The Effect of Low Ionic Strength Extracellular Solutions on the Resting Potential in Skeletal Muscle Fibers

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ABSTRACT Intracellular measurements of the resting potential were made in fibers of the frog sartorius muscle in solutions of varying salt composition and concentration to determine the effects of low ionic strength extracellular solutions on the resting potential. Changes in the glass microelectrode tip potential in low ionic strength solutions were minimized by adding $ThCl_4$ to the extracellular solution. These experimental conditions allowed measurement of the relationship of the resting potential to the concentration of the salt in the extracellular solution by replacing it with the nonionic substance, sucrose. Substitution of sucrose for the extracellular NaCl produced a stable depolarization which was logarithmically related to the NaCl concentration. Substitution of sucrose for choline Cl, instead of NaCl, produced the same degree of depolarization. When Na salts of anions less permeable than chloride (Br, I, NO_3) were used, the resting potentials in 116 mM solutions were close to those with chloride (±3mv). The depolarizations produced in low ionic strength solutions of these salts were significantly less than those with chloride.

INTRODUCTION

Recent reviews have dealt with the role of anions in the electrical and contractile properties of skeletal muscle (Horowicz, 1964; Tsofina and Liberman, 1964). It has been concluded from electrical and flux measurements that the skeletal muscle membrane is highly permeable to chloride (Boyle and Conway, 1941; Hutter and Padsha, 1959; Hutter and Noble, 1960; Adrian and Freygang, 1962; Harris, 1965). However, attempts to demonstrate a stable effect on the resting potential produced by changing the ratio of extracellular to intracellular chloride concentrations have been unsuccessful (Adrian, 1956; Hodgkin and Horowicz, 1959). Previous experiments involved replacement of chloride by an impermeable anion with only transient changes in the resting potential observed (Hodgkin and Horowicz, 1959). The resting potential of

the skeletal muscle fiber was shown to be logarithmically related to the ratio of extra- to intracellular potassium concentrations (Adrian, 1956). From these results it was concluded that the resting potential in the skeletal muscle cell is determined only by the distribution of potassium ions, with chloride "passively" distributed in accordance with this potential.

The experiments reported here provide a measurement of the effects on the resting potential in skeletal muscle fibers produced by replacing the extracellular NaCl by the nonionic substance, sucrose. Under such conditions stable depolarizations were produced which were logarithmically related to the concentration of NaCl in the extracellular solution. Similar experiments were performed using, instead of NaCl, choline Cl or Na salts of anions less permeable than chloride (NaBr, NaI, or $NaNO_3$). The relations of the extracellular concentration of each of these salts to the membrane potential are compared graphically.

The large tip potentials associated with glass microelectrodes in low ionic strength solutions (Adrian, 1956) were minimized by using $ThCl_4$ in all experimental solutions (Agin and Holtzman, 1966). All experimental solutions were K-free since such solutions seem to accentuate effects on the membrane potential produced by changing the concentration of other salts. The dilution experiments were designed to insure that all measurements were made on viable cells. Control experiments were designed to insure that the depolarizations observed with low extracellular salt concentrations were stable with time. To avoid confusion the experimental designs are described under Results.

METHODS

Experiments were conducted between the months of October and March using the sartorius muscle of the grass frog, *Rana pipiens*. The resting potentials of fibers on the deep surface (underside) of the bundle were measured with intracellular micropipettes filled with 3 M KCl in contact with a Ag-AgCl wire. The microelectrode resistances were 5–10 MΩ. The reference electrode was a Ag-AgCl wire in the bath solution. The recording setup included a Grass P6 preamplifier with cathode follower and an oscilloscope. Potentials were also monitored with an audiomonitor to insure that a sharp potential change occurred upon penetration of a cell. Junction potentials were zeroed out with both electrodes in the bath solution by adjusting the potential trace to an arbitrary base line before penetration of the cell. A resting potential measurement was accepted only if the potential returned to the original base line when the electrode was withdrawn from the cell. If the electrode resistance changed during an experiment, the electrode was replaced before continuing the experiment.

Immediately after the dissection the muscle was placed in the recording chamber, deep side facing up. The muscle was left in the bath solution 30 min to allow equilibration before any potentials were measured. Fibers were then found which had rest-

ing potentials greater than 100 mv in the 116 mM solution being tested and which could be easily and repeatedly recognized by nearby landmarks, such as blood vessels. Perfusion of the bath was maintained at a constant rate throughout the course of all experiments, but was speeded up when solutions were changed to allow complete replacement of the bath solution within ½ min. The solutions used are described below.

Electrode Artifacts

The junction potentials between the bath solution and reference electrode, between the bath solution and the 3 M KCl in the microelectrode, and between the 3 M KCl and the Ag-AgCl wire were not included as part of the recorded membrane potentials. These were zeroed out after introducing the new solution and before penetration of a cell by always measuring the membrane potential as a deflection of the potential trace from a single arbitrary base line upon penetration of the cell. The difference in the junction potential between the 3 M KCl and the bath solutions and that between the 3 M KCl and the intracellular solution does add to the recorded potential and is necessarily included in the results given below. This difference is dependent upon the ionic strength of the extracellular solution. The difference in the junction potential in 116 mM NaCl solution and in 1 mM NaCl solution is approximately 1.6 mv as calculated from the Henderson equation (MacInnes, 1961). This difference is comparable for all the anions used in these experiments since all have mobilities close to that of potassium (MacInnes, 1961). The calculated error introduced by these junction potentials in comparing resting potentials in these solutions is 2–3% for the chloride solutions and up to 6% for the nonchloride solutions.

The tip potential is defined as the difference between the total circuit emf before and after breaking the tip of the microelectrode. The magnitude of the tip potential is inversely related to the ionic strength of the solution outside the electrode and may be as high as 50 mv in 1 mM NaCl (Adrian, 1956; Agin and Holtzman, 1966). However, the dependence of the tip potential on the ionic strength of the bath solution is markedly decreased when calcium and thorium are present (Agin and Holtzman, 1966).

In the experiments reported in this paper, 1 μ M $ThCl_4$ and 2 mM $CaCl_2$ were used in all solutions. In 116 mM solutions made up in this way, the tip potentials in electrodes with resistances of 5–10 MΩ are between −7 and −9 mv (inside of the electrode negative with respect to the outside). At the completion of each dilution experiment, the tip potential was measured by breaking the electrode in the 1 mM solution and observing the potential change. The tip potentials measured in the lowest ionic strength solutions were between −7 and −10 mv. Therefore, the tip potential in these experiments was not dependent upon the ionic strength of the extracellular solutions in the concentration range used. Because tip potentials exist in the extracellular solutions and to an unknown extent in the intracellular solution, quantitative determination of the resting potential with intracellular glass electrodes is still not possible. However, since the magnitude of the tip potential can be kept constant in both these solutions, differences in the resting potentials in extracellular solutions of different ionic strengths can be measured without significant artifactual contribution from tip potential differences.

Solutions

All solutions used in these experiments contained the following salts:

$CaCl_2$	2 mM
NaH_2PO_4	0.43 mM
Na_2HPO_4	1.08 mM
$ThCl_4$	1 μM

The solutions of highest ionic strength contained 116 mM of the respective experimental salt (NaCl, NaBr, NaI, $NaNO_3$, or choline Cl). The solutions of low ionic strength contained 58, 29, 10, 5, or 1 mM of the experimental salt and were made osmotically equivalent with sucrose. Osmotic coefficients were obtained from Robinson and Stokes (1959). The pH of all solutions was 7.1–7.2. All solutions were potassium-free.

The thorium salts of the anions used are very soluble. Some difficulties were encountered due to the low solubility of the phosphate salts of thorium. With 1 μM $ThCl_4$ no precipitation or clouding was observed. Phosphate stock solutions were made up fresh once a week. Sucrose stock solutions were made fresh every 3–4 days. The experimental solutions were made from stock solutions the day of an experiment.

RESULTS

1. *Resting Potentials in 116 mM NaCl and Other 116 mM Solutions*

In each experiment resting potentials were recorded in five fibers after equilibration in 116 mM NaCl solution. The 116 mM nonNaCl solution was then washed in and potentials were measured in the same fibers after 3–5 min (after all spontaneous contractions ceased). Junction potentials were zeroed out before resting potentials were measured. The resting potentials were measured in fibers again after 10 and 20 min in the nonNaCl solution. In experiments involving choline Cl, potentials were measured every 10 min for 40 min in the choline Cl solution because of transient potential changes.

After completing the potential measurements in the nonNaCl solution, the microelectrode was left in one fiber while the 116 mM NaCl solution was washed back in. The electrode was left in the fiber for at least 1 min after reintroducing the NaCl solution in order to observe any transient changes in the potential which might occur upon changing the ionic content of the bath solution. The change in potential due to the change in junction potential between the bath solution and the reference electrode was noted after removing the microelectrode from the fiber. 10 min after reintroducing the NaCl solution, the resting potentials were recorded in the same five fibers.

Two experiments were performed on separate muscles with each nonNaCl 116 mM solution. Thus, measurements were made on 10 fibers in each salt solution and compared with values recorded before and after in the same fibers in 116 mM NaCl solution. Potential changes were monitored continu-

ously in eight fibers during replacement of a nonNaCl solution by the NaCl solution. The results are given in Table I.

When the 116 mM NaCl solution was replaced by the 116 mM solution of a Na salt other than chloride, all the fibers on the surface of the bundle contracted spontaneously, usually for 2–3 min. After the contractions ceased, resting potentials were measured and, in most fibers, were within 4 mv of the potentials recorded in the same fibers in the NaCl solution. No changes in the resting potentials were observed at 10 and 20 min after introducing the nonchloride solution.

Some fibers were irreversibly depolarized in the nonchloride solutions. In the 116 mM NaBr solution half the fibers had stable resting potentials between

TABLE I

MEAN RESTING POTENTIALS MEASURED IN 116 mM NaCl AND THEN IN THE 116 mM SOLUTION OF ONE OF THE OTHER EXPERIMENTAL SALTS

Values are only from those fibers whose resting potentials in 116 mM NaCl, after exposure to a nonNaCl solution, were within 4 mv of the potentials recorded initially in NaCl. The potentials given are those recorded after 40 min in choline Cl or after 10 min in the other solutions. The number of fibers, which met the above criterion, of the 10 measured in each solution, is given.

	Resting potential		No. of fibers
		NaCl	
	mv	*mv*	
NaBr	110	110	4
NaI	115	112	7
$NaNO_3$	111	109	10
Choline Cl	109	112	8

40–70 mv. These fibers remained depolarized during the 20 min in which they were exposed to the NaBr solution. Many of the fibers on the surface were in a state of contracture; all these fibers were depolarized. No fiber which was depolarized in a nonchloride solution was repolarized when the fiber was again exposed to the NaCl solution. Such fibers were considered damaged and the values discarded.

Replacing the nonchloride solutions with the NaCl solution produced no spontaneous contractions. In each experiment there was a change in the recorded potential during this change in solutions, which was stable for the 1 min the electrode was in a fiber. The magnitude and direction of this change were, in each case, within 4–5 mv of the change in the base line due to the change in the junction potential between the bath solution and the reference electrode. Therefore, no transient changes in the resting potentials of six fibers were observed during the first minute after replacing a foreign anion by chloride in the extracellular solution. 10 min after reintroducing the NaCl so-

lution, all those fibers which were not depolarized in the nonchloride solution had resting potentials within 4 mv of those potentials recorded in the nonchloride solution and, initially, in the NaCl solution.

When the NaCl solution was replaced by a 116 mM choline Cl solution, no spontaneous contractions were observed. However, all the fibers were depolarized 15–20 mv. This depolarization was transient with most of the fibers recovering within 30 min. After 40 min in the choline Cl solution, the resting potentials were within 2–4 mv of those recorded in NaCl. Those fibers which did not repolarize in the choline Cl remained depolarized when the 116 mM NaCl solution was washed back in. No change in the resting potential was observed during the first minute and at 10 min after NaCl replaced choline Cl.

2. *Resting Potentials in Cells Exposed for 60 Min to Low Ionic Strength Solutions*

Resting potentials were measured in five fibers after equilibration in the 116 mM solution of the salt being tested. The 1 mM solution of the same salt was introduced into the bath and resting potentials were measured in the same five fibers every 10 min for 1 hr. After the last set of measurements in the 1 mM solution, the microelectrode was left in one fiber for at least 1 min while the 116 mM solution was washed back in, in order to observe any transient potential changes. 10 min after reintroducing the 116 mM solution, the potentials were recorded in the same five fibers. Two such experiments were performed on separate muscles with each of the experimental salts.

Stable depolarizations were observed in the 1 mM solution of each of the five experimental salts. All fibers were depolarized when potentials were recorded after 10 min in a 1 mM solution. Some of the fibers appeared to be in a state of contracture. The fibers remained depolarized to the same extent during the entire 60 min exposure to the 1 mM solutions.

In each of the experiments, no transient potential changes were observed in the first minute after reintroducing the 116 mM solution. The resting potentials returned to approximately the same values observed initially in the 116 mM solutions after correcting for the junction potential changes. The time course of the repolarization was not followed, but in each case it was completed within the 1 min observation period. 10 min after reintroducing the 116 mM solution, the resting potentials in most of the fibers were within 4 mv of the values recorded at the start of the experiment. Some fibers did not repolarize completely in the 116 mM solution and were considered damaged. Two such fibers were observed for ½ hr in the 116 mM NaCl solution and did not repolarize.

3. *Dilution Experiments*

Resting potentials were measured in solutions containing 116, 58, 29, 10, 5, and 1 mM of the salt being tested. After equilibration in the 116 mM solution,

one fiber was chosen with a resting potential above 100 mv. A low ionic strength solution of the same salt was then washed in. The potential trace was returned to the base line and the resting potential was recorded in the same fiber after 10 min. The 116 mM solution was then washed back in and the resting potential measured in the same fiber after 10 min. If the resting potential recorded in the 116 mM solution was greater than 100 mv and was within 4 mv of the potential recorded before the exposure to the low ionic strength solution, the experimental value recorded in the low ionic strength solution was accepted. These criteria were used to insure that all experimental

TABLE II

MEAN RESTING POTENTIAL ± 1 SE (IN MILLIVOLTS) IN SOLUTIONS OF DIFFERENT SALTS IN DIFFERENT CONCENTRATIONS

Osmotically equivalent quantities of sucrose replace the salt in low ionic strength solutions. All solutions are potassium-free and contain 2 mM $CaCl_2$. All solutions are buffered with Na_2HPO_4 and NaH_2PO_4 and contain $ThCl_4$ to eliminate tip potential differences. Parentheses indicate the number of resting potentials recorded from different fibers to make up the mean resting potential.

Salt	Concentration of salt in extracellular solution, mM					
	116	58	29	10	5	1
NaCl	110.9±1.0 (24)	102.0±0.8 (9)	92.9±2.0 (15)	76.3±2.8 (14)	64.3±3.1 (13)	50.0±3.2 (10)
Choline Cl	109.0±1.3 (9)	97.3±1.5 (9)	89.3±1.2 (9)	81.6±2.2 (9)	72.0±3.0 (9)	58.0±3.8 (8)
NaI	117.0±1.2 (23)	105.6±1.9 (10)	100.2±1.7 (9)	91.0±2.7 (10)	88.3±1.6 (12)	83.6±1.6 (11)
NaBr	108.8±1.5 (19)	103.6±2.4 (9)	95.4±2.0 (10)	87.8±1.8 (10)	89.6±3.4 (11)	80.4±4.0 (10)
$NaNO_3$	114.9±0.8 (19)	106.8±1.3 (10)	100.4±1.5 (10)	93.2±1.6 (10)	91.6±2.1 (9)	90.0±1.5 (10)

values were from viable cells. Successive experiments on one fiber in low ionic strength solutions of the same salt were conducted as long as these criteria were met. No more than one measurement in each low ionic strength solution of the same salt was made on a single fiber.

The mean resting potentials measured in solutions of different concentrations of each experimental salt are given in Table II. Enough measurements were made on different fibers at each concentration to reduce the standard error to less than 4 mv. The number of values for each point is given in parentheses. The values used for the 116 mM solutions are the resting potentials recorded at the beginning of the experiments. Fibers from eight muscles were tested in NaCl, from four muscles in choline Cl, from eight muscles in $NaNO_3$, from five muscles in NaBr, and from five muscles in NaI.

The results in Table II are given graphically in Fig. 1. The use of choline

Cl instead of NaCl did not appreciably change the relationship of the potential to the extracellular salt concentration. The values for choline Cl are not included in Fig. 1. In 1 mM NaCl solution (5 mM total extracellular chloride) there was a mean depolarization of 60 mv with a range of 52–69 mv. The mean depolarization in 1 mM choline Cl was 51 mv. The slopes are −33 mv/log [NaCl] and −29 mv/log [choline Cl] from 100 to 10 mM. One bundle was observed which showed depolarizations of 10–15 mv in 8 of 20 fibers impaled in 1 mM NaCl. The values from this bundle are not included in the results reported here.

The results with the other anionic solutions differ markedly from those with chloride solutions. Depolarizations in 1 mM solutions were 33 mv in NaI, 28 mv in NaBr, and 25 mv in $NaNO_3$. No fibers were found which did not show approximately this degree of depolarization. The slopes of the curves for

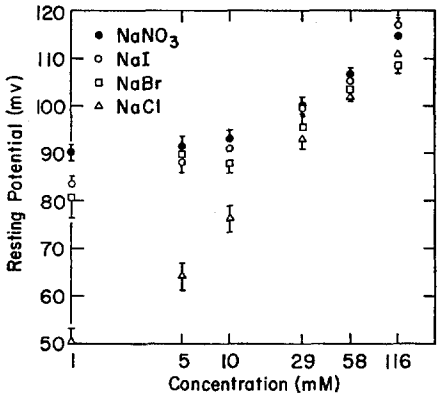

FIGURE 1. The relationship of the resting potential to the logarithm of the concentration of extracellular NaCl, $NaNO_3$, NaI, and NaBr. Each point represents the results of measurements on at least nine fibers. The bar is one standard error of the mean. The data are taken from Table II. All solutions are K-free and contain 2 mM $CaCl_2$. The range of concentration of monovalent anion is, thus, 5 to 120 mM.

these salts are −23 mv/log [NaI], −20 mv/log [NaBr], and −20 mv/log [$NaNO_3$] between 100 and 10 mM.

DISCUSSION

The experiments reported in this paper were designed to investigate the relationship of the resting potential in skeletal muscle to the concentration of various salts in the extracellular solution. The resting potential is logarithmically related to the extracellular NaCl concentration. The effect of anions, other than chloride, on the resting potential is a complex one which cannot be explained simply in terms of replacing chloride by a less permeable anionic species. In 116 mM solutions the resting potential appears to be independent of the extracellular anion, but the depolarization produced by low ionic strength chloride solutions is much greater than that produced by low ionic strength solutions of other anions.

The first set of experiments was conducted to determine the nature and time course of the potential changes produced by substituting equivalent concen-

trations of nonchloride salts for NaCl. The resting potentials were the same in 116 mM chloride solutions and in the nonchloride solutions. No changes in the resting potential were observed during exposure to the nonchloride solutions in recordings made 3, 10, and 20 min after introducing the new solution.

Adrian (1956, 1961) reported that substitution of sulfate or nitrate for extracellular chloride produced no change in the resting potential in frog skeletal muscle fibers. Hodgkin and Horowicz (1959) also observed no stable potential change with sulfate substitution. Kitamura (1958) reported results that differ markedly from those reported here and those of Adrian and Hodgkin and Horowicz. In Ringer's solutions with low potassium concentrations (0.5–2.0 mM), he observed depolarizations when chloride was replaced by sulfate, nitrate, carbonate, or bromide. However, many of the fibers tested may not have been viable. Cells were chosen which had resting potentials above 70 mv in Ringer's solution with 2 mM KCl. Adrian (1956) gives the mean resting potential of skeletal muscle cells in Ringer's solution with 2.5 mM KCl as 92 mv. In addition, Kitamura did not measure resting potentials in NaCl solution after each experiment to determine whether the depolarizations were reversible.

Szaimi and Tomita (1963) reported a bimodal distribution of resting potentials when the extracellular chloride was replaced by sulfate, ferrocyanide, or glutamate. One group showed a resting potential near that of fibers in Ringer's solution. The other group was depolarized by about 50 mv. The depolarized fibers were not tested again in Ringer's to determine whether this potential change was reversible. As reported here some fibers were stably depolarized in the nonchloride solutions. This was most frequent in NaBr solution in which about half the fibers were depolarized. However, in no case was a fiber observed which was depolarized in a nonchloride solution and then repolarized in the chloride solution. These fibers were considered to be damaged, perhaps by the dissection or by repeated microelectrode impalements.

Hodgkin and Horowicz (1959) reported that substitution of sulfate for extracellular chloride produced a transient depolarization in muscle cells. They also reported that replacement of the extracellular sulfate by chloride produced a comparable transient hyperpolarization. Their measurements were made on isolated single cells from the frog semitendinosus muscle with potentials continually recorded with an intracellular electrode. In the experiments reported here on the whole frog sartorius, resting potentials were not recorded while replacing the chloride solution with another anionic solution. Spontaneous contractions were observed with each solution which may indicate a depolarization at least great enough to reach the contraction threshold, about 65 mv (Sandow, 1955). However, the occurrence of contractions does not necessarily indicate the occurrence of a depolarization, since it is also

known that foreign anions affect the contractile properties independently of any effects on the cellular potential (Hodgkin and Horowicz, 1960; Horowicz, 1964). Spontaneous contractions when chloride is replaced with another anion have been reported by many authors (Hodgkin and Horowicz, 1959; Hutter and Noble, 1960; Szaimi and Tomita, 1963).

An attempt was made to observe the transient hyperpolarization upon replacing each foreign anion by chloride in the whole sartorius preparation. The microelectrode was left in a single cell during and for 1 min after the change in solution was effected. In no case was a transient hyperpolarization observed. The observed potential change could be accounted for by the change in junction potential between the reference electrode and the bath solution. The potential was still unchanged when recorded 10 min after reintroducing the chloride solution. Failure to observe this transient hyperpolarization has also been reported by Giebisch et al. (1957) in the gracilis muscle of the cat and by Szaimi and Tomita (1963) in the frog skeletal muscle. There is no apparent explanation for the difference between these results and those of Hodgkin and Horowicz (1959).

A transient depolarization was observed when the 116 mM NaCl solution was replaced by 116 mM choline Cl. Most fibers were completely repolarized within 30 min after introducing the choline Cl. Upon reintroducing the NaCl solution no potential changes were observed either within the first minute or after 10 min. Ochs (1966) observed a similar transient depolarization upon replacing NaCl by choline Cl, but did not measure the potentials again in NaCl.

The second set of experiments reported here was conducted to determine the effects on the resting potential of a prolonged exposure of the muscles to an isotonic 1 mM solution of each salt. All fibers were depolarized when the potentials were measured 10 min after introducing the 1 mM solutions. These depolarizations were stable for the entire 60 min during which the muscles were exposed to the low ionic strength solutions.

Giebisch et al. (1957) reported a stable depolarization, when extracellular NaCl was replaced by sucrose, in the cat gracilis muscle. Adrian (1956) also observed a stable depolarization in the frog sartorius when one-half the extracellular NaCl was replaced by sucrose. The magnitudes of these depolarizations are comparable with those reported here in the dilution series. Hodgkin and Horowicz (1959) reported that in one isolated fiber replacement of NaCl by sucrose resulted in a transient depolarization with a return to the potential recorded in Ringer's solution. Replacement of the low ionic strength solution by NaCl Ringer's produced a transient hyperpolarization. In the experiments reported here the potentials were monitored continuously in two fibers for 1 min after the 1 mM NaCl solution was replaced by the 116 mM NaCl solution. No transient potential changes were observed. In addition, the

depolarizations in the 1 mM NaCl solution were stable for at least 60 min. 60 min was the duration of the longest transient depolarization reported by Hodgkin and Horowicz. Again, there is no clear explanation for these discrepancies.

Mullins and Noda (1963) observed no potential change when replacing a 60 mM sulfate solution with a 5 mM sulfate solution, both solutions containing 1.25 mM K_2SO_4. With K-free solutions a hyperpolarization was observed. However, it is questionable whether the resting potential values given for the various solutions can be compared because resting potentials were measured in selected fibers from different bundles in each solution. Sulfate was not used in the experiments reported here because of the low solubility of $CaSO_4$.

The dilution experiments were designed to demonstrate the dependence of the resting potential on the concentration of the extracellular NaCl and to compare this dependence with that on the concentration of salts of other anions and choline Cl. The experiments with the different salts were all conducted in the same way. The criterion for a viable cell, resting potential greater than 100 mv, was the same for each 116 mM solution since the resting potential in a given cell is unchanged by substituting one of the other salts for NaCl. The resting potential measurements were made 10 min after introducing a solution since with each salt the depolarization recorded at 10 min is stable for at least 60 min. Some of the inevitable differences in the condition of individual fibers were avoided by requiring that the resting potential in the 116 mM solution be over 100 mM and be unchanged before and after each measurement in the low ionic strength solutions. Therefore, comparisons can be made of measurements of the resting potentials of different cells in different solutions.

The results of the dilution experiments, given in Fig. 1, show that the change in resting potential for a change in extracellular salt concentration is much greater for NaCl than for the Na salts of the other anions. This effect on the resting potential shows a similar difference with different anions as do other electrical properties of the membrane such as conductance (Hutter and Padsha, 1959; Hutter and Noble, 1960) and permeability (Conway and Moore, 1945). Changing the cation has little effect on the relationship of the resting potential to the extracellular salt concentration.

These results suggest that the effect on the resting potential is produced by changing the concentration ratios of intra- and extracellular anions, with the effect modified by using anions with different physical properties. However, at high extracellular concentrations (116 mM), the anionic series does not show quantitatively different effects on the membrane potential. In addition, the 1 mM solutions of the nonchloride salts have a higher concentration of chloride than of the foreign anion, due to the presence of 2 mM $CaCl_2$ in all solutions. The fact that the resting potentials in the 1-mM solutions do not approach a

common value would indicate that the foreign anion has more of an effect on the membrane potential than the effect due to its replacing a more permeable anion.

The use of K-free solutions in the experiments reported here probably increased the magnitude of the depolarizations produced in the low ionic strength solutions. Adrian (1956) reported that the depolarization produced by partial replacement of NaCl by sucrose is larger in low potassium solutions (0.5 and 1.0 mM KCl) than with 2.5 mM KCl in the solution. The use of K-free solutions also accounts for the high resting potentials recorded in the 116 mM solutions. Any error due to increase in cell volume or loss of intracellular potassium with the use of K-free solutions was avoided since both these effects should result in depolarization of the resting potential in the 116 mM solutions.

The main innovation in these experiments was the use of $ThCl_4$ to eliminate changes in the tip potential in solutions of low ionic strength. Some preliminary experiments were conducted without $ThCl_4$, with stable depolarizations observed in low ionic strength NaCl solutions. $ThCl_4$ has also been reported to have no effect on the magnitude or stability of the resting potential or on the characteristics of the action potential in skeletal muscle (Agin and Holtzman, 1966). Therefore, the depolarizations reported here were not due to any effect of $ThCl_4$ on the membrane. In addition, sucrose in the low ionic strength solutions did not produce the depolarizations, since resting potential measurements have been done in 116 mM NaCl solutions made hypertonic with sucrose, with no depolarizations observed (Holtzman, unpublished observations).

This manuscript was submitted to the Department of Physiology, the University of Chicago, in partial fulfillment of the requirements for the Ph.D. degree.

This work was supported by grants to Dr. D. Agin from the United States Public Health Service (NB-05457-01) and the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. The work was done during the tenure of a training grant from the National Institute of Child Health and Human Development (No. 5T1 HD-1).

Received for publication 9 September 1966.

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