolarization, as in the rhythmic activity in the heart or in fibers subjected to low $[Ca]_o$; (b) that arising following a single spike with no obvious change in the level of E_m', as in low $[Ca]_o$ and in low concentrations of DDT; and (c) that arising from a sustained cathodal depolarization of sufficient intensity.

1. Repetitive activity during prolonged depolarization. Cole *et al.* (30) have shown that the characteristics of type (c), *viz.*, that the frequency and duration of the train of impulses increases with the intensity of the depolarization (*e.g.*, 21*I*), are predicted by the Hodgkin-Huxley equations. Apparently, each impulse restores the membrane (by virtue of the positive potential) despite continued cathodal current so that permeability and activation changes are recurrent. Obviously, if inactivation (or the prolonged refractory period) were not reversed, repetitive firing could not take place. Since repolarization will decrease the inactivation, the increase in G_K at the end of the spike must play a particularly important role in the sustained repetitive activity. Also, depolarization increases G_K and hastens its rise during the spike (and in subthreshold oscillations) in those fibers where G_K is susceptible to change (Section IV); this no doubt contributes to the rhythmicity of firing as well as to subthreshold oscillations.

It is important for sustained repetitive activity that development of inactivation be slow compared to the rate of increase of G_{Na} during cathodal depolarization. In keeping with this, it has long been known that repetitive firing during sustained depolarization is enhanced as λ is increased (*e.g.*, 122), an observation substantiated by comparison of invertebrate motor fibers with different values of λ (2, 97, 236).

The frequency of repetitive activity will be a function of the rate of development of G_{Na} (reflected by the speed of development of the active subthreshold response, as observed by 97). This, in turn, will be reduced by stabilizers or augmented by labilizers. The frequency will also depend on how close E_m' is to E_T as long as the state of inactivation is not greatly affected. In keeping with this, Brink *et al.* (21*I*) have described the increased rate of spontaneous firing with depolarization in vertebrate nerve that subsides somewhat with time (presumably due to increased inactivation) and converse effects with hyperpolarization.

In rhythmic heart fibers the same factors are operative with the additional features of the diastolic depolarization (512*I*). Thus, the rate can be slowed by delaying the latter [*e.g.*, with low temperature (59*I*), low $[Na]_o$ (96*I*), and Ach (245*I*)] or by lowering E_T [*e.g.*, with elevated $[Ca]_o$, local anesthetics, and quinidine, shown by (511*I*) and (35)]; it can be increased by accelerating the diastolic depolarization [as with epinephrine (245*I*)].

Another type of repetitive activity is exhibited by TEA-treated muscle (191*I*) and invertebrate (25) and vertebrate (40) nerve fibers. In these, when the slower repolarization

that occurs following the spike (apparently due to failure of P_K to rise, as discussed in Section IV E) reaches the critical level, another spike arises. The slower the repolarization, the lower the frequency of firing (191*I*). Evidently, when inactivation has subsided sufficiently during the rise in E_m, the regenerative increase in G_{Na} can occur again—apparently because G_K is so low in TEA. Hagiwara and Watanabe (191*I*) make the additional important observations that by keeping the fiber depolarized electrically one can suppress or make weaker the subsequent responses, whereas with hyperpolarization one can augment them, in accord with the characteristics of inactivation. The action of TEA on the heart may provide interesting data on the importance (or lack of importance) of P_K in its rhythmicity.

2. Repetitive activity following brief threshold shocks. This requires that there be a large depolarizing oscillation, following the spike, adequate to attain $\bar{E}_T$, which must be back nearly to normal. Thus, inactivation must also subside quickly during the first spike. These requirements are evidently met in low $[Ca]_o$, veratrine alkaloids, DDT, and many other compounds (Section III D). Veratrine was pointed out to differ from low $[Ca]_o$ and DDT in giving rise to a negative after-potential, which is not a necessary concomitant of repetitive activity.

A comparison of the action of low $[Ca]_o$ with the other agents has suggested that the latter do not exert an effect, or as large an effect, during the resting state as appears in the repetitive activity following the first impulse. The growth of oscillations when successive shocks are delivered to fibers in veratrine suggests a progressive depletion of calcium, which ordinarily stabilizes by being in or on the membrane, and that this is but slowly restored. Such a process also is suggested by the progressive shortening of the interval between successive spontaneous spikes, of sciatic nerves in veratrine, towards the value obtained in calcium-free Ringer (413*I*). This conclusion is in keeping with previous considerations. The modification of an event during the spike, such as has been proposed, appears necessary to account for the failure of agents such as veratrine and DDT to produce spontaneous activity although they cause intense repetitive activity following a single spike.

Sato (187) has called attention to a considerable lengthening in λ of nodes of Ranvier in 5 times hypertonic NaCl that is not associated with repetitiveness. Threshold is not greatly affected (323*I*). However, the absence of repetitiveness is not surprising in the light of the considerable delay in subsidence of inactivation indicated by the greatly prolonged relatively refractory period (187). It will be recalled that hypertonicity with sucrose also increases spike duration (which corresponds to the increased absolutely refractory period also seen by Sato); consequently, the possibility merits exploration that hypertonicity rather than Na^+ (or Cl^-) is the responsible factor, and that at nodes it slows both the development and subsidence of inactivation. The effect of hypertonicity on muscle spikes is negligible (438*I*), perhaps because muscle fibers are better osmometers.

Lüttgau (324*I*) has most recently reviewed several parameters of possible relation to rhythmical activity from the standpoint of the action of Ba^{++}, Ca^{++}, Na^+, K^+, and electrotonus, *viz.*, rheobase, resting potential, λ, and the degree of "damping" of the decline in excitability following a brief subthreshold shock. The action of Ba^{++} is interesting in that it first causes a fall in excitability on

which is superimposed a hyperirritability; the fall is probably due to lowered P_{Na}, the rise most likely the consequence of a depolarization (Part I), and this apparently is due to a decrease in P_K, for G_m is reduced (325*I*) (*cf.* the action of Ba^{++} as a stabilizer in Part I). Although elevated $[Ba]_o$ resembles low $[Ca]_o$ with respect to its action on the parameters discussed by Lüttgau, only the latter appreciably increases spontaneous activity. However, Ba^+ exerts an opposite effect on the permeabilities (although this is not evident from the measurements summarized by Lüttgau), and this can be expected to account for the difference. The potency of small quantities of stabilizers in suppressing after-discharge, pointed out earlier, is in keeping with this.

3. Spontaneous repetitive activity. Little can be said on this score except to point out that lowering of $[Ca]_o$ is particularly effective in bringing about spontaneous activity. For this to occur, at least 2 conditions in addition to the above are necessary, *viz.*, an E_T which is close to E_m with little change in the state of inactivation (*i.e.*, a low threshold of excitability), and spontaneous (thermal?) fluctuations in G_{Na}. These conditions are indeed satisfied, for we have seen that E_m' is little changed by low $[Ca]_o$; also, rheobase is greatly decreased and spontaneous fluctuations in E_m are increased (21*I*). The spontaneous fluctuations may be expected from increased effectiveness of thermal agitation of membrane molecules as a result of reduced rigidity (Part I).

Depolarization, electrically or by drugs, may itself enhance spontaneous activity, presumably by contributing to the removal of Ca^{++} from the sodium sites by the postulated formation of ion pairs; this cannot be as effective as low $[Ca]_o$ because of the greater development of inactivation, as seen in the transitory elevation of spontaneous activity during depolarization (21*I*).

In conclusion, after-discharge can be anticipated under conditions that augment sodium and potassium permeabilities and do not interfere with or enhance recovery from inactivation. In addition to these factors, a slow rate of inactivation development (λ or utilization time long) is required for repetitive activity during sustained depolarization. An evaluation of the mechanism of action of an experimental condition cannot be regarded as complete without data related to these phenomena, such as E_m', E_T, rising and falling phases of the action potential, including the positive potential, as well as utilization time or λ, and refractory period.

B. Skeletal and heart muscle contraction

A full understanding of the action of physiological and pharmacological agents on contraction in living muscle cannot be obtained without a determination of the extent to which alterations in excitability and the action potential as well as in the contractile mechanism contribute to the results. Unfortunately, the former is not always considered, although in many studies, for example in metabolic inhibition, this may well be important. An extended discussion of this subject is beyond the scope of this review, but more obvious relationships between the bioelectrical phenomena and initiation of contraction will be pointed out.

It has long been recognized that under normal physiological conditions the

spike triggers muscle contraction. Propagation and associated longitudinal currents as such have been ruled out as of any importance by stimulation with "massive" electrodes, whereby the entire fiber is excited at once with the usual contraction (183, 184), and by demonstration with microelectrodes that longitudinal currents in the myoplasm have no effect, whereas depolarizing transmembrane currents cause shortening (191*I*). Thus, one is led to suspect the depolarization itself as an important element in the process. In keeping with this is the wealth of evidence under a wide variety of conditions that depolarization—by electrical means, by drugs, by ions—causes contraction or contracture of skeletal, heart, and perhaps smooth muscle; and when this depolarization is prevented or reversed, electrically or by drugs or ions, relaxation follows (*e.g.*, 26*I*, 99, 113, 147*I*, 149*I*, 151*I*, 152*I*, 153*I*, 162, 285*I*, 346*I*).

On the other hand, depolarization itself is not the most intimate link. Thus, the shape of the action potential, especially in the heart, bears no obvious relation to the strength of contraction. For example, while the spike is reduced and shortened by lowering $[Na]_o$, the contraction of the heart may be greatly improved (155, 346*I*). Trautwein and associates (55a, 493*I*–495*I*) find little correlation between spike duration and strength of the heart contractions under a variety of other conditions, *e.g.*, varied heart rate, stretch, level of $[Ca]_o$, strophanthin, and post-anoxic recovery. Contraction (contracture) can occur without depolarization, *e.g.*, in TEA-treated insect muscle (191*I*) and in smooth muscle (113*I*), and it can fail to occur with depolarization, as when Ca_o^{++} is lacking (*e.g.*, 346*I*). In skeletal muscle, iodoacetate causes contracture to begin immediately, but depolarization is delayed for two hours; moreover, contracture appears in isotonic $CaCl_2$ without a change in E_m' (306*I*). Fast "twitch" fibers as well as heart and smooth muscle recover from K^+ contracture despite continued presence of K^+ and without change in the depolarized state (184, 289*I*). Although the depolarizing effectiveness of K^+ may be little changed, twitch fibers go into contracture at lower $[K]_o$ as Cl^- is replaced with more polarizable anions (99). Action potentials of skeletal fibers are practically unchanged when Cl^- is replaced by anions only slightly more polarizable, yet maximal twitches are augmented (144).

Thus, another link is present between depolarization—whether by spike generation or by K^+—and shortening of the contractile elements of muscle. This is most likely to be ionized Ca^{++}, for only it, of all the physiological ions that have been injected in small quantities—K^+, Na^+, Cl^-, Mg^{++}, and ATP—causes contraction in skeletal muscle (90, 92), a point stressed by Heilbrunn (91). Direct evidence is at hand of increased calcium influx, I_{Ca}, during activity and K^+ depolarization of squid axons (69*I*, 126).[4] Indirect evidence of the importance of Ca^{++} for contraction and contracture is substantial, a point especially emphasized by studies on the heart. Thus, in the absence of extracellular Ca^{++}, a heart will gradually fail, but if the surrounding volume is small,

[4] Since submission of the manuscript, Ca^{45} measurements made in our laboratory by Dr. C. P. Bianchi have shown that calcium influx is increased by stimulation of frog sartorius muscle, and this is of the order of 0.3 $\mu\mu$mole/cm^2 per twitch.

leakage from the heart will cause $[Ca]_o$ to rise and restore the strength of contraction (284*I*). Potassium contracture of the heart also requires extracellular calcium (162, 346*I*).

The more extreme conditions of heart failure in low $[Ca]_o$ are probably due to failure of spike production. Attention was called in earlier sections to inactivation and the increased G_{Na} and G_K which develop. This inactivation was suggested earlier to be due to saturation, by Na^+ and K^+, of sites normally occupied by Ca^{++} and necessary for increased sodium entry during excitation. However, the dependence of K^+-contracture, which is independent of the excitable mechanism, on $[Ca]_o$ shows a specific role of Ca^{++} in the shortening of the muscle protein.

That the role of $[Ca]_o$ is to supply Ca^{++} to the myoplasm via the membrane, which also obtains some of its calcium from an intracellular store of Ca^{++}, is shown by the following:

(a) When Ca_o^{++} is removed at the time $[K]_o$ is raised, the initial contracture of heart fibers is practically normal, but declines faster than when Ca_o^{++} is present; if Ca_o^{++} is removed minutes before K_o^+ is added, contracture is weak and slow in developing unless Ca^{++} is added again (162).

(b) When Ca_o^{++} is first removed from single vertebrate and invertebrate fibers, the depolarization or rise in excitability is slow; subsequent application and removal of Ca^{++} acts very quickly (21*I*, 324*I*, 461*I*).

(c) After removal of Ca_o^{++}, but before contractility and spike production completely fail, repetitive stimulation of frog skeletal muscle at low rates causes rapid progressive decline in multifiber spikes and in the shortening; this can be repeated a number of times, each time after a brief rest. After failure is complete in zero $[Ca]_o$, replacement of Cl^- with SCN^- immediately restores contractility and the action potential, which again show progressive decline with slow repetitive stimulation and recovery with rest [see (e) below]; in the continued absence of $[Ca]_o$, failure finally is permanent. These experiments were recently carried out in our laboratory specifically as a test of the hypothesis that the calcium of the membrane, $[Ca]_m$, is released and depleted with excitation and restorable from intracellular reserves (201).

(d) Agents which form complexes with Ca^{++} and with other multivalent ions can be expected to improve retention of Ca^{++} in the membrane [see (e) below]. From this standpoint, Loewi (140) accounts for improved contractility of depressed hearts by F^- and oleate, as well as Ca^{++}. Improved cardiac contraction with organic and inorganic phosphates and bentonite (152) is probably the consequence of the same mechanism. The reduced permeability of skeletal muscle to ions and water in certain Ca-complexing agents (410*I*) and the resistance of nodes of Ranvier to Ca^{++}-lack when phosphate is used as a precipitant (72) may involve the same action.

(e) Substitution of more polarizable anions (Br^-, NO_3^-, I^-, SCN^-) for Cl^- enhances skeletal muscle twitches so rapidly that a surface effect is necessarily involved (120, 121, 173); the effectiveness of the anions increases with polarizability in the same sequence as their ability to reduce G_m and raise E_m, and inversely as their ability to penetrate the membrane (Part I). These effects are explainable as the consequence of increased $[Ca]_m$, as in the case of (d).

Studies currently in progress in our laboratory by Dr. Bianchi have already been successful in demonstrating augmentation of Ca^{45} movement in skeletal muscle by more polarizable anions.

The studies by Scatchard and associates (191, 192) reveal that the binding of anions to serum albumin is in the expected increasing series $Cl^- < Br^- < NO_3^- < I^- < SCN^-$. F^- behaves anomalously, for it is comparable to SCN^- at higher concentrations, more being bound at about 0.3 M, and less below this concentration; more F^- is bound than I^- down to 0.02 M. This behavior of F^- is probably related to its tendency to form complexes (*e.g.*, p. 467 in 153) and is of interest in the light of the improvement in contractility that it also induces [(d) above]. Thus, the reactions of the anions with albumin appear to be related to their effectiveness in improving contractions and to their action on passive membrane characteristics.

Saroff and Simpson (186) find that binding of Zn^{++} to serum albumin is substantially increased by SCN^- over that in NO_3^-. Even though the binding of Ca^{++} undoubtedly involves somewhat different and weaker bonds (*i.e.*, "ionic bonds", such as with carboxyl groups, rather than "homopolar" bonds or "valence bond resonance"; see 125, 153), Dr. Saroff (personal communication) points out that enhancement of calcium binding of this type is also to be expected with more polarizable anions.

Such studies and considerations show that augmented interaction of calcium with suitable molecules is possible, but do not indicate whether the same reactions or similar membrane components are involved. But the essential ionic properties would appear to be those outlined here. The considerable information available on many different metal complexes, and the systematic methods available for their study—pH, ion series, *etc.*—give promise of clarifying the details for the living membrane (*cf.* chapters 3–6 in 153).

Not only may more polarizable anions raise $[Ca]_m$, but their polarizability should favor association during depolarization (Part I), which could also contribute to the lower potassium concentrations that induce contracture of skeletal muscle in their presence (99). This would be comparable to the lower potassium requirement for contracture of heart fibers when $[Ca]_o$ is elevated (162).

(f) Niedergerke and Lüttgau (346*I*) have shown that the positive inotropic action (*i.e.*, improvement of contractility of weakened hearts) of Ca_o^{++} and low $[Na]_o$ reflect a stoichiometric competition for surface sites, improved contraction (or contracture in elevated $[K]_o$) being favored with greater $[Ca]_o/[Na]_o^2$ ratios. This conclusion was supported by the demonstration of increased influx and reduced outflux of Ca^{45} when $[Na]_o$ (or $[K]_o$) was lowered (346*I*); also, the kinetics of emergence and of contractile effects of Ca^{++} appears to require a substantial amount of the alkaline earth in the membrane (163).

(g) The augmentation of contractility or of Ca^{++} effects by cardiac glycosides is consistent with an increased $[Ca]_m$. Thus, strophanthin counteracts the effects of low $[Ca]_o$ on heart rate and on the configuration of the action potential (225); the effects of digitalis on spike and contractility are remarked to be similar to low $[Na]_o$, as observed by Daly and Clark for strophanthin (472*I*), which from (f) (and previous sections) is consistent with elevated $[Ca]_m$.

It remains to be determined whether cardiac glycosides do indeed raise $[Ca]_m$ and with it the availability of Ca^{++} to induce contraction; and, if so, whether by an action like anions, directly, or indirectly by keeping Na^+ from the sites. The additional many indirect observations described by Wilbrandt (517*I*) point to increased $[Ca]_m$ and with it increased entry of Ca^{++}, although he prefers an interpretation in terms of decreased active calcium outflux.

Wilbrandt has described experiments which appeared to show reduced O_{Ca} in glycosides; subsequent experiments in his own laboratory and by Harris fail to confirm this (personal communication). Another explanation has been in terms of a lowered Na^+ and K^+ content of the fibers (85), but Reiter (365*I*) actually finds an opposite change in net ionic content accompanying improved contractility with ouabain.

It should be pointed out that the tendency of digitonin to form complexes [with steroids (41)] may, like that of anions, be significant for the effect of cardiac glycosides on $[Ca]_m$. Moreover, if the sites affected also include those which transport sodium, the failure of active transport under the influence of the cardiac glycosides would follow as a natural consequence of their being occupied by Ca^{++}.

(h) Quinidine and quinine act like the more polarizable anions on maximal muscle twitches (133). Whether their effect is due to stabilization, which decreases outward leak of $[Ca]_i$ or reduces membrane sodium, thereby raising $[Ca]_m$, or to a reaction similar to that of anions, remains to be determined. The action of yohimbine and other stabilizers would be of interest in this connection.

It is of interest that quinine (59a), like SCN^- and I^- (17, 59b, 144), increases the negative after-potential in muscle. While this appears to be in keeping with the general observation that muscle shortening depends on depolarization, Lubin (144) points out that the prolonged muscle activation substantially outlasts NAP in SCN^--treated muscle fibers, and the augmented contraction with NO_3^- occurs with a questionable enhancement of NAP. The more likely view would therefore seem to be that the changes in NAP and muscle contractility are signs of the same or related processes, rather than one the cause of the other.

Thus, many observations strongly favor the view that a high level of $[Ca]_m$ is intimately concerned with skeletal and heart muscle contraction; others appear to be interpretable from this standpoint. Hence, the proposal that excitation and spike generation constitute a depolarization that causes Ca^{++} to be displaced from sites by ion pair formation with an anion, and that this ion pair diffuses into the cell, provides a basis for the link between electrical and mechanical phenomena. Sandow (183) has pointed out that in Arbacia eggs increased $[K]_o$ causes the transfer of calcium from the cortical layer to the interior. We may note, too, that improved heart contractility in low $[Na]_o$ immediately rules out increased $[Na]_i$ or decreased $[K]_i$ resulting from the spike as of any importance in contractility in this system. In "twitch" fibers, as well, K^+-contracture is not interfered with by replacement of Na_o^+ with $choline^+$ (99), hence sodium is unimportant for the shortening.

At this point one cannot state whether the Ca^{++} effective in contraction is derived solely from the sites concerned with Na^+ and K^+ movement or is released from many others as well during the depolarization. The latter is the more likely situation in muscle, a point that remains to be verified by comparing calcium influx in contractile tissue with that in nerve.[5]

[5] Our estimate of an increased calcium influx of 0.3 $\mu\mu$mole/cm^2 per twitch (footnote 4) is probably on the low side for technical reasons that cannot be gone into here. Nevertheless, it is 50 times greater than that obtained per impulse in squid giant axons (109), a difference which is especially significant in view of the ten-fold smaller calcium concentration of Ringer compared to the medium used for squid axons.

Although the possibility of entry of ionized calcium as a factor in contraction of skeletal muscle has been previously noted (*e.g.*, 183), it has been generally discounted because diffusion from the surface would be too slow to affect the entire fiber rapidly enough.

However, a metastable state in muscle—either with respect to the "relaxation factor" (88, 158) or to some of the bound calcium itself—is conceivable whereby the entry of ionized calcium functions like the "seeding" of a supersaturated solution, in which a chain-reaction acts over large distances. Or the radial hollow tubes which have been suggested to compose the Z-bands in skeletal muscle and to descend into the myoplasm from the fiber surface (98) may suffice to reduce the diffusion distances so as not to present a problem. The kinetics of K^+-contracture and muscle twitches must be analyzed and compared with Ca^{45} movements before an attempt can be made to analyze the specific manner in which Ca^{45} could trigger shortening. The initial part of the sequence of reactions worked out by Sandow to account for the latency relaxation as well as for the twitch (182) may be helpful in this respect.

In any case, it is important for the hypothesis that increased free Ca^{++} in myoplasm be demonstrable. In view of the evidence for the low level of unbound Ca^{++} in nerve and muscle already given, a substantial increase in calcium outflux during stimulation might be expected if there is such a rise in ionized calcium. In giant axons, it will be recalled, no appreciable increase in O_{Ca} occurs with stimulation (109).

Recent experiments in our laboratory (201) confirm the preliminary report by Woodward (234) that the release of Ca^{45} is accelerated during stimulation of muscle. In these studies it was found important to tetanize for repeated short periods to avoid fatigue; single twitches (*e.g.*, 1 to 2/sec.) were ineffective, as noted by Harris (201*I*). These results are consistent with lack of a net change in intracellular calcium during tetanic stimulation of muscle (64), for the increased influx of activity would tend to cancel the increased outflux.

From this standpoint, "treppe", the improvement in contraction with the first few maximal twitches, especially in low $[Ca]_o$ (*e.g.*, 161, 192*I*), is due to the augmented intracellular ionized calcium, which favors its entry into the fiber surface and thereby raises the available Ca^{++} for release during the next impulse. Raising $[Ca]_o$ or lowering $[K]_o$, which by its reduced competition with $[Ca]_o$ also raises $[Ca]_m$ (346*I*), makes the initial twitches larger (161); this would follow from the additional $[Ca]_m$ supplied by the medium under these conditions.

The fact that Ba^{++} and Sr^{++} can substitute for calcium to maintain heart contraction (128, 346*I*) is in keeping with their effectiveness in causing contraction on injection into skeletal mucle (90, 92).

The prolongation of the "state of muscle activation" by all conditions that improve maximal twitches (*e.g.*, 133, 144, 184) and the prolongation of potassium contracture in "twitch" fibers by more polarizable anions (133) may be due to a more prolonged entry of Ca^{++} into the myoplasm or to a greater "slug" of Ca^{++} that requires more time to be bound by the intracellular constituents.

The increased uptake of Ca^{45} by deteriorating muscle shows that there is potentially a considerable reserve of binding sites (171*I*), as might be expected to enable the tissue to handle many contractions until active transport can take over. The existence of active calcium transport remains to be demonstrated experimentally. Metabolic inhibition experiments failed to reveal a change in O_{Ca} in squid axons (109). It would be interesting to determine whether one factor in the contracture of fatigue may be excessive accumulation of intracellular ionized calcium.

We emerge, therefore, with the postulate that the appearance of ionized calcium in myoplasm triggers contraction. Depolarization of any kind, including that during the spike, has been suggested to allow calcium in the membrane to associate with available anions (by depression of the "electric field effect") and to diffuse as ion pairs into the protoplasm, where they dissociate. From this standpoint, the importance of the calcium content of the membrane lies in providing an adequate source of calcium that will ionize on entering the fiber. The rate of entry or the quantity of ionized calcium entering the myoplasm may be expected to be a factor in delaying the rebinding of Ca^{++}. Differences in the duration of contracture in different muscles reflect important differences in subsequent kinetics that remain to be resolved. These cannot be discussed here.

It must be emphasized again that the idea of Ca^{++} entry has appeared many times before. It has now been presented in a detailed context that appears susceptible to tests from the standpoint of contraction, of spike generation, and of ion fluxes in the resting and excited state. The details are not regarded as unassailable nor necessarily as completely general. They can hardly be expected to stand unmodified as new facts emerge. They are offered, however, in order to focus attention on those variables which appear to integrate numerous phenomena and as a concrete basis for further experimental study.

C. Smooth muscle

In this tissue, particularly, the dissociation between effects via the excitable system and E'_m and that directly on the contractile mechanism has been little studied on the cellular level (see 75). The outstanding recent work is that of Bülbring and her associates. The technical difficulties involved in microelectrode measurements on such small cells have been pointed out; to this must be added the technical problem of correlating tension or shortening due to many cells with electrical phenomena observed in individual cells. The parallelism that has been observed in guinea pig taenia coli between increased tension and the decrease of E'_m and increased spontaneous activity under a wide variety of conditions (22, 25*I*, 26*I*) suggests remarkably uniform behavior of the fibers. Whether the phase difference between frequency of spikes and tension in rat pregnant uterus (514*I*) or the absence of correlation reported between depolarization and tension in cat myometrium and rodent taenia coli (46) represents solely a lack of coordination among the short fibers sampled and the major population contributing to the tension cannot be answered at present. The studies of Prosser *et al.* (168–170) suggest "ephaptic," *i.e.*, fiber to fiber transmission, probably electrical, rather than the mediation of nerve fibers, in the rat ureter, chick amnion, and cat intestine; if the safety factor is low [Section V 3 b 2)], as it well may be especially *in vitro*, lack of coordination would not be surprising when so many fibers are involved. Effectiveness of transmission in such preparations should be checked.

In certain respects, spike production in smooth muscle resembles that seen in the previously discussed systems. Thus, depolarization, electrically or with ions or drugs, reduces the amplitude and increases the duration of the spikes

and the frequency of repetitive discharge; hyperpolarization has opposite effects (23, 169). Also, lowering $[Na]_o$ by replacement with sucrose to ⅓ or 9/10 depresses spike production; however, choline$^+$ can replace all but 17 mM Na_o^+ with little effect on the spikes or repetitive activity (113). Only when all Na_o^+ is replaced with choline$^+$ does spike generation fail (113). It remains to be determined whether choline$^+$ can replace Na^+ as a penetrating cation during the spikes, as guanidine appears to act at nodes of Ranvier and perhaps choline in heart fibers.

That the contractile mechanism may be inhibited independently of electrical activity is indicated by the loss of tension development in dinitrophenol and azide before electrical activity and the latter's response to drugs (acetylcholine, histamine) are greatly affected (24).

Activity in smooth muscle resembles that in other excitable cells in that its initiation or increase by various means (*e.g.*, Ach, neostigmine, TEA, histamine) leads to a loss of intracellular potassium (16*I*, 46, 149, 301*I*). In a preliminary report Daniel (46) states that this release is obtainable when contraction is induced in the absence of Na_o^+. This is of interest in the light of the observations by Hughes *et al.* (114) that lowering $[Na]_o$ quickly restores the contractile response to Ach and histamine in guinea pig uteri that have either gained sodium by staying in Krebs' solution or have been rendered insensitive (tachyphylactic) by large doses of the same drugs; moreover, strophanthin acts like low $[Na]_o$ in tachyphylaxis.

The last observations suggest that the same interaction between $[Na]_o$ and calcium in the membrane surface as occurs in the heart can play a part in governing $[Ca]_m$ and hence smooth muscle contraction. The increase in tension with depolarization found by Bülbring and Holman would be in keeping with ion pair formation as suggested above for skeletal and heart muscle. A study of the effect of substitution of more polarizable anions for chloride on smooth muscle contractility and electrical activity is therefore desirable, as is an examination of Ca^{++}-flux and its relation to potassium loss in contracting smooth muscle, especially under conditions in which depolarization apparently is not involved (*e.g.*, 113*I*).

D. Myoneural junctions

The initiation of an impulse at myoneural junctions in "twitch" or "fast" fibers has been shown to be dependent on the endplate potential, e.p.p., attaining the critical firing potential, E_T, of the electrically excitable membrane of the muscle cell (*e.g.*, 119*I*, 120*I*, 342*I*, 488*I*, 489*I*). Thus, in the light of the details discussed in Part I and here, transmission across endplates can be altered by changes (a) in the release of the transmitter (by interference with production of the transmitter, with its storage in the terminals, or with the release mechanism), (b) in the electrical reactivity of the endplate (through "stabilization" and perhaps "competition" for sites), (c) in the enzymatic activity of the transmitter esterase, and (d) in the excitability of the muscle membrane. Nicholls (345*I*) reports that denervation leads to an increase in the

critical firing potential, the depolarization required for stimulation, V_T, being about ½ that of controls; this therefore contributes somewhat to the Ach threshold being $\frac{1}{100}$ of that of intact controls.

It has been suggested (109) that the entry of Ca^{++} into nerve terminals during activity may disrupt the vesicles seen in this region which may contain Ach (86*I*), thereby causing Ach release. The augmentation of Ach release by raised $[Ca]_o$ (Part I) and by increased $[K]_o$ (243*I*) and the effects of presynaptic depolarization (49, 139) are in keeping with this proposal. Evidence for disruptive effects by the calcium ion on protoplasm and its organelles also is available (109).

E. Central nervous system

Transmitter release and synaptic reactivity as well as the excitability of the pre- and postsynaptic cells, indicated as factors in studies of myoneural junctions, represent only part of the pattern requiring analysis for interpretation of central effects. For we have to deal with excitatory and inhibitory synapses, with the relative activity of each, with their geometry relative to each other, with their relative sensitivity to physiological and pharmacological agents, with the presence of negative and positive after-potentials because of continual activity, *etc.*

Phenomena observed with either nerve or muscle alone have long been known to be insufficient for a description of events in the central nervous system. However, consideration of factors governing the operation of junctions, especially with respect to transmitters, such as their synthesis, their release, and their effectiveness as affected by the sensitivity of the synaptic membrane and the activity of the transmitter-splitting enzymes, will probably prove enlightening. It is interesting, for example, that at myoneural junctions magnesium suppresses acetylcholine release whereas calcium enhances it and counteracts magnesium; such action, at least with respect to other transmitters, may be involved in the central anesthesia induced by magnesium which can be counteracted by calcium. Such antagonism between the alkaline earth ions certainly is not demonstrable on peripheral nerve or on curarized muscle.

One is inclined to suspect that many central convulsants and agents which increase central "excitability", particularly those that act as depressants in the periphery, actually depress in the central nervous system, but act predominantly at inhibitory pathways or junctions. Evidence has been obtained for such action by strychnine (Part I). Conversely, block of excitatory pathways or synapses may lead to depression. Many of the possible mechanisms are indicated in (184a*I*) and (231). Not until more gross studies are supplemented by research on single cells can conclusions be regarded as final. Further use of double-barrel microelectrode-pipettes may be expected to clarify numerous problems. Coaxial microelectrode-pipettes offer the additional possibility of observations during drug or ion applications outside single cells (159).

From considerations presented in Part I, local anesthetics may be expected to exhibit selectivity for different pathways or for synaptic junctions in the

central nervous system by virtue of differences in membrane solubility. From this standpoint, radioisotopically labeled drugs may ultimately prove convenient for radioautography of affected regions; in this way, for example, inhibitory pathways believed to be blocked during the convulsive action of local anesthetics may be demonstrable.

F. General

The demonstration that stabilizers can slow the running down of cells produced by impaired metabolism raises the question whether it may be possible to provide some protection to organs deprived of adequate circulation or in certain disease states. The problem is one of finding agents that do not markedly affect other processes or the functioning of other vital organs. The possibility of delaying cerebral damage, such as may result from cardiac arrest, or of minimizing cardiac hyperirritability and arrhythmia that can arise from potassium release during ischaemia (87), might be explored from this standpoint.

A frequent observation is that tissues *in vitro* retain potassium (and exclude sodium) less readily than *in situ*, although the ionic environment closely duplicates that of plasma, and that this may be corrected by raising $[K]_o$ (*e.g.*, 18*I*, 365*I*). While the usual explanation in terms of impaired metabolism (and hence active transport) may indeed be involved under certain conditions, the possibility should be examined that organic components in the plasma (*e.g.*, fatty or amino acids) function like stabilizers to reduce passive ionic leak. It will be recalled that oleic acid may act in this way in the heart.

The more extensive knowledge of junctional transmission and excitability has obvious implications for certain diseases (52, 76). Thus, whether the resemblance of *myasthenia gravis* to curarization is actual, *viz.*, due to reduced reactivity of the endplate to Ach, or superficial, *i.e.*, the consequence of a limited store or deficient release of Ach, still remains to be established. To these possibilities should be added still another, *viz.*, an excessive "stabilizing" action of Ach itself on the endplate (Part I, see also 124).

In myotonia, the increased sensitivity of skeletal muscle to potassium appears to be at least partly responsible for the prolonged response to indirect stimulation; indeed, this would appear to be of more significance than dysfunction at the junction, for elevation of the blood level of Ca^{++}, which should exacerbate the condition by improving Ach release without affecting endplate sensitivity (Part I), can diminish or abolish myotonia (76). Depolarizability of the muscle by potassium may therefore be enhanced (P_K elevated), or, more likely, the association constant of muscle membranes for Ca^{++} may be low. The action of raised $[Ca]_o$ in reducing P_K and P_{Na} or in improving membrane calcification would account for its protective action. Denny-Brown (52) favors the possibility of low $[K]_i$, but evidence for this is not presented. Measurements of membrane potentials during responses should prove enlightening, for they could serve to determine whether repetitive firing of the muscle is due to the membrane potential being low following an impulse, as in TEA- or SCN^--treated muscles, or oscillatory as in low $[Ca]_o$.

The correlation of the muscle inexcitability of familial periodic paralysis with the fall in $[K]_o$ and its response to potassium therapy (76) may indicate an excessive rise in E'_m in low $[K]_o$, either because P_K is higher or P_{Na} lower than in normal individuals. A generally lower P_{Na} could also contribute to excessive fluctuations in plasma K^+ under conditions of modified metabolism, since this would render active transport more effective in transferring K^+ to the cells. Again, measurements of E'_m and of spike configuration in single cells may prove enlightening.

VII. CONCLUSIONS

The phenomena that have been examined in the unexcited and excited cell have revealed (a) that the membrane permeability to one ion may be changed more than to another, (b) that *changes* in permeability, during excitation and by drugs, can be more strikingly affected than the permeability at rest, and (c) that permeability changes to one ion may also be affected more than to another.

These permeabilities and their changes—predominantly with respect to sodium and potassium—and the equilibrium potentials of the ions—determined by the ion concentrations inside and outside the cells—have been found necessary and sufficient to account for the vast majority of observations on the resting potential, action potential, and excitation and for their correlations with membrane conductance and ion fluxes.

The establishment of this unifying view has been possible in part through recognition of the dependence of the permeabilities on the membrane potential itself and on a process designated as yet only in terms of its interference with electrically induced increases of sodium permeability, *viz.*, inactivation.

An additional principle, tentatively identified on the basis of a reinterpretation of available data and contributing to an integration of the facts, is the dependence of the decline in sodium entry or of the rise in potassium exit towards the end of a spike, under certain conditions, on the individual ionic currents—a competition, as it were, for passage through the same channels such as was previously described between the unidirectional fluxes of K^+.

The alterations in the behavior of excitable cells in response to certain physiological and pharmacological agents have been found to be consistent with action on these variables.

Thus, excitation and the rising phase of the spike normally reflect an increase in sodium permeability, hence agents that prevent or slow this process (*e.g.* stabilizers, ultra-violet light) can be detected by their effect on these phenomena and on associated conductance changes, frequently with little change in the resting membrane potential and conductance.

Sustained depolarization (by anoxia, potassium, drugs) can either increase or decrease excitability. If the normal membrane potential, E'_m, is high, depolarization will increase excitability since the state of "inactivation" and potassium permeability (if it tends to increase with depolarization) are not greatly affected, thereby leaving the critical firing potential, E_T, little changed while E'_m is lowered towards E_T. If membrane potential is low to start with, de-

polarization will increase inactivation (and P_K, when this is affected), thereby decreasing E_T more than E'_m, and hence depressing excitability and spike production.

During the spike, increase in potassium permeability frequently follows the increase in P_{Na}, in which case it hastens termination of the spike. Interference with its increase, such as occurs when Ba^{++} or certain quaternary ammonium compounds are present, is reflected by prolongation of the spikes.

The delay in the rise in P_K varies in different fibers, being very long in heart fibers and infinite at nodes of Ranvier under certain conditions. When this delay is substantial, the inactivation that normally shuts off the increase in P_{Na} during the spike is incomplete, so that P_{Na} remains somewhat elevated and comparable in order of magnitude to that of P_K, and thereby produces the "plateau". It was pointed out that available data can be interpreted as indicating that the entry of Na^+, when P_{Na} is about equal to P_K, interferes with exit of K^+, as would occur if the same narrow channels available to K^+ were occupied by the Na^+. It remains to be seen whether the cessation of sodium entry, perhaps by the slow completion of the return of calcium to the fiber surface, explains the termination of the plateau with an increase in G_K in heart muscle and in TEA-treated axons. The response of the plateau in heart fibers to a large variety of experimental conditions was shown to be accounted for by the requirement that sodium entry (I_{Na}) approximately equal potassium exit (I_K); those that decrease the former or increase the latter hasten the end of the plateau.

Emphasis has been placed in this review on the postulate that the increase in P_{Na} (and P_K) during excitation is due to the displacement of Ca^{++} from sites on the fiber surface that can be occupied by Na^+ (and K^+). The effect of lowering $[Ca]_o$ on P_{Na} (and P_K), and the evidence in the heart for such competition, are in keeping with this hypothesis. The similarity of the crystal ionic radii of Ca^{++} and Na^+ leads one to expect such competition, and the greater charge, polarizability, and strength of coordinate bonds of the alkaline earth should make it normally much more effective than the alkali metal in occupying such sites as well as a negligible contributor to the conductance.

It is suggested that during depolarization Ca^{++} is displaced from these sites by combining with an anion to form ion pairs. As pointed out in Part I, such ion pair formation normally could be prevented by the intense electric field in the membrane, as occurs in low dielectric solvents, but becomes possible by virtue of (a) the decrease in E_m, (b) the charge, polarizability, and perhaps coordinate bonds of Ca^{++}, and (c) the low "effective" dielectric constant of the membrane.

The anion might be an organic one residing in the membrane. However, in the case of muscle, chloride appears to be involved. The greater excitability described in more polarizable anions follows as a natural consequence of the improved ion pair formation to be expected.

These considerations lead immediately to a previous proposal for the mechanism of muscle contraction, *viz.*, the appearance of ionized Ca^{++} in the myo-

plasm by entry and dissociation of the ion pairs in the cell. In addition to the evidence for increased calcium influx and outflux during muscle activity and for the high sensitivity of myoplasm to Ca^{++} compared to all physiological ions, some of the wealth of indirect evidence from studies of skeletal and heart muscle have been outlined. Moreover, recent findings on the effects of more polarizable anions as well as of Ba^{++} and Sr^{++} on muscle contraction also seem explainable on this basis.

This hypothesis as to the link between excitation and contraction calls for a quantitative examination of the effects of the alkali metal, alkaline earth, and halide series on the behavior of G_{Na}, G_K, inactivation, contraction, and ion movements during depolarization and activity. Whether the cardiac glycosides, quinine alkaloids, and other conditions that affect contractility act via Ca^{++} movements also remains to be determined. Such studies may be expected to provide information on the sites involved and on the interactions among the ions in the light of current knowledge of complex formation (153). They may also offer a clue to inactivation and the delayed increase in G_K. Tentatively, it has been suggested (a) that inactivation reflects a depletion of anions from the membrane, perhaps in part through formation of ion pairs with some of the many sodium ions entering during excitation, accompanied by occupation of additional Na^+ sites by Ca^{++}, and (b) that the delayed increase in G_K is the consequence of removal of Ca^{++} from still other sites, possibly located closer to the intracellular surface of the membrane. It has been pointed out that certain interrelationships that have been described among inactivation, the increase in P_K, the shut-off of P_{Na}, and $[Ca]_o$ may be accounted for if the Ca^{++} removed during depolarization from the potassium sites proceeds to occupy sodium sites.

Whether entry of Ca^{++} during excitation or depolarization occurs in other systems remains to be demonstrated. It has interesting implications should it prove more general. Intracellular calcium is so strongly bound in the cells where it has been studied carefully that the appearance of ionized calcium could function as an exquisitely delicate trigger for a number of processes. Thus, as suggested for Ach at nerve terminals, vesicles that may function for its storage (or for storage of other physiological agents, *e.g.*, histamine, 5-hydroxytryptamine, in other cells) may be disrupted as has been described for axoplasm and organelles, thereby releasing the contents. Or the "mobilization" (release from a bound form) of physiological agents, such as those mentioned, might be activated by ionized calcium. It would be interesting to determine whether, as in Ach release at nerve terminals and in muscle contraction, extracellular calcium exhibits a modifying influence. Attention has already been called to improved reactivity of smooth muscle to drugs when extracellular sodium is lowered; this effect seems comparable to the improved contractility of the heart in low $[Na]_o$, which enhances the uptake of Ca^{++} by the cells.[6]

The positive potential, oscillations, and negative after-potential and accompanying excitability changes fit into the general scheme that has been outlined. The positive potential occurs when the resting potential is substantially below the potassium equilibrium potential and the spike terminates with G_K increased. Oscillations arise when G_{Na} and G_K are elevated somewhat and to about the same extent so that E'_m and hence inactivation are not excessively affected. The negative after-potential may have its origin as (a) a delay in the

[6] After the manuscript was submitted, Mongar and Schild (157a) reported that the anaphylactic release of histamine *in vitro* by guinea pig lung tissue is dependent on $[Ca]_o$, whereas the concentrations of the other ions of the milieu are of considerably less importance for this process. Attention has been called to the importance of calcium for the contraction of guinea pig uteri in response to antigens (39a).

complete return of G_{Na} to normal (hence its augmentation by the alkaloids of veratrine and the increased excitability during this period), (b) the diffusion away from the fiber surface of potassium liberated during the spike, and (c) a slow decline of P_{Cl}, which is elevated during the spike.

The positive after-potential is shown by inhibition studies to be related to the active transport process, which raises E_m (a) by depleting potassium from the fiber surface and (b) by a process of sodium exclusion, revealed in studies of vertebrate tissues (Part I) to reduce the inward leak of sodium. The latter process affords a basis for the accompanying reduced excitability, which may exceed that to be expected from the changes in E_m, especially in yohimbine. It remains to be seen whether mild metabolic impairment or a cardiac glycoside can mitigate the prolongation of the relatively refractory period. Unfortunately, earlier observations with inhibitors were complicated by the effects of potassium accumulation in the interstitial spaces, as shown by the effects of washing (146*I*).

These considerations indicate that an understanding of the action of a particular agent on excitable cells requires that we know at least the following:

(a) Is active transport interfered with (*e.g.*, as by metabolic inhibitors or by cardiac glycosides)? If so, it will first change E_{Na} and then E_K because the relative increase of $[Na]_i$ is greater than the decrease of $[K]_i$; as a result, processes dependent on sodium entry (active subthreshold response, spike, plateau, negative after-potential) will be depressed, followed by those dependent primarily on E_K (resting potential, negative after-potential). When sodium exclusion transport is appreciable, its decrease will lead to an early depolarization and loss of processes dependent on it (*e.g.*, positive after-potential). If depolarization is large enough, inactivation is increased and affects the spike and active subthreshold response.

(b) Is resting permeability altered? If P_{Na} is increased, (*e.g.*, with veratrine), E_m' will decrease and will increase P_K (hence G_m) if P_K is sensitive to E_m; this depolarization, if large enough, may also increase inactivation, and hence reduce the spike, but the effect on E_T will depend on the relative magnitudes of the increase in G_{Na} and inactivation. If P_{Na} is decreased (*e.g.*, with cocaine), it will raise E_m' if the latter is appreciably below E_K and tend to raise R_m; if E_m' is not greatly changed, G_m will change little for the contribution of P_{Na} to it is generally small. If P_K is decreased (*e.g.*, with Ba^{++}) so that it is appreciably closer to P_{Na}, depolarization will occur; since P_K is a major factor in G_m, the latter will be reduced. If P_K is increased, E_m' will increase if it was originally appreciably below E_K. If P_K and P_{Na} are increased (by low $[Ca]_o$) or decreased (by stabilizers) almost equally, E_m' may not change, but G_m will reflect the changes in P_K.

The finding that the transference number for Cl^- in frog muscle is twice that of K^+ (98) indicates that G_m changes will not be quite as large for a given alteration in G_K as originally expected in this tissue.

(c) Is an increase in permeability altered? If the increase in P_{Na} is reduced (*e.g.*, by stabilizers other than Ca^{++}), the rise, and to a lesser extent the amplitude of the spike, and the maximum increase in conductance, will be reduced; E_T will be lowered, too. If an increase in P_K is delayed (*e.g.*, with TEA, perhaps Ba^{++}), spike duration will be prolonged more than expected from a reduction in spike amplitude alone (as by inactivation).

(d) Is the secondary decrease in sodium permeability during the spike modified? When the decline in P_{Na} is slowed (*e.g.*, in veratrine and low $[Ca]_o$), especially when the increase in P_K is interfered with (as in the heart normally or in TEA-treated fibers), the exceptionally long delay in repolarization known as the plateau appears. Under these conditions, changes that increase potassium exit more than sodium entry, or that decrease sodium entry more than potassium exit, hasten the end of the plateau.

An outstanding accomplishment of the studies of the past decade has been to refocus attention on the physicochemical factors in the maintenance and excitation of excitable cells. This has been made possible by the development of powerful techniques for the study of both slow and rapid electrical (potential and conductance) characteristics and of ionic movements under conditions which provide adequate control of secondary factors. The degree of correlation that has been achieved among the electrochemical variables—potential, conductance, and ionic concentration and flux—provides ample basis for the conclusion that ion diffusion processes do indeed underlie many of the phenomena of resting and excited cells. It remains to be seen whether in special situations, as in crab muscle or in cells subjected to certain Na^+ substitutes, other ion species besides Na^+ may function as the diffusing ions. It should be remembered that the concept of permeability includes two parameters—a distribution coefficient and mobility (Part I); when the latter is very low, we still are left with the former, which could now produce similar electrical changes, but these would be termed phase boundary potentials. Although no commitment has been necessary regarding which of the parameters is the primary factor in the changes of permeability, the detailed postulates that have been made in this review are probably best considered in terms of the distribution coefficients.

A clarification of the role of metabolism in these phenomena is regarded as another contribution of this decade. The discovery of the importance of accumulation of potassium in extracellular spaces during metabolic inhibition in the effects observed has been helpful in this respect. Even with single cells care is necessary to distinguish electrical effects due to transitory changes in $[K]_o$ from those due to the permeability changes. In any case, it is now clear that a major part of the action of metabolism is in the maintenance and restoration of the ion distributions rather than in a direct contribution to membrane potentials or to their changes. The energy for the ionic interchanges is derived from the potential energy stored by metabolism in the form of ion concentration differences and in the attendant membrane potential. Recent studies on vertebrate nerve show that metabolism can also raise E_m by reducing the inward leak of sodium in exchange for intracellular potassium and thereby can function in producing hyperpolarizations such as the positive after-potential and post-anoxic recovery in oxygen.

It remains to be seen whether certain of the principles elaborated for the electrochemical phenomena of excitable cells may have bearing on contraction and other processes in these and other cells. Thus, the concept that energy is released from a store of potential energy during a muscle twitch rather than delivered directly by metabolism during the shortening phase is gaining support from observations such as the absence of expected changes in ATP (67, 68, 157), the inverse relationship between the rate of ATP splitting and the degree of shortening of contractile elements (156), and the many anions besides ATP that can act on the contractile elements (158). The findings of Park and his associates (164, 165, 165a) show that insulin enhances the entry of carbohydrate independent of any effect on metabolic reactions; in fact, this effect on entry may constitute a means of regulating carbohydrate metabolism.

In any case, it is apparent that future studies of cellular phenomena and of ion, drug, and perhaps hormone action must consider the possibility of physicochemical interactions even when such obvious indices of metabolism as respiration, heat production, or acid production are altered. Sufficient examples have already been given of "specific" inhibitors that exhibit the less specific effects of stabilization to serve as a warning against a disregard of such factors in attempts to interpret effects on cellular functioning. Indeed, the possibility has been pointed out that the metabolic changes in intact cells can be secondary to other effects, such as an alteration in the spontaneous activity of rhythmic tissues or in the intracellular ionic milieu (Part I); when care is exercised to use reasonable concentrations and to control heart rate, for example, ouabain exerts no effect on oxygen consumption until the improved contractility begins to fall off (136).

And, finally, we have seen that the selectivity of the permeability changes and of the agents that modify them require a much broader concept of membrane structure than provided by "homogeneous" membranes, whether rigid or fluid. A preliminary picture was presented in Part I of a semi-rigid structure, with channels of the dimensions of unhydrated ions and relatively large inter-channel regions which could be entered by "soluble" molecules, *i.e.*, those that can interact with and displace the membrane components. Such entry was proposed to increase lateral pressure in the membrane, as in surface films, thereby interfering with the changes in the channels during increases in permeability. Since the increase in permeability to one ion may be affected more than to another, the entry of some stabilizers may occur closer to certain channels than to others, perhaps by virtue of inhomogeneities in the interchannel region. We have seen that such selectivity of action may differ among cells, even among those from different parts of the heart.

A distinction has also been drawn between the action of multivalent ions, particularly Ca^{++}, and other stabilizers. The former appear to compete with sodium and potassium for the sites along which the monovalent ions move during excitation

Present information on inclusion compounds, with excellent evidence for formation of channel structures, such as around anions or between choleic acid and fatty acids or steroids, together with accumulating knowledge of the bonds involved in complexes, give promise of providing a basis for understanding better the results of future as well as past studies of physiological and pharmacological agents.

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