THE EFFECTS OF ANIONS ON EXCITABLE CELLS

PAUL HOROWICZ

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina

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I. INTRODUCTION

Much experimental work in recent decades has been focused on the role of cation movements in excitable cells. As a result, there is now a solid experimental base supporting the theory that rapid changes of membrane permeability to cations provide an adequate ionic description for the action potential in excitable cells. First the movement of sodium ions followed shortly thereafter by the movement of potassium ions down their respective electrochemical gradients are the essential events resulting from the permeability changes that occur in the membrane during the action potential. Recently, however, attention has gradually been turned to a study of the movement of chloride ions across the membranes of excitable cells. The results which have so far been obtained, although less extensive and detailed than those obtained for the cations, clearly show that chloride movement significantly affects and modulates the activity of excitable cells; this is so despite the fact that the movement of chloride is not primarily responsible for the characteristic all-or-none propagated action potential in animal cells. In certain types of synaptic inhibition, an increase in the chloride permeability of the postsynaptic membrane is a prominent and essential part of the inhibitory process.

Late in the nineteenth century, Hofmeister was the first carefully to study the concentration of electrolytes required to salt-out egg albumin (87). He found that both the cations and the anions exerted effects and he arranged a series for the two groups indicating the relative molar concentrations of the ions required for salting-out egg albumin. Any series of cations or anions arranged in the order of their ability to alter some measurable property or function and similar in order to the sequence of cations or anions originally found by Hofmeister is known as a lyotropic or Hofmeister series. Such ionic sequences have a general significance and have been found for such properties as the solubility of gases in solutions of electrolytes, the solubility of nonelectrolytes in water, and many other similar phenomena (47). The effects produced by substituting anions of a lyotropic series for chloride ions constitute one experimental device widely used in the study of the role of chloride ions in excitable cells.

Substitution of foreign anions for chloride affects excitable cells in several ways. First, since the membrane permeability is not the same for the different anions, substitution of any anion for chloride alters the current carried across the membrane by anions for any given transmembrane potential disturbance. Second, some foreign anions can alter the membrane permeability to the chloride ions. Third, from the evidence presently available, foreign anions appear to have little effect on the potassium permeability but appear to have a small effect on the sodium permeability of the membranes in excitable cells.

In recent years considerable attention has also been given to the problem of how excitation of the sarcolemma brings about the contraction of muscle proteins (95, 149, 162, 163). Substitution of foreign anions for chloride markedly affects this excitation-contraction coupling process. The effects which have been observed are not yet fully explained. In part they can be ascribed to changes in transmembrane potentials resulting from the altered membrane conductance induced by the specific anion replacement for chloride. However, other effects are not easily ascribed to the permeability changes induced by the altered anion composition of the extracellular fluid.

The first part of this review is concerned with the problems raised by the early workers in their studies on the changes in excitability produced when foreign anions are substituted for chloride. Then, the more recent data on anion effects in amphibian skeletal muscle are considered. Since this work is the most extensive, it provides a framework of fairly well studied effects which is useful for interpreting the somewhat less extensively explored effects of anions on other excitable tissues. Finally, the role of chloride movement in synaptic inhibition is reviewed.

II. EARLY STUDIES ON THE LYOTROPIC SERIES OF ANIONS

Several experimental procedures were used by early workers in studying the effects of foreign anions on excitable cells. Because contractions are relatively easy to measure, muscles were extensively employed in these studies.

One frequently used technique was to test the responsiveness of muscles to chemical stimuli as they gradually become spontaneously active after being

placed in solutions free of calcium and potassium ions. The appearance of spontaneous rhythmic contractions in muscles exposed to a solution containing 86 mM NaCl, made slightly alkaline by Na_2CO_3, was first observed and studied by Biedermann (17, 18). He concluded that these spontaneous contractions were caused by direct action of the sodium salt solution on the muscle fiber because the contractions occurred in fully curarized muscles. In 1886 Ringer (147) reported that spontaneous activity also appears when partially isolated sartorius muscles are placed in a pure isosmotic sodium chloride solution without the addition of Na_2CO_3. In one of the first studies on the effect of using anions other than chloride, Zoethout (178) showed that a muscle placed in a 125 mM sodium iodide solution for 10 minutes was sensitized to give contractions on subsequent exposure to a solution containing 6.3 mM potassium chloride. This potassium concentration ordinarily does not produce contractions. A similar pretreatment with 125 mM sodium bromide gave a smaller effect on exposure to 6.3 mM potassium. Subsequently, in a more extensive series of experiments using various chemical stimuli, Lillie (123) found that the contractile response of muscle was potentiated by a short 4-minute preliminary exposure to pure isosmotic sodium salt solution. The order of the relative potency of the salts was $NaCl < NaBr < NaNO_3 < NaClO_3 < NaI \leq NaSCN$. Gellhorn (60), studying only the potentiation of potassium contractions, found a similar sequence with sulfate placed between nitrate and iodide. Gellhorn also showed (61, 62) that the potentiating action of NaSCN solutions was so large that mere reimmersion in a physiological salt solution having a normal potassium concentration was sufficient to produce contractions. In later experiments, Chao (29) found that the action of any given anion was antagonized by HCl and by chlorides of the alkaline earth metals in the order $SrCl_2 < MgCl_2 \approx CaCl_2 < HCl$. For any given antagonist the minimal concentration needed was least for chloride and gradually increased in the order of the lyotropic sequence.

In addition to the changes in their excitability, muscles also swell when they are immersed in pure isosmotic sodium salt solutions. Chao and Chen (32) found that the rate of swelling is influenced by the anion. The swelling rate in sodium iodide was 80% greater than it was in sodium chloride; the rates for bromide and nitrate were in their usual intermediate positions for the lyotropic series.

Another method used in these early studies on the action of anions developed out of Overton's original findings which showed that muscles become electrically inexcitable when they are placed in isotonic solutions of sucrose, glucose, or mannitol (137). Under these conditions, the addition of 12 mM NaCl restored excitability. After rendering muscles inexcitable by soaking them in isotonic sucrose solutions, Schwartz (155) tested the action of anions by transferring these muscles to isotonic solutions in which 68 milliosmoles of sucrose were replaced by equivalent amounts of various sodium salts. The action of an anion was determined by measuring its ability to restore and maintain contractile responsiveness under constant low frequency stimulation. Thiocyanate was able to maintain the contractile responsiveness for the longest time, citrate, the shortest. In the sequence citrate, $SO_4^=$, CH_3COO^-, Cl^-, Br^-, I^-, CNS^- a given anion restored the

response of a muscle made inexcitable in a solution containing the anion preceding it in this series. That these results are dependent neither on the relatively low ionic strengths of the solution used nor on the presence of sucrose, was demonstrated by Gellhorn (63). In his experiments, muscles were stimulated at a low frequency until they were mechanically unresponsive in solutions which had 30% of the normal chloride replaced by osmotically equivalent amounts of foreign anions. The relative effectiveness of the anions in restoring and maintaining mechanical responsiveness was the same as that originally found by Schwartz. At this point it is important to note that muscle membranes rapidly depolarize and eventually become inexcitable in low Ca and Ca-free solutions (22, 37, 127). Since citrate and sulfate bind calcium, the relative effectiveness of these anions in excitability studies depend not only on their lyotropy but also on their chelating properties whenever the ionized calcium is not maintained by some buffer system.

The results of experiments with muscles using other types of stimuli for testing contractile responsiveness have given the usual lyotropic series. For example, Chao (30) found that the reversible contracture produced on sudden cooling of frog muscle after a brief soak in a pure isotonic sodium salt solution depended not only on the temperature difference but also on the anion present; the usual sequence was found for the sensitizing effectiveness of the anions. The same potentiating sequence is also obtained when muscle is stimulated by light after staining with eosin (124).

In a very interesting study by Chao (31), two different methods were used to determine the action of anions on the electrical excitability of muscle fibers. In one method, the rheobase of the most excitable fibers was determined by stimulating the muscle with condenser discharges having a long time constant. In the second method, changes in excitability were presumed to have occurred when there were changes in contraction strength in response to constant stimuli that were above threshold but submaximal. With this second method, an increase in the mechanical response was interpreted as a recruitment of fibers resulting from an increase in excitability. The lyotropic sequences obtained using these two methods were the same and were in agreement with the series obtained in the earlier work. As will be seen, the increase in mechanical response in Chao's second method is the result not only of recruitment of fibers but also of an increased twitch height in fibers which are excited.

Out of all these early experiments, only those results obtained from the determination of rheobase, just quoted, are easy to evaluate. In these measurements the output, a just barely detectable mechanical response, is fairly directly related to the stimulus input. In all the other measurements, the output variable generally measured was the extent and duration of muscle shortening. Such measurements are difficult to evaluate with precision because in muscle there are many factors which intervene and play a significant role between the stimulus input and the output measured as shortening or tension development. From what follows, it will be seen that nearly every known step between excitation and contraction is altered when the anionic composition of the bathing medium

is varied. These early findings gave such consistent sequences because most of the factors in the chain of events leading to contraction vary in a synergistic manner.

III. AMPHIBIAN SKELETAL MUSCLE

A. Resting membrane properties

Most of the work concerned with the effects of anions on the resting electrical and ionic properties of striated muscle has been done on the "fast" or "twitch" fiber system in frog muscles. For comparison of this system with the physiologically and anatomically distinct "slow" striated muscle system in the frog, the reader is referred to the articles by Kuffler (115) and Peachey (140). For the twitch fiber system, it was generally accepted prior to 1941 that the muscle membrane is permeable to potassium and essentially impermeable to anions and sodium (53). At that time, Conway advanced the hypothesis that the muscle membrane is permeable to potassium and cations of the same or smaller diameter in aqueous solutions and also to the smaller anions such as chloride (20). Boyle and Conway showed that when the external potassium and chloride ion concentrations were varied over a wide range the muscle potassium and chloride conformed with the concentrations to be predicted for a Donnan equilibrium system in which the muscle membrane is permeable to potassium and chloride ions. More recently, Adrian (2, 3) has measured by direct and indirect methods the steady state internal chloride concentration in muscles exposed to solutions with normal and raised potassium content. His findings are consistent with the view that chloride reaches the equilibrium predicted from the measured membrane potential (1) in any given solution.

In order to determine the relative conductance or permeability of the potassium and chloride ions, Hodgkin and Horowicz (84) measured the effect on membrane potential of sudden changes in the concentration of external potassium or chloride, using single fiber preparations. At constant external potassium concentrations, variation of external chloride, by replacing chloride with the impermeant anion sulfate (84), produces no permanent displacement of membrane potential. There are, however, large transient changes lasting from 10 to 60 minutes, in the direction expected for a chloride electrode. Thus, when the external chloride was reduced by replacement with the impermeant anion sulfate, there was a transient depolarization of the membrane; when the external chloride concentration was increased to its normal level after equilibrating in the low chloride solution there was a transient hyperpolarization of the membrane followed by a return to the resting membrane potential. The explanation of these results is fairly simple. When the external chloride is suddenly reduced, starting from an equilibrium condition, there is an unbalanced leakage of chloride ions out of the cell which produces the depolarization; when the equilibrium is disturbed by an increase in external chloride concentration the movement of chloride ions into the fiber causes a hyperpolarization. For a wide range of external potassium and chloride concentrations the chloride permeability remains at a constant value of about 4×10^{6} cm/sec. In resting fibers equilibrated in a physiological salt

solution, experiments such as those described above indicated that the chloride conductance was about twice the potassium conductance; the absolute magnitude of the resting potassium and chloride conductances was about 100 and 200 μmho/cm^2, respectively. These same experiments also showed that the potassium and chloride systems together accounted for 90% of the total membrane conductance in resting frog muscle. Later, Hutter and Noble (92), measuring the fall in membrane conductance on replacing chloride by the impermeant monovalent anions methylsulfate and pyroglutamate, found that in the resting state chloride ions accounted for two-thirds of the total membrane conductance. Adrian and Freygang (4), using an elegant electrical method and substituting the impermeant sulfate ion for chloride, also found a similar contribution of chloride to the total membrane conductance. Both groups of investigators were also able to fit their findings with a constant membrane permeability to chloride for a wide range of experimental conditions. All investigators, however, have found that the *relative* contribution of chloride ions to the membrane conductance varied with the membrane potential and the external potassium concentrations. The reason for this is that the potassium conductance is highly dependent on the membrane potential and on the direction of net potassium movement in any given situation. For inward movement of potassium ions the membrane permeability is much greater than for outward movement of these ions (4, 5, 84, 107). This property of the potassium system seems to be needed to explain the low resting membrane potential found in some fibers when they are placed in chloride- and potassium-free solutions (52, 166).

Hutter and Padsha (90) measured the membrane resistance of muscle when other permeant anions were substituted for chloride in the bathing medium. When the chloride normally present in physiological salt solution is replaced by bromide, nitrate, or iodide the membrane resistance is increased. The relative magnitude of the membrane resistance when the chloride is replaced by one of these anions was: $Cl^-:Br^-:NO_3^-:I^- = 1.0:1.5:2.0:2.3$. Since Edwards *et al.* (46) found earlier that Br^-, NO_3^-, and I^- do not greatly influence the movement of K^{42} in muscle, these experiments indicate that the increase in membrane resistance was due to a reduction in the anion conductance of the muscle membrane. The interpretation is also consistent with the earlier findings of Conway and Moore (34) which showed that when muscles were placed in solutions containing high concentrations of potassium salts the rate of swelling was fastest for chloride, slower for bromide, and slowest with nitrate.

One consequence of the increase in membrane resistance produced by these other anions would be a reduction in the current threshold of the muscle fibers. This expectation is in good agreement with the older findings of Chao (31) in which the rheobase was reduced by treatment with these anions.

Since nitrate crosses the membrane less easily than chloride one might expect that replacing chloride with nitrate would give a transient depolarization of the type observed with sulfate or with a salt solution in which some NaCl has been replaced by sucrose. Using whole sartorius muscle and single fibers several observers have found that the resting potential after equilibration was the same

in nitrate as in chloride salt solution and could detect no transient depolarization on first applying nitrate. This observation can be explained on the assumption that the effect of nitrate is to reduce the chloride permeability until it about equals the nitrate permeability. Under these circumstances there would be no change in resting potential on applying nitrate. The measurements of Adrian (3) and Harris (71) on chloride movement in the presence of other anions make the above assumption reasonable. In radioisotope experiments, they have shown that when a muscle is loaded with Cl^{36} the outflow of isotope is reduced when chloride in the external fluid has been replaced by Br^-, NO_3^-, I^-, ClO_4^-, or SCN^- in order of increasing effect. Adrian's value of 11 minutes for the time constant for loss of Cl^{36} from muscles in normal chloride solutions agrees reasonably with the earlier results of Levi and Ussing (122).

In some respects it appears as if the anion SCN^- behaves in a somewhat anomalous fashion, particularly when used at high concentrations. Thus, Hutter and Padsha (90) have observed that at low concentrations of SCN^-, there is an increase in the membrane resistance but at high concentrations of SCN^- the resistance begins to fall again. In some earlier work by Höber and his colleagues (78, 79), in which measurements on demarcation currents were made, SCN apparently caused an initial transient hyperpolarization. These observations are difficult to evaluate because a liquid junction potential was present in the measuring circuit which was not allowed for. The experiments of Lubin (125), however, in which the transmembrane potential was measured with microelectrodes, also indicate a small hyperpolarization of the membrane in SCN^- solutions. These findings might be explained on the assumption that at high concentrations of SCN^- the membrane becomes rather permeable to SCN^-.

Recently, Hubbard (89) has measured the membrane constants of normal and denervated frog sartorius muscles. The total membrane conductance is decreased in denervated fibers. This decreased conductance is due to a decrease of potassium conductance; the chloride conductance is unchanged after denervation. As a result, the fraction which chloride contributes to the total membrane conductance increases from two-thirds in normal fibers to three-quarters in denervated fibers.

B. Action potential

According to the now classic theory of Hodgkin and Huxley, which accounts for action potentials in various excitable tissues, the local currents in front of an action potential depolarize the membrane to a level at which the normally low permeability of the membrane to sodium increases in a regenerative fashion so that the membrane becomes highly and selectively permeable to sodium (81, 82, 94). As a result, sodium ions can now move down their electrochemical gradient. In this theory, the rising phase of the action potential is ascribed to a large entry of sodium ions. The falling phase is caused by a delayed increase in potassium permeability and a decrease in the sodium permeability subsequent to its initial rapid increase. The falling phase is thus ascribed to a large exit of potassium ions over and above any residual entry of sodium ions.

In support of this theory for striated muscle, Nastuk and Hodgkin showed that the peak of the action potential varied as if it were largely a sodium equilibrium potential when they varied the external sodium concentration (132). This theory was also checked by measuring the movement of labeled potassium and sodium ions during activity in single muscle fibers (83). On stimulating the fibers, there was a net entry of 15 $\mu\mu$mol of Na per cm^2 surface membrane per impulse. The net potassium exit amounted to about 10 $\mu\mu$mol per cm^2 per impulse. It was suggested that the difference might be due to a net chloride entry during each impulse.

After the peak of the muscle action potential repolarization occurs in two distinct phases. Initially there is a rapid repolarization which is complete within approximately 2 or 3 milliseconds after the peak of the spike and leaves the membrane depolarized by about 15 to 20 millivolts. After the initial repolarization, the potential more slowly approaches its resting value. This second phase of slow repolarization is referred to as the negative afterpotential and generally lasts about 30 to 50 msec. In early experiments, Frank (55) reported that the decline of the negative afterpotential was exponential with a time constant similar to that determined from the decay of hyperpolarizations. More recently, Persson (141) has shown that although the later phase of the negative afterpotential decays exponentially, during the first 10 to 15 msec the behavior of the membrane potential deviates significantly from its terminal time course. During the earlier part of the afterpotential, the membrane resistance is much lower than it is at rest. Consonant with this are the earlier findings of Benoit and Coraboeuf (14) that the membrane potential during the early phase of the afterpotential is much less altered by polarizing currents than is the later phase of the afterpotential. Desmedt (42) found that the absolute magnitude of the negative afterpotential remained constant when the potassium equilibrium potential was made more negative (*i.e.*, inside more negative to outside) by either decreasing the external potassium concentration or increasing the internal potassium concentration. On the other hand, both Frank (55) and Persson (141) found that the afterpotential becomes less negative when the potassium equilibrium potential is made less negative by increasing the external potassium concentration. Thus, the available evidence favors the view that after the first 10 to 15 msec the negative afterpotential is largely passive. However, the initial portion of the afterpotential is associated with a low membrane resistance which is probably related to the terminal phases of the increased cation permeabilities from the spike. The initial value of the afterpotential seems to depend in part on the potassium equilibrium potential.

The effects on the action potential of various anions used to replace chloride in the external solution have been the subject of a number of reports. Hutter and Noble (92), using the impermeant anion methylsulfate as a substitute for chloride, showed that above 17°C the fast phase of repolarization is not appreciably affected, while the negative afterpotential is increased in size and duration. At about 17°C, the negative afterpotential begins to show a "hump" which at higher temperatures gives rise to repetitive firing. The lengthening of

the afterpotential can be attributed to the increased membrane resistance produced by the removal of the chloride conductance system. These authors suggested that the prominent "hump" at the high temperatures may be caused by a rapid recovery of the Na conductance system coupled with the removal of the shunting effect of chloride; together, these conditions could give rise to a subthreshold regenerative potential change before the afterpotential has decayed. It is noteworthy, in this connection, that Persson (141) found that the membrane resistance during the afterpotential increases when methylsulfate replaces chloride in direct proportion to the change in the resting membrane resistance produced by this replacement. At temperatures below 17°C, Hutter and Noble found that the rapid phase of repolarization was also prolonged when methylsulfate was substituted for chloride. Harris and Martins-Ferreira (72) had previously noted a slowing of the fast phase of repolarization when nitrate replaced chloride in the muscles of a South American frog. An increase in the magnitude and duration of the negative afterpotential has been observed for the anions, Br^- (50), NO_3^- (49, 50, 72), I^- (49), SCN^- (15, 125), thiosulfate (51), moniodomethane sulfonate (51), and acetate (51).

C. Mechanical response

Using single supramaximal shocks while measuring tension development under isometric conditions, Kahn and Sandow (101, 102, 103) showed that when chloride is replaced by nitrate the peak twitch tension is greatly increased; the contraction and relaxation times are prolonged; and the maximum rate of tension change for both contraction and relaxation is increased. The responses were twitches, since only a single action potential was recorded when the muscle was stimulated by a single shock. Since the stimuli were maximal for both chloride and nitrate, the twitch potentiation was not caused by fiber recruitment. This finding has been confirmed in other experiments which show that nitrate potentiation of the twitch also occurs in single fibers (86).

It has been demonstrated in whole muscle that there is no change in the maximum tetanic tension (77, 103), and in single fibers that there is no change in the maximum contracture when high concentrations of SCN^- replace chloride. For most of the anions it has been demonstrated that the velocity of shortening of the contractile component under isotonic loads has not appreciably changed (77, 125, 150). In terms of the phenomenological model developed by Hill and his colleagues over the years (75, 174), the reason why the tension in an isometric twitch is less than in a tetanus is that the active state for the contractile element lasts too short a time in a twitch for it to be able to stretch the series elastic component enough to exert full tension. Since the maximum tension development and the force-velocity relation for the contractile element is unaltered by the anions of the lyotropic series, Ritchie (148), as well as Hill and Macpherson (77), suggested that these anions produce their effects on the twitch by increasing the duration of activity in the contractile element. By increasing the duration of activity, the contractile element can have more time to shorten and thereby to stretch the series elastic component more completely. This allows the ability

of the contractile element to bear a load and develop tension to be revealed more fully during the twitch. In conformance with this expectation, most of the anions on the lyotropic series, as well as methylsulfate, have been shown to increase the duration of the active state of the contractile component during muscular contraction (77, 92, 125, 148).

Sandow and his colleagues have shown that the twitch tension in a series of twitches is reduced by fatigue more quickly in nitrate solutions than it is in chloride solutions (103). The nitrate effect on fatigability can be simulated in chloride solutions by stimulating at a low frequency with pairs of maximal shocks 20 msec apart.

Hill and Macpherson (77) have noted that the heat production in an isometric twitch was greater when nitrate replaced chloride. This increase in heat production was nearly proportional to the increase in peak tension. In an isometric fused tetanus during maximum tension maintenance the heat production was the same for both anions.

Other anions of the lyotropic series have similar mechanical effects and all workers agree that the potentiating effectiveness of anions on the twitch is $Cl^- < Br^- < NO_3^- < I^- < SCN^-$ (77, 86, 103, 128).

D. Excitation-contraction coupling

A number of investigators have reached the conclusion that the event which normally induces contraction in skeletal muscle is a change of membrane potential (85, 114, 149, 162, 163). Also, there seems to be general agreement that in normal muscle fibers, placed in chloride-containing solutions, the degree of depolarization required to activate the mechanical system is close to the threshold for the propagation of excitation (85, 114, 145, 168). An important advance has been made by the work of Huxley and his associates (95, 96, 97) who have studied the activation of the mechanical system in muscle fibers by the use of small electrodes placed against the external surface of the sarcolemma. By passing current pulses through these electrodes they were able to restrict the area of membrane which was polarized and thus could explore the surface membrane for patches particularly sensitive in producing contractions. Only at certain areas were pulses found to be effective in producing local contractions when the membrane was depolarized; and from a comparative study it was found that these areas were located over regions associated with the triads of the endoplasmic reticulum (144).

In the early work on the effects of anions in the Hofmeister series by Kahn and Sandow (103), as well as Hill and Macpherson (77), it was shown that the rate at which the effects developed in whole muscle when an anion was applied and the rate at which the effects were reversed when the anion was removed were limited by the rate at which the anion could diffuse through the interspaces. In later experiments with single fibers when the anions could be rapidly changed at the surface it was shown by Hodgkin and Horowicz (86) that the effect of the anions on twitch amplitude took place with a delay of 1 to 3 seconds; this delay

was several times longer than that found for the blocking action of Na-free solutions and thus could not easily be attributed to diffusion through an unstirred layer outside the fiber. That a membrane site is involved in these anion effects is clear from the fact that the effect develops fully before any significant quantities of the anions appear inside the cell and from the fact that the effects disappear rapidly after long equilibration in solutions containing the various anions. In the latter situation, the effects disappear when the anions are removed from the outside before there is any significant decrease in the anions which, after equilibration, are inside (77). In order to explain why the effects of nitrate on twitch amplitude are slower than those of sodium on propagation, it was suggested that the sites responsible for the action potential are probably on the surface, whereas those concerned with activating the contractile system, which are affected by the anions, might be located in a tubular component of the endoplasmic reticulum.

Several workers (15, 49, 128, 170) have suggested that the effect of foreign anions on the twitch may be partly due to their action in prolonging the spike or the afterpotential, or both, and in increasing the amplitude of the afterpotential. Another factor, which is probably also important, is revealed in the experiments on the effects of foreign anions on potassium-induced contractures. Thus it has been recently found that Br^-, NO_3^-, and low concentrations of SCN^- reduce the potassium concentration and the membrane depolarization required to induce a given amount of tension (56, 86). From these observations, the potentiating effect of foreign anions on the twitch can be attributed partly to increased activation during the spike and partly to a reduction of the mechanical threshold below the level of the afterpotential.

It has been suggested (86) that since the order in which the anions act ($Cl^- < Br^- < NO_3^- < I^- < SCN^-$) corresponds to their absorbability (151, 152, 160), the shift of the mechanical threshold towards the resting membrane potential might be accounted for by their absorption at the outer edge of the membrane. Such absorption of the anions would introduce locally an electric field inside the membrane which subtracts from that contributed by the resting membrane potential. Thus the foreign anions could alter the distribution of other charged groups or dipoles within the membrane without changing the potential difference across the membrane. If a reduction in the electric field at some location within the membrane is the critical event initiating contraction, such absorption of anions will shift the membrane potential which is just threshold for mechanical activation towards the resting membrane potential.

Another suggestion concerning the mechanism by which foreign anions potentiate contractions was advanced by Shanes (157). This suggestion rests on the specific hypothesis that calcium entry into skeletal fibers is the important link between excitation and contraction. Much of the evidence for this hypothesis has already been reviewed by several workers (16, 57, 149, 157). It is assumed that calcium which is found at certain sites in the membrane is released into the myoplasm as the result of membrane depolarization. Shanes suggested that since

the foreign anions are more polarizable they might be expected to increase substantially the binding of calcium in the membrane at rest and enhance the release of calcium into the cell when the membrane is depolarized.

E. Other metabolic effects

In 1936, Solandt found that in frog sartorius exposed to a modified physiological salt solution containing 10 to 20 mM potassium ions there is a large increase in the rate of heat production (159). Earlier work by Hegnauer *et al.* (74) had shown that these concentrations of potassium had a similar effect on the oxygen consumption. Recently Hill and Howarth (76) have confirmed and extended the observations on heat production. From the effects that increased Rb and Ca have on this stimulated heat production, they concluded that a partial depolarization of the membrane, insufficient to produce contraction, releases some of the chemical processes normally activated by excitation. Substitution of NO_3^- and I^- reduced the concentration of external potassium needed to induce these high rates of heat production. These effects are analogous to the effects of the foreign anions on potassium-induced contractures mentioned above.

Axelsson and Thesleff (9) have shown that caffeine produces contractures without depolarization of the surface membrane. That this caffeine contracture is also potentiated by the foreign anions in their usual order of potency, has been demonstrated by Matsushima *et al.* (129).

IV. MAMMALIAN SKELETAL MUSCLE

Very little information exists on the internal chloride concentration in normal mammalian muscle cells. It is, in fact, current practice to equate the chloride space with the extracellular space in most work done on mammalian skeletal muscle. Twenty-two years ago, Conway and Fitzgerald (33) abandoned this procedure. In 1945, Wilde (173) reported that in two groups of nephrectomized rats having plasma potassium concentrations of 8.9 and 12.4 mM, the internal chloride concentrations were 7.3 and 10.1 mM, respectively. Although the internal chloride increased with an increase in the external potassium the results do not agree quantitatively with the Conway theory since the internal [K] $\times$ [Cl] product was about twice the external product. To test whether the chloride ion is distributed passively it is necessary to know the membrane potential at the various external potassium concentrations. The Conway treatment assumes potassium is in equilibrium and that the membrane behaves as a potassium electrode. Since it has been shown (177) that the membrane potential does not behave as a potassium electrode in rat muscle, it would be hazardous to state definitely whether or not chloride is distributed passively on the basis of Wilde's data.

Indirect evidence that mammalian muscle is permeable to chloride and that chloride is normally present within the cells comes from the experiments of Giebisch *et al.* (64) in which membrane potentials were measured on single fibers of the gracilis muscle of the cat in a perfused hindlimb preparation. Partial re-

placement of NaCl by saccharose was accompanied by a depolarization from a mean resting potential of −92 mV and a sudden increase of concentration of potassium and lactic acid in the venous outflow. These effects were not accompanied by contractures and were reversible. Much larger depolarizations were produced by sulfate substitution. These were accompanied by fibrillations and contractures. These effects can be ascribed to the reduction of extracellular chloride producing a net loss of chloride from the cells. The larger effects produced by sulfate are probably the result of the decreased calcium ion concentration in the perfusate.

In other experiments (113), it was shown that loss of potassium upon replacing chloride in the perfusion solution was dependent on the foreign anion. The loss of potassium was greatest for sulfate, with the other anions being ordered $SO_4^{=} > NO_3^{-} > Br^{-}$. However, the tension developed by the gastrocnemius was not changed during perfusion with solutions having nitrate and bromide replacing chloride.

When rat diaphragm muscle is placed in chloride-free solution, in which chloride is replaced by methylsulfate, a single shock to the nerve gives rise to repetitive firing (126). This effect is similar to the repetitive firing found in frog muscle exposed to chloride-free solutions at comparable temperatures (92). Chronically denervated diaphragms fibrillate in chloride-free solution. In such denervated preparations, acetylcholine is more effective in depolarizing the muscle membrane in chloride-free solutions (126). Stormann *et al.* (164) have also shown that in normally innervated muscle of the cat replacement of chloride by sulfate, nitrate, or bromide increased the sensitivity of the muscle to intraarterial injections of acetylcholine. It can be inferred from all of these effects that chloride ions contribute to the membrane conductance of mammalian muscle and thereby have a stabilizing effect.

V. CARDIAC MUSCLE

A. Cation mechanisms and the action potential

It is generally agreed that the initial rapid depolarization in the cardiac action potential is produced by a regenerative increase in sodium permeability as in nerve. As for the mechanism of the much slower repolarization there is still some debate. The kernel of the dispute is whether or not the repolarization is dependent on the membrane potential *and* time or time alone. The disputants agree that the repolarization is determined mainly by the combined effects of the sodium and potassium conductance systems so that the current carried outward by the potassium exceeds the current carried inward by the sodium during the falling phase. For the details concerning the experimental data and theoretical interpretations the reader is referred to the papers of Johnson, Noble, Woodbury, and their associates (21, 99, 100, 134, 135, 136, 175). Excellent general reviews of the ionic mechanisms involved in cardiac action potentials have recently been provided by Woodbury (175) and Trautwein (169).

B. Internal chloride concentration in quiescent cells

Page (139) has presented evidence that Cl^- can enter heart muscle cells. Using quiescent cat papillary muscle and incubating for 1 hour in a normal physiological salt solution containing Cl^{36} and C^{14}-mannitol (the latter for determining the extracellular space) he obtained a value of 17 mM for the internal chloride concentration. A value of 18 mM for internal bromide concentration was obtained after incubating for 2 or 3 hours in Br^{82}-labeled solution. The resting membrane potential was found by microelectrodes to be 80 mV, with the inside negative (138). If chloride was in equilibrium this value of membrane potential corresponds to an internal chloride concentration of only 7.4 mM. Although this discrepancy might be taken to indicate that there is active transport of chloride into the cell, other alternatives such as inhomogeneities in the composition of the extracellular compartment, a lower activity coefficient for the intracellular Cl, or both, provide possible explanations which cannot be excluded at present. Lamb (121) has noted a similar discrepancy in auricles from rat hearts, for which similar explanations can be advanced.

C. Effects on membrane conductance and spontaneous activity

When foreign anions replace chloride in the bathing medium several striking effects are produced in cardiac muscle. These effects have been attributed to the sensitivity of certain phases of the cardiac action potential to changes in membrane conductance associated with changing the anions. As in skeletal muscle, replacing chloride by large impermeant anions like sulfate and methylsulfate reduces the membrane conductance. In contrast with skeletal muscle, however, replacement of chloride by bromide, nitrate, or iodide increases the membrane conductance (93).

Hutter and Noble (91, 93) have shown that, in spontaneously active sheep or dog Purkinje fibers, when chloride is replaced by nitrate, the spontaneous activity initially slows down and then accelerates to a steady frequency about twice the original frequency. On returning to the chloride solution a further transient increase in frequency occurs before the original frequency is attained. With a large impermeant anion such as methylsulfate replacing chloride (11) the reverse effects were obtained. On changing to the larger anion, there was an initial transient increase in frequency followed by a slowing in which the frequency eventually fell to values of 40 to 90% of that in chloride solution.

From their conductance measurements when methylsulfate replaces chloride, Hutter and Noble concluded that chloride ions carry from 20 to 30% of the total membrane current in the resting fiber. The simplest explanation is that chloride ions move through the cardiac muscle membrane, and therefore, in the absence of any active transport process the chloride equilibrium potential, E_{Cl}, should become equal to the resting potential in a quiescent preparation. In a constantly active cardiac muscle preparation the distribution of chloride ions would take on an equilibrium potential value intermediate between the maximum positive membrane potential and the maximum negative membrane potential. The exact value of E_{Cl} depends on the time course of the membrane potential variations

and on the current-voltage function for the chloride conductance system. Hutter and Noble estimate that the chloride equilibrium potential is very close to the threshold membrane potential, so that during diastole when the membrane potential is more negative inside than E_{Cl}, chloride ions would move out and contribute to the slow diastolic depolarization. On the other hand, during the action potential when the membrane potential is more positive inside than E_{Cl}, chloride ions would move in and hasten repolarization.

With a more permeant anion such as nitrate, in the steady state situation with spontaneous activity, the nitrate equilibrium potential, E_{NO_3}, will also take on a value intermediate between the maximum positive and negative membrane potentials. The greater permeability of the membrane to nitrate than to chloride will tend to increase the rate of depolarization during diastole and thereby increase the frequency. During the action potential it will move more readily into the cell and thus hasten repolarization, producing a shorter action potential. The observations conform to this hypothesis. On the other hand, the larger impermeant anions will eliminate the anion movements during diastole and the action potential. In this case, the diastolic depolarization will proceed at a slower rate, producing a lower frequency of activity, and the action potential will be longer.

The *initial* changes in frequency can be explained on the basis of transient anion movements between steady state situations. When a large impermeant anion first replaces external chloride, the chloride ions inside the cells will move out and produce a depolarization current until they have been completely washed out of the cell. This will clearly increase the rate of firing of these cells until the cells become chloride-free. With the more permeant nitrate anion replacing chloride, the inward movement of nitrate will exceed the outward movement of chloride and thus produce a transient hyperpolarization. This should decrease the rate of firing of the cells until the nitrate gradually replaces the chloride inside the cells.

Carmeliet (26, 28) has in many respects obtained similar results. Of particular interest is his finding that in potassium-free solutions the permeant anions can have large effects. This is related to the fact that in potassium-free media the K permeability is decreased (27). Thus he found that while nitrate ions produce little hyperpolarization when normal potassium concentrations are present, they produce a hyperpolarization of more than 20 mV when the membrane is first depolarized by K-free solutions. When fibers are equilibrated in media containing the relatively impermeant acetylglycinate anion, K-free solutions also cause depolarization and readmission of K-free solutions containing chloride now causes a large hyperpolarization. On the basis of his results, Carmeliet suggested that the Purkinje fiber membrane is permeable to the anions in the series $P_{NO_3} > P_{Cl} > P_{acetylglycinate}$.

De Mello (41) has also presented evidence that chloride ions contribute appreciably to the total membrane conductance of atrial muscle fibers, Purkinje fibers, and fibers of the S-A nodal pacemaker in rabbits. Of particular interest is his finding that the S-A nodal fibers seem to be especially sensitive to chloride

movements. For example, he finds that the transient depolarization when sucrose replaces chloride is greater in fibers of the S-A node than in fibers from atrial muscle. Since he found the internal chloride concentration in pacemaker cells was 37 mM as compared with 2.2 mM in atrial muscle fibers, removal of the external chloride should produce a larger loss of chloride from the nodal fibers and hence cause a greater depolarization in these fibers provided the total membrane conductance is about the same for both the nodal and atrial fibers. De Mello also demonstrated an appreciable fall of the internal chloride concentration when the S-A node was placed in low chloride solutions. His other findings are largely compatible with those of Hutter, Noble, and Carmeliet.

Sekul and Holland (156) have estimated the unidirectional fluxes of chloride using Cl^{36} in spontaneously beating atrial preparations. Both the influx and efflux of Cl^{36} are considerably increased when the preparations are rapidly stimulated and when fibrillation is induced by acetylcholine.

Feigen and his associates (142, 143) have studied the effect of nitrate substitution for chloride in contracting guinea pig atria stimulated electrically at rates of 3 to 4 per second. With high concentrations of nitrate they find a reduction in the magnitudes of the resting potential and the action potential, and the rate of initial depolarization of the action potential. With only partial nitrate replacement for chloride there was no decline in the resting potential. The amplitude of contraction decreased in proportion to the amount of nitrate used for replacement. In the presence of nitrate there was an increase in internal sodium concentration and an equal decrease in internal potassium concentration. These findings on internal concentrations are based on calculations in which the inulin space is equated to the extracellular space. Page (139) has shown that the inulin space in cat papillary muscle is much less than the mannitol space, although mannitol behaves osmotically as if it does not enter the cell. If the inulin does not distribute itself throughout the extracellular space in guinea pig also, then the experimental findings of Feigen are not easily evaluated.

VI. SMOOTH MUSCLE

A considerable amount of effort in recent years has been placed in studying smooth muscle of various sorts. Two excellent reviews covering the physiology and pharmacology of smooth muscle have recently appeared (23, 48). The reader is referred to these for summaries on the general physiology of smooth muscle.

Concerning the internal concentration of chloride, most workers assume that the chloride is distributed passively across the membranes of smooth muscle (39) while others, assuming the inulin space to be equal to the extracellular space, find that the internal chloride concentration is greater than predicted for passive distribution (104, 106). The uncertainties in the determination of the extracellular space, however, make it hazardous to draw any definite conclusions from this latter finding. Another factor complicating this issue is that most smooth muscle preparations either are not quiescent or do not have stable membrane potentials.

Membrane potential changes associated with alterations of the extracellular chloride concentrations indicate that the chloride conductance contributes sig-

nificantly to the total membrane conductance of smooth muscle. Thus, when external chloride is replaced by the larger impermeant $SO_4^=$ and $C_2H_5SO_3^-$ ions there is a transient depolarization in the muscle cells of the taenia coli (24, 88, 118). The ethanesulfonate anion has been shown to be impermeant in a number of different muscle tissues (67). After a short period in the altered solution the membrane potential does not completely return to normal values. The initial depolarization produced when various foreign anions replace chloride is in the order $SO_4^= > Br^- > NO_3^- \geq I^-$. With nitrate and iodide solutions, after 20 to 30 minutes the membrane potential is more negative inside than it is in chloride media for any given external potassium concentration. Kuriyama (118) found that during the initial depolarization there was an increase in the rate of spontaneous discharge. An interesting observation was that even though the sulfate depolarization was greater than that associated with either nitrate or iodide, the maximum frequency of spike discharge was greater for the latter ions. Although it is possible that the greater depolarization produced by sulfate may make the fibers somewhat less excitable by inactivation of the spike generating system, it is also possible that the threshold for firing is decreased in nitrate and iodide. That the latter possibility may also contribute to this phenomenon is seen in the records of Kuriyama (118), in which the frequency of discharge in the nitrate solutions is considerably larger than in chloride solutions at times when the membrane potential is more negative inside in the presence of nitrate. Eventually the spike discharge ceases in nitrate solutions when the membrane becomes more hyperpolarized.

When preparations are driven electrically, Axelsson (8) has found that replacement of chloride with nitrate results in repetitive firing to a single stimulus when in chloride solution each stimulus gave only one action potential. When the frequency of spike discharge was made high by either direct electrical stimulation or by increasing the temperature, an increase in tension development was observed without any further change in frequency of firing when nitrate replaced chloride. Under these conditions the repolarization phase of the spike was lengthened.

Similar increases in membrane activity and tension have also been observed in uterine muscle and other smooth muscles (36, 105) when nitrate replaces chloride. In guinea pig ileum replacement of chloride by ethanesulfonate induces tonic contractions (66) while replacement of chloride by methylsulfate does not have this effect (120). The reason for this difference is not understood.

VII. INVERTEBRATE MUSCLE

Boistel and Fatt (19), as well as Reuben *et al.* (68, 146), have presented evidence that crayfish muscle fibers are permeable to chloride ions. Both groups have observed depolarizations, similar to those found with frog skeletal muscle, when an impermeable anion was substituted for external chloride. Boistel and Fatt (19) showed that γ-aminobutyric acid (GABA) increased the membrane conductance in the presence of external chloride more than in its absence. GABA also increased the depolarization associated with chloride-free solution. From

these findings it can be inferred that GABA increases the membrane permeability to chloride. In a study of the effects of various drugs on the magnitude and time course of the membrane potential changes caused by chloride-deficient solutions, Reuben *et al.* (68, 146) have confirmed these results with GABA and in addition have shown that the chloride permeability of the membrane is decreased by picrotoxin. They ascribed these effects to actions on the nonsynaptic membranes (68).

In a more recent publication (65), this group finds that there is formation of vesicles in the transverse tubular system of the sarcoplasmic reticulum whenever the conditions are such as to produce exit of KCl. They conclude that the transverse tubules are permeable almost exclusively to chloride.

VIII. NERVE

A. Giant axons of Loligo[1]

Soon after the rediscovery of the giant axons in cephalopods measurements were made on the electrolyte content of the axoplasm in these fibers. The internal chloride content is relatively high in axons when measured one or more hours after death of the animal. For *Loligo pealii*, Bear and Schmitt (12) found an internal chloride concentration of 130 mM. After their initial report, the value for internal chloride has been redetermined and found to be 88 mM by Steinbach (161), 140 mM by Koechlin (112), and 151 mM by Deffner (40). For *Loligo forbesi*, the internal chloride concentration has been reported to be 109 mM by Webb and Young (171), 83 mM by Keynes and Lewis (111), and 125 mM by Keynes (110). Steinbach's early report (161) indicated that in freshly dissected squid giant axons the internal chloride concentration was 42 mM and that this increased rapidly during the first half hour and then remained nearly constant at 88 mM. Hodgkin, in his early review (81), pointed out that the higher value for chloride is not compatible with electrochemical equilibrium for isolated axons with membrane potentials of -50 to -60 mV. On the other hand, if one takes the lower value and allows for a short dissection time, then the value *in vivo* could be close to 30 mM. This extrapolated value agrees reasonably with a passive distribution for chloride in intact axons whose membrane potentials range from about -68 to -77 mV (82, 131). However, recent experiments by Keynes (108, 109, 110) have not confirmed the low values of Steinbach. Axoplasm extruded from axons *in situ* soon after live squid were caught had internal chloride concentrations close to 100 mmol/kg axoplasm.

In isolated axons, Mauro (130), using Ag–AgCl electrodes to measure the transmembrane potential, found that the inside potential was still -35 mV. Keynes (110), using a similar technique, has also shown that the activity coefficient for chloride in extruded axoplasm is not appreciably different from what it is in sea water. From these findings it appears that chloride is not appreciably bound

[1] To facilitate comparison, values in the literature not reported in units of mmol/kg of water have been converted to these units by using the value of 865 g of water/kg of axoplasm given by Koechlin (112).

inside the axons. Thus the electrochemical potential for chloride is higher inside than outside the axon and the chloride is not in equilibrium.

Measurements on unidirectional chloride fluxes have been made by several investigators. For *Loligo pealii*, Shanes and Berman (158) reported that the value for the chloride influx measured with Cl^{36} is somewhere between the limits of 14 and 31 pmol/cm^2 sec. For *Loligo forbesi*, the first determinations of Caldwell and Keynes (25) gave 13 pmol/cm^2 sec for the chloride influx; the more recent results of Keynes (110) give an average influx of 23 pmol/cm^2 sec. For both species, the rate constant for loss of Cl^{36} injected into the axoplasm varies from 0.0002 min^{-1} to 0.001 min^{-1} (25, 110, 167). Taking a mean value of 0.0006 min^{-1} for the efflux rate constant and the measured internal chloride concentration, the estimated absolute efflux is about 21 pmol/cm^2 sec (110). This value is close to the estimated influx for chloride.

After poisoning axons for two hours with 0.2 mM dinitrophenol, Keynes found that the influx was reduced by 58% while the efflux was not much altered. Unfortunately, similar experiments using 2 mM cyanide gave such variable results that they could be consistent with cyanide having either the same effect as dinitrophenol or no effect at all. Ouabain at a concentration of 0.01 mM had no effect on the chloride influx. On the basis of the above findings Keynes (110) concluded that the high internal chloride concentration "results from the operation of a mechanism for uphill inward transport of chloride." If this conclusion is correct, it is extremely interesting that an animal tissue with a high plasma chloride should have an active transport system for chloride which is inwardly directed.

On the basis of these chloride flux measurements, the calculated chloride conductance can account for only a small fraction (about 0.2) of the resting leakage conductance found in isolated giant squid axons. Baker *et al.* (10) have also concluded that ions other than chloride must contribute to the leakage current since there was only a relatively small difference between the resting potential of axons perfused with isotonic KCl and those perfused with isotonic K_2SO_4.

B. Crustacean nerve

In nonmyelinated fibers from the leg nerve of the spider crab, Wilbrandt (172) found by measuring changes in demarcation potential that there was a small hyperpolarization when NO_3^- or SCN^- replaced chloride. Lactate and pyruvate also cause a small hyperpolarization. Although this effect suggests that these anions are somewhat more permeant than chloride, other interpretations are possible.

C. Amphibian myelinated nerve

Adequate measurement of the internal chloride concentration in myelinated fibers is not presently available. There is, however, some indirect evidence to show that the membrane of the frog node is permeable to chloride and that chloride is present in the axoplasm. The early observation of Straub (165) that there is a membrane depolarization when chloride is replaced by sulfate or phosphate

is insufficient evidence on which to base a claim for chloride movement because no provision was taken to keep the ionized calcium concentration constant. Schmidt and Stämpfli (153) have shown that calcium deprivation causes marked depolarization in frog myelinated fibers. In the solutions used by Straub the sulfate and phosphate should have lowered the ionized calcium considerably. In other experiments (154), changes in membrane potential were measured when saccharose or glucose replaced the external NaCl; these experiments, too, are, for several reasons, difficult to use for assessing chloride movement. The most significant reason is that the reduction in the resting inward leakage of sodium on removal of external sodium more than compensates for the small increase in chloride leakage on removal of external chloride; the net effect is a hyperpolarization of the membrane. The most direct experiments on the effect of chloride removal are those reported by Frankenhaeuser (58), in which he observed that the membrane depolarized by a few millivolts when chloride was replaced by the large methylsulfate anion. Since methylsulfate does not alter the ionized calcium (92), this finding suggests that the membrane is slightly permeable to chloride in the resting condition.

In early work, in which changes in demarcation potentials were measured, there was no detectable change in the resting potential when foreign monovalent ions were substituted for chloride (80, 133). Straub (165) found no significant difference in the membrane potential when nitrate replaced chloride. On the other hand, Hashimura and Osa (73) find that NO_3^- and SCN^- replacement of chloride produces a small hyperpolarization. The hyperpolarization which they measured was in both cases greater than the calculated junction potential which was present in their measuring system. Although this might indicate that these ions are slightly more permeant than chloride, the effects, discussed below, of NO_3^- and SCN^- on action potentials altered by cobalt suggest another explanation. Since the frog nerve membrane is so sensitive to the changes in the resting sodium leakage, and since these authors present indirect evidence that NO_3^- and SCN^- produce some inactivation of the sodium conductance system it is possible that these anions reduce the resting sodium conductance and thereby produce a small hyperpolarization.

In these experiments of Hashimura and Osa, the action potential, in untreated fibers, is somewhat reduced in height when NO_3^- and SCN^- replace chloride. The duration of the action potential, however, is not appreciably altered. In line with this observation, Frankenhaeuser (58) has shown, using the voltage clamp technique, that the delayed currents associated with a reduction of the membrane potential are not changed when chloride was replaced by methylsulfate or isethionate ions. Since anodal polarization can restore the height of the action potential in NO_3^- and SCN^- solutions, the effects can be attributed to a partial inactivation of the sodium conductance system. With addition of cobalt, the action potential is prolonged and this prolongation is increased when NO_3^- and SCN^- replace chloride. This finding suggests that chloride ions may aid in the repolarization phase of the action potential in cobalt-treated fibers and that NO_3^- and SCN^- are less permeant than chloride. In the cobalt-treated fibers, the height of the action potential is also reduced in solutions containing NO_3^- and SCN^- and can

be restored by anodal polarization. This observation, again, suggests a partial inactivation of the sodium conductance system.

These findings can help to rationalize the variable results reported for the effects on excitability in frog nerve when foreign anions replace chloride. Some time ago, Höber and Strohe (80) reported that the electrical excitability was slightly increased immediately after Br^-, NO_3^-, or I^- replaced chloride in the bathing solution. After a short time, the excitability decreased in the presence of these anions. With SCN^- there was either an immediate decrease in excitability or no initial effect followed by a slow decrease in excitability. On the other hand, Hashimura and Osa report that the threshold potential for firing the action potential is closer to the resting potential in solutions with NO_3^- or SCN^- and the fibers are more excitable. If chloride contributes to the resting membrane conductance and both NO_3^- and SCN^- are less permeant than Cl^-, and if both NO_3^- and SCN^- reduce the resting sodium permeability and partially inactivate the sodium conductance system, then the above findings can be explained. In some cases, the decrease in membrane conductance when NO_3^- or SCN^- replaces chloride might more than compensate for the effects on the sodium conductance system and thereby decrease the threshold. Such may be the case with the fibers of Hashimura and Osa, since the action potential in their fibers does not increase under anodal polarization and hence the sodium conductance is probably fully activated in the resting condition. However, in nerves which initially may have a somewhat inactivated sodium conductance system, further inactivation of the sodium conductance by NO_3^- and SCN^- could make the fibers less excitable despite a decrease in the membrane conductance associated with anion substitution for chloride. The time variation in excitability noted by Höber and Strohe can be accounted for if the effect on anion permeability comes on more rapidly than does the effect on the inactivation of the sodium conductance system.

IX. CHEMORECEPTORS

From the work of Beidler (13) and others (54, 176) the ability to stimulate those chemoreceptors which have low thresholds to solutions containing salts is determined by the cation portion of the salt. There are no significant differences among the anions in their stimulating action on chemoreceptors of rats and frogs. Kusano and Sato (119) have also shown that the response pattern or the threshold to various solutions does not change during the first 30 minutes after replacing chloride by foreign anions in solutions bathing a frog tongue. However, after 30 to 60 minutes the thresholds to the various stimulating solutions begin to increase and the magnitude of response begins to decrease; eventually the sensitivity to all solutions is abolished. The depressing action of the anions was $SCN^- > NO_3^- > I^- > Br^-$. For the "water" receptors the sequence of depressing action was reversed. These effects are irreversible.

X. SYNAPTIC TRANSMISSION

There are several excellent reviews which summarize experimental work on the mechanisms involved in excitation and inhibition in various synaptic structures (44, 116). Excitation of postsynaptic membranes, whether by chemical

transmitters or by electrical coupling, involves predominantly cation permeability mechanisms. On the other hand, some of the inhibitory mechanisms involve, at least in part, alterations in anion permeability in the postsynaptic membrane. For the purposes of this review, the latter mechanisms will be briefly discussed. Curtis (38) has recently provided a summary of the pharmacology of inhibition.

When inhibition by chemical transmitters directly involves the postsynaptic membrane, it is manifested by an increase in the postsynaptic membrane conductance (44, 116). When there is a change in the postsynaptic membrane potential, it may be of either sign and is generally only a few millivolts in magnitude. In all cases, if the membrane is at first sufficiently hyperpolarized by passing current through the membrane a depolarization is produced by inhibitory impulses; if the membrane is initially depolarized by current a hyperpolarization is produced by inhibitory impulses. Thus, there is a definite transmembrane potential to which the inhibitory volley drives the postsynaptic membrane; this potential is generally rather close to the resting membrane potential. Since there is an increase in membrane conductance during inhibition, this means that the postsynaptic membrane becomes more permeable to one or more ions. Because the potential to which the membrane is driven is rather close to its resting value, this implies that the ion or ions involved have equilibrium potentials close to the resting transmembrane potential. The two ions which are distributed between the extracellular and intracellular phases with the proper chemical gradients and in sufficiently large concentrations are potassium and chloride. In general, where chemical transmitters are involved it has been found that the postsynaptic membranes become more permeable to either one or both of these ions during inhibitory action. To test whether chloride ions are involved, either the extracellular or the intracellular chloride concentration is altered and observations are made to see if the transmembrane potential associated with an inhibitory volley follows the equilibrium potential for the chloride ions.

It was first shown by Coombs *et al.* (35) that electrophoretic injection of chloride ions into motoneurons causes the normally small hyperpolarization response of the inhibitory postsynaptic potential to change to a depolarizing response. Similar results have been obtained in other cells of the central nervous system. Electrophoretic injection of another foreign anion such as Br^-, NO_3^- or SCN^- produces the same effect as does injection of Cl^- ions. Electrophoretic injection of larger anions like sulfate or phosphate does not alter the hyperpolarizing response of the inhibitory postsynaptic potential. These early observations have been repeated and many other anions have been tested (6, 98). When anions whose diameters in the hydrated state are greater than 1.32 times the diameter of hydrated potassium ions are injected into the motoneuron cells, no observable changes in inhibitory postsynaptic potential was recorded. Eccles (44) has interpreted these findings to mean "that the inhibitory transmitter has converted the specific inhibitory patches to a sieve-like membrane having pores of a precisely standardized size." For the evidence that the motoneuron also becomes more permeable to K during the inhibitory process, the reader is referred to the review of Eccles (44).

Inhibition in the crustacean stretch receptor cell seems to involve an increase in the postsynaptic membrane permeability to both potassium and chloride since the inhibitory equilibrium potential follows alterations in both the chloride and potassium equilibrium potentials, induced by altering the external potassium and chloride concentrations (45, 70, 117).

Inhibition *via* alterations in the postsynaptic membrane in crustacean muscle fibers seems to involve an increase in the membrane permeability primarily to chloride, since the inhibitory equilibrium potential follows the changes in chloride equilibrium potential (19, 69) but is not significantly altered by changes in the potassium equilibrium potential (19, 43).

Furukawa and Furshpan (59) have shown that there are two types of inhibition which act upon the Mauthner cell of the goldfish. One type is the result of certain extracellular potential changes which passively hyperpolarize the axon-hillock region of the Mauthner neuron. Another type of inhibition involves the increase of the conductance in the postsynaptic membrane for ions which are close to electrochemical equilibrium at the resting membrane potential. These authors, as well as Asada (7), have shown that the inhibitory conductance change is due to an increase in the postsynaptic membrane permeability to chloride ions. Asada also injected various other anions into the Mauthner cell and on the basis of the effects on the inhibitory postsynaptic potential found essentially the same permeability characteristics as others have found for the cat spinal motoneurons. The activated inhibitory postsynaptic membrane is permeable to Br^-, Cl^-, NO_3^-, SCN^-, ClO_3^- and $HCOO^-$, and nonpermeable to HS^-, BrO_3^-, HSO_3^-, HCO_3^-, CH_3COO^-, $SO_4^=$, and other large anions. He also presents evidence which suggests that potassium ions do not play a major role in this inhibitory conductance increase.

XI. SUMMARY AND CONCLUSION

There is now a considerable amount of evidence, both direct and indirect, that most excitable cells are permeable to chloride ions. The relative permeability to chloride varies from cell to cell and consequently its physiological significance varies. Where the relative permeability to chloride is low, as for example in giant squid axons, chloride ions play a minor role in the electrical events associated with activity. Where the relative permeability is high, as in skeletal muscle and to a lesser extent in cardiac muscle, chloride ions play a significant role in the electrical events during activity. In the latter situation, the contribution of currents caused by chloride is less important when the sodium and potassium currents are high during certain phases of activity.

In the absence of active transport, the actual concentration of chloride inside the cell is a function of the membrane permeability to chloride, the chloride concentration outside the cell, and the behavior of the membrane potential as a function of time. In cells which are either periodically or continually active, the instantaneous internal chloride concentration is related to the past history of the cell rather than to the momentary or even short-term average membrane potential. For cells in a steady state of constant activity the internal chloride concentration is a function of the steady state. The actual concentration depends on

precise temporal behavior of the membrane potential and the chloride permeability of the membrane. The chloride equilibrium potential is somewhere between the limits set by the maximum and minimum values of the membrane potential during activity. In quiescent cells where the membrane potential remains constant, and in the absence of active transport, the chloride equilibrium potential should assume a value equal to the membrane potential. To a first approximation this seems to be the case in skeletal striated muscle. On the other hand, this appears not to be the case in squid giant axon where an inwardly directed pump for chloride ions appears to be present. The influence of foreign anions in the lyotropic series on the electrical events in excitable cells is largely ascribable to either 1) the fact that their permeance is different from that of chloride and consequently they contribute a different amount of anion current, or 2) the fact that they alter the passage of chloride through the membrane, or both of these mechanisms. The effects that foreign anions have on the sodium conductance seem to produce minor consequences.

In the case of contractile tissues, the evidence indicates that there is a change in the ability of the membrane potential to control the contractile process when other anions replace chloride in the external medium. To the extent that one can generalize to other processes controlled by the membrane potential, it is tempting to suppose that neurosecretion and other processes may be altered by changes in the external anion composition. To recognize such analogies and to pursue them experimentally might prove fruitful in furthering our understanding of the role that chloride ions play in the functioning of excitable cells.

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