Effect of Detergent on Electrical Properties of Squid Axon Membrane

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ABSTRACT The effects of detergents on squid giant axon action and resting potentials as well as membrane conductances in the voltage clamp have been studied. Anionic detergents (sodium lauryl sulfate, 0.1 to 1.0 mM; dimethyl benzene sulfonate, 1 to 20 mM, pH 7.6) cause a temporary increase and a later decrease of action potential height and the value of the resting potential. Cationic detergent (cetyl trimethyl ammonium chloride, 6×10^{-5} M or more, pH 7.6) generally brings about immediate and irreversible decreases in the action and resting potentials. Non-ionic detergent (tween 80, 0.1 M, pH 7.6) causes a slight reversible reduction of action potential height without affecting the value of the resting potential. Both anionic and cationic detergents generally decrease the sodium and potassium conductances irreversibly. The effect of non-ionic detergent is to decrease the sodium conductance reversibly, leaving the potassium conductance almost unchanged.

INTRODUCTION

A detergent is generally considered to be a surface-active agent. Usually it is a fairly large molecule (molecular weight about 300) containing a hydrophilic moiety at one end and a fairly long chain lipophilic group at the other end. It adheres to boundaries between two different phases such as exist between living membranes and extracellular fluid. Micelles may be formed under these conditions if the concentration of the detergent exceeds a certain limit. This characteristic of detergent action seems to be useful in the study of some aspects of the functional structure of living membranes.

Schulman and Rideal (1937 *a*, *b*) and Ponder and Ponder (1954) have studied the hemolytic action on human red blood cells of some detergents, namely certain sodium and potassium salts of some fatty acids. According to them, the detergent molecules are adsorbed first at the surface of the red cell membrane by forming complexes with the membrane substructure.

The adsorption can be expressed by means of Langmuir's adsorption isotherm. Hemolysis has been shown to occur with more concentrated detergent. Höber, Andersh, Höber, and Nebel (1939) and Wasano, Ogata, and Goto (1956) have observed a decrease in the demarcation potential of frog muscle fibers in ionic detergents. Kishimoto (1959) has reported that an irreversible reduction of resting potential of *Chara* internodes occurs in cationic, anionic, or amphoteric detergents, but that there is almost no reduction in resting potential upon exposure to a non-ionic detergent.

The aim of the present paper is to report in some detail the action of detergents on the relations between the membrane potential and the permeabilities to sodium and potassium in the squid giant axon.

METHODS

The giant axons were taken out of the squid (*Loligo pealii*) and were cleaned of surrounding fibers and loose connective tissue. The axons were, however, not necessarily completely clean, because of the necessity of leaving several tiny nerve branches attached to the giant axon. Pulling of such tiny branches or cutting them off too close usually reduced the survival time of the axon.

The experiments were carried out by the use of the space- and voltage-clamp techniques which were developed by Marmont (1949), Cole (1949), and Hodgkin, Huxley, and Katz (1952). The ionic currents which occurred during short times following step changes of membrane potential were recorded with the improved point control system of Cole and Moore (1960 *b*). All step changes in membrane potential were preceded by step hyperpolarizations of 5 msec. duration and 50 mv to insure complete removal of inactivation of the sodium conductance prior to the main voltage-clamp pulses. A correction of 4 mv for the liquid junction potential in our microelectrode system (Cole and Moore, 1960 *a*) was made where applicable.

The composition of the artificial sea water in mM was NaCl: 430, KCl: 9.18, $CaCl_2$: 9.46, $MgCl_2$: 23.4, $MgSO_4$: 26.0, and $NaHCO_3$: 2.19. The detergents used here were anionic, cationic, and non-ionic, molecular weights of which are around 300. Ionic detergent was dissolved in artificial sea water without any correction for its osmolarity, since the concentrations of the ionic detergents used were usually small. 30 cc of polyoxyethylene sorbitan mono-oleate (tween 80) dissolved in 1 liter of artificial sea water has an osmolal concentration of about 0.1 M. The pH of the solutions was adjusted to 7.6. The solutions were oxygenated before they were circulated around the axon as routine at a speed of about 1 ml per minute. The temperatures of the solutions were maintained to within ± 1 °C.

RESULTS

Anionic Detergents Anionic detergents used in these experiments were sodium lauryl sulfate, 0.1 to 1.0 mM, and dimethyl benzene sulfonate, 1 to 20 mM. There was no essential difference in the effect on squid axons of these two anionic detergents except for their effective concentrations. A typical experiment using 0.1 mM sodium lauryl sulfate is shown in Fig. 1. Membrane

potentials at the peak of the action potential (E_{AP}) and at rest (E_{RP}) are plotted against time for exposure periods initially to artificial sea water (ASW), to detergent solution, and finally to artificial sea water. The equilibrium potential for Na ion (E_{Na}) (Moore and Adelman, 1961) was obtained from records of the membrane currents in response to voltage clamping for brief intervals during each of the test periods and is shown at the top of Fig. 1. These will be discussed later.

Anionic detergents generally cause a temporary increase and a later decrease of value for the peak of the action potential if the concentration of detergent in the sea water is not excessive. The resting potential changes almost parallel the action potential changes. The temporary increase of the

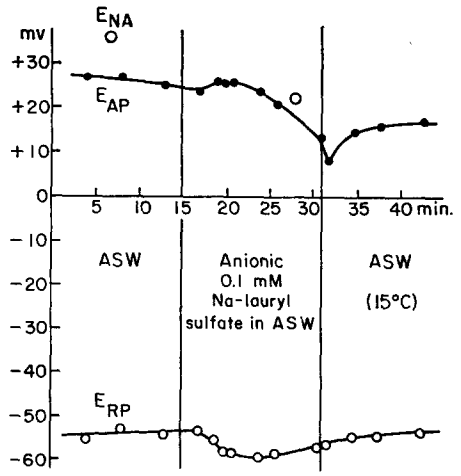

FIGURE 1. Effect of 0.1 mM sodium lauryl sulfate, an anionic detergent, on resting (E_{RP}) and action (E_{AP}) potentials. E_{AP} is the membrane potential at the peak of the spike. The equilibrium potential for Na ion, E_{Na} (open circles at the top of the figure) decreases in sodium lauryl sulfate solution. Axon 60-21.

value of the peak of the action potential, however, never exceeds the value of the sodium potential. The temporary increase (about 8 mv) of the resting potential is considered to be not the result of the electrode potential change inasmuch as measurements of microtip *vs.* reference electrode potential differences for isotonic KCl *vs.* detergent sea water were the same as for isotonic KCl *vs.* ASW. These temporary increases probably occurred immediately upon exposure to the anionic detergent inasmuch as it took about 2 minutes to clear the dead space of the fluid system and flush out the experimental cell. It is conceivable that the change in action potential amplitude may have been related to the increase in resting potential as this would tend to decrease any resting sodium inactivation or refractoriness.

Long exposure of an axon to anionic detergent results in an irreversible depression of the action potential and resting potential. The reduction of the sodium equilibrium potential suggests an increase of Na concentration inside the membrane (Moore and Adelman, 1961). When more concentrated

anionic detergent is applied, the reductions of action and resting potentials are very rapid and the temporary increases can barely be observed.

According to Hodgkin and Huxley (1952 *b*) the sodium conductance is defined as $g_{Na} = I_{Na}/(E_p - E_{Na})$ where I_{Na} is the current carried by the sodium ions, E_p the value of the clamped membrane potential. Similarly, the potassium conductance is defined by $g_K = I_K/(E_p - E_K)$ where I_K is the current carried by K ions and E_K the membrane potential at which no net potassium current flows. I_{Na} is determined from the peak value of the transient early component of membrane current and I_K is determined from the

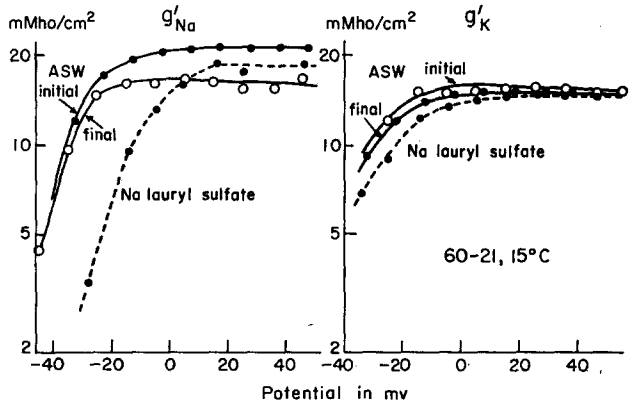

FIGURE 2. Sodium conductance at the peak of the transient inward current, g'_{Na}, and potassium conductance at the steady outward current, g'_K, are plotted against the potential of the clamped membrane. The dashed lines show the effects of 12 minutes' exposure to 0.1 mM sodium lauryl sulfate. A longer exposure to or use of a higher concentration of anionic detergent causes more marked reductions of peak sodium and steady-state potassium conductances.

value of steady outward current. Since the equilibrium potential for K ion was not determined for each axon, E_K is approximated by the resting potential (E_{RP}). Since E_{RP} must be considered to be generally a few millivolts less than E_K, the approximation can be inaccurate for small depolarizations, but is reasonably accurate for larger depolarizations. In the determinations of the peak inward Na current and of the steady outward K current the contributions of the leakage current were subtracted under an assumption that the latter did not change with time during step depolarization. This was done by assuming a linear current voltage relation for the leakage and that I_L is zero at E_{RP} and determinable at E_{Na}. In fact there is evidence (Adelman and Taylor, 1961) for a curvilinear I_L *vs.* V relation and therefore taking a linear I_L correction for I_{Na} and I_K introduces a small error in these values. At the right of Fig. 2, g'_K is plotted against the value of the membrane potential during each step depolarization. At the left of Fig. 2, the sodium

conductance at the peak of the transient inward current is plotted in a similar manner.

The peak sodium conductance, g'_{Na}, *vs.* voltage curve is shown in Fig. 2 to be shifted about 20 mv to the right upon exposure of the axon to a solution of anionic detergent. The potassium conductance *vs.* voltage curve is virtually unchanged or is decreased slightly in this axon, and in some axons the potassium conductance is increased at all voltage values. However, such changes in g'_K are generally not marked and may be considered insignificant. The shift of the curve for g'_{Na} toward the right in the direction of depolarization in the solution of anionic detergent would be expected to produce an

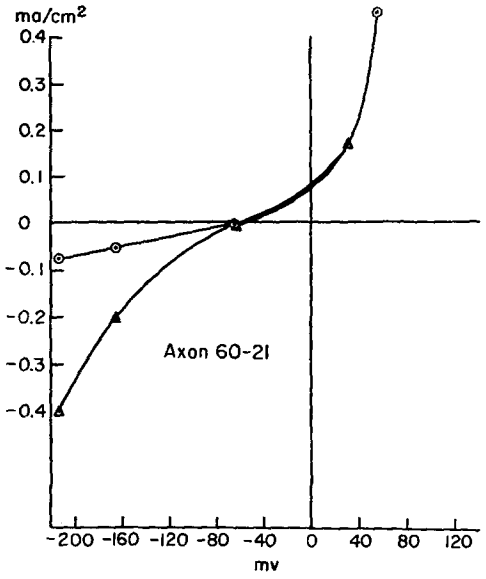

FIGURE 3. Leakage current *vs.* membrane potential. Circles, in ASW before detergent application. Triangles, in 0.1 mM sodium lauryl sulfate. See text.

increase of threshold for excitation inasmuch as the critical voltage for firing is more positive. g'_{Na} and g'_K tend to recover after the detergent solution is replaced with the artificial sea water, though the recovery is not always complete. A long exposure to a high concentration of anionic detergent causes marked irreversible reductions of the peak sodium and the steady state potassium conductances.

One of the more interesting effects produced by anionic detergents is a change from normal in the leakage current *vs.* voltage curve, especially in the region of hyperpolarization. In Fig. 3 are plotted values for the leakage current *vs.* membrane potential. The values of current to the right of the y-axis (membrane potential = 0) were obtained at 0.4 msec. after the onset of a pulse to the sodium potential. The membrane current at this time and at this voltage is expected to contain no contributions from sodium and

potassium charge-carriers because at the sodium potential there is no net sodium current and the hyperpolarizing prepulse delays the onset of the potassium current to such an extent that a negligible potassium current is expected during the first half millisecond (Cole and Moore, 1960). The values of current corresponding to voltages more hyperpolarized than the holding potential (equal to the resting potential) have no sodium or potassium components because the total current does not depend on the external sodium and potassium (Hodgkin and Huxley, 1952 *b*). The curve obtained initially in artificial sea water (circles) is similar to that obtained by Adelman and Taylor (1961) and is plotted as a curved line on the basis of their experiments. The curve obtained after 10 minutes in 0.1 mM sodium lauryl sulfate (tri-

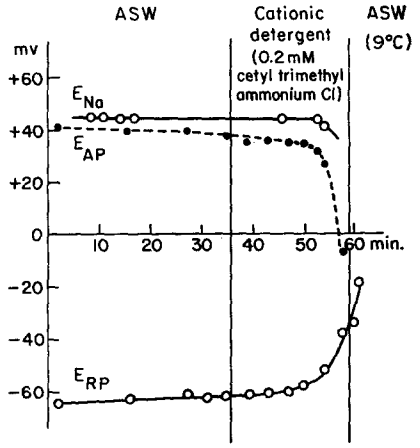

FIGURE 4. Effect of 0.2 mM cetyl trimethyl ammonium chloride, a cationic detergent, on resting potential (E_{RP}) and action potential peak voltage (E_{AP}) of squid giant axon. The sodium equilibrium potential, E_{Na}, decreases in parallel with E_{AP}. The effect of cetyl trimethyl ammonium chloride is irreversible even at a concentration as low as 6×10^{-5} M. Axon 60-37.

angles) shows considerable deviation from the normal curve in that the leakage current is considerably increased for hyperpolarized potentials. We may assume that this phenomenon implies a non-specific leak or an increased general permeability of the membrane to all ions and may in part be responsible for the general decline in resting potential and sodium potential seen at this time.

Cationic Detergents The effect of cationic detergents on the axon is generally very drastic. A cationic detergent solution as low as 6×10^{-5} M in concentration has a significant depolarizing effect. In Fig. 4 the effects of 0.2 mM cetyl trimethyl ammonium chloride on the resting and action potentials are shown. This detergent decreases the resting potential and the peak value of the action potential very rapidly and irreversibly. In more than ten experiments such temporary increases of resting potential as observed in anionic detergent were never observed in cationic detergent. The threshold for excitation to short duration depolarizing currents increases rapidly and the axon soon becomes inexcitable. The sodium equilibrium potential de-

creases in parallel with the peak voltage of the action potential. In Fig. 5 (left) sodium conductance at peak inward sodium current may be seen to decrease greatly at any membrane potential, upon exposure of the axon to 0.2 mM detergent for 18 minutes. Potassium conductance at steady outward potassium current also decreased markedly. However, in some axons the potassium conductance *vs.* voltage curve showed a shift to the right in cationic detergent initially just before the entire conductance curve was depressed. Current measurements for different clamped membrane voltages take at least 1 minute. During this period the rapid effect of cationic detergent progresses, causing some time-dependent errors.

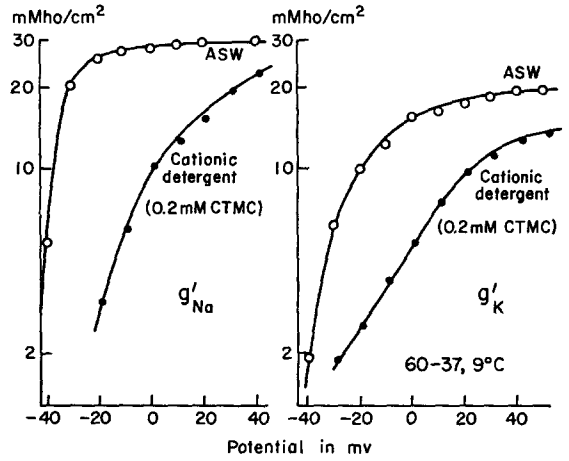

FIGURE 5. Sodium conductance at the peak of the transient inward current, g'_{Na}, and potassium conductance at the steady outward current, g'_K, are plotted against the potential of the clamped membrane. g'_{Na} and g'_K decrease for all depolarized values of membrane potential, upon 18 minutes' exposure to the detergent.

Fig. 6 illustrates the effects of a more severe cationic detergent treatment (0.4 mM cetyl trimethyl ammonium chloride) on the sodium and leakage currents. Current voltage relations are plotted for the peak values of the initial transient inward component of membrane current (sodium) as well as the leakage current values obtained in the manner described earlier for Fig. 3. In this case the entire leakage current *vs.* voltage curve is altered showing a rather general rise in the non-specific ion permeability. In Fig. 6 we have not attempted to draw a curvilinear relation for the leakage current in view of the fact that the value of I_L at E_{Na} for detergent treatment is so elevated above normal that the effect becomes predominantly clear. It is our general experience that whenever the leakage currents become as large as these, irreversible effects soon become apparent. Indeed, this axon became irreversibly inexcitable soon after these measurements.

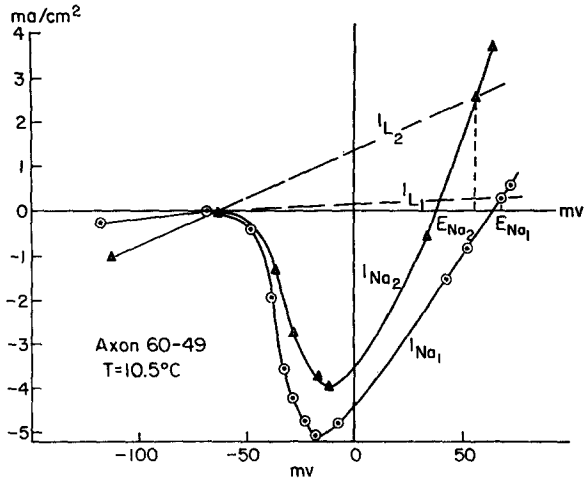

FIGURE 6. Peak transient currents (I_{Na}) and leakage currents (I_L) plotted against membrane potential. Circles, in ASW before detergent. Triangles, in 0.4 mM cetyl trimethyl ammonium Cl for 10 minutes.

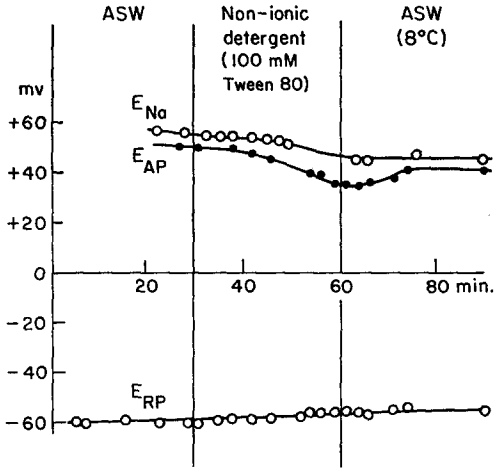

FIGURE 7. Effect of 0.1 M polyoxyethylene sorbitan mono-oleate (tween 80) on resting (E_{RP}) and action (E_{AP}) potentials. Decreases of E_{RP} and E_{AP} in non-ionic detergent are not as marked as those in ionic detergent even at a concentration as high as 0.1 M. If the period of application of non-ionic solution is not long, the height of the action potential recovers more or less reversibly upon return to sea water. If the period of application is long, E_{AP} decreases first and later E_{RP} decreases irreversibly. Axon 60-45.

Non-Ionic Detergents A concentration of tween 80 of 10 mM or less has no observable influence on the membrane characteristics of the squid axon.

In Fig. 7, the effect of 100 mM tween 80 on the resting potential and the peak value of the action potential is shown. The peak voltage of the action potential was decreased slightly and the threshold for excitation to short

duration depolarizing currents increased upon exposure to this solution. When we replaced the detergent solution with the original artificial sea water, the action potential recovered toward its initial value. The equilibrium potential for Na ion changed in parallel with the potential at the peak of the action potential, indicating an increased resting net sodium influx. On the other hand, the resting potential remained almost unchanged.

When the axon was kept in 100 mM tween 80 solution for a long time, *i.e.* 1 hour, or more, the action potential decreased irreversibly, excitability was lost irreversibly, and the leakage current increased markedly. Both g'_{Na} and g'_{K} decreased irreversibly.

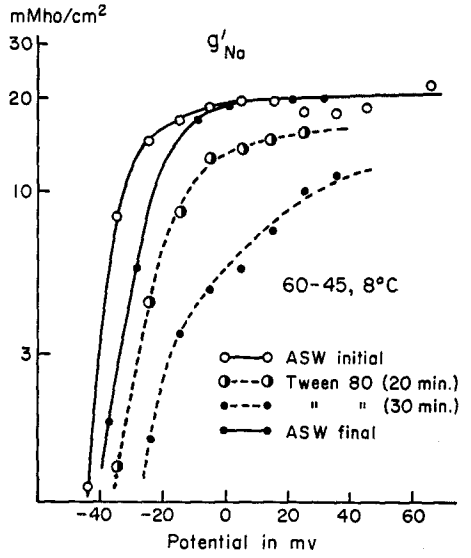

FIGURE 8. Sodium conductance at the peak of the transient inward current, g'_{Na}, is plotted against the potential of the clamped membrane.

Inasmuch as the potassium conductance in tween 80 was almost unchanged from normal at any clamped membrane potential, only the sodium conductance at peak inward current is plotted against clamped membrane potential in Fig. 8.

The osmotic effect of 100 mM tween 80 solution unfortunately was not tested on squid axons. But 100 mM sucrose in sea water had no appreciable effect on the resting and action potentials of single lobster axons. In general, the effects of non-ionic detergents on the squid axon are much milder than those of cationic and anionic ones, and require concentrations a number of orders of magnitude greater than the effective concentrations of ionic detergents.

DISCUSSION

The minimum concentrations of ionic detergents needed to depolarize the squid giant axon are about 10^{-4} M for Na-lauryl sulfate and 6×10^{-5} M

for cetyl trimethyl ammonium chloride. At the early stage of application of an anionic detergent temporary increases of the values of the resting potential and action potential were observed. If applied anionic detergent is of minimum effective concentration, its depolarizing effect can be made more or less reversible as is shown in Fig. 1. However, the effect of more concentrated anionic detergent is progressive and irreversible. This effect is similar to the depolarizing effect of the cationic detergent on the squid axon. The latter effect occurred more rapidly and no temporary increases of resting and action potential values during the early stage were observed. On the other hand, a much more concentrated solution was needed for the non-ionic detergent to have a depolarizing effect.

Schulman and Rideal (1937 *a*, *b*) suggested on the basis of their experiments on lipoprotein monolayers that there are two types of complex formation between injected molecules and molecules forming the monolayer. The first consideration is that mutual association takes place by means of polar groups alone. The other consideration is that the association is the result of mutual interaction of both the polar and the non-polar portions of each molecule. The anionic and cationic detergents used in our experiments are of the latter type and have a long chain of fatty acid. According to Kishimoto (1959) the action on *Chara* cells of an amphoteric detergent, which has both positive and negative charges, is almost the same as that of either cationic or anionic detergent alone. Therefore, it appears that the long detergent molecules adhere to the reactive groups of the axon membrane by more than one point of contact. In other words, an ionic detergent molecule will be adsorbed to the membrane with its long chain in parallel with the axon membrane. Therefore, the area covered by one ionic detergent molecule will be larger than the area occupied if it is assumed that the detergent molecule is adsorbed at only one point of contact with its long axis perpendicular to the membrane surface. Such a condition might manifest itself in two ways. First, such binding either directly at or close to specific ion-permeable sites might produce a screening effect (with respect to the effect of the electric field). This would generally depress the ionic conductances, as is somewhat apparent for cationic detergent. Second, such a condition might produce a depolarization of the membrane through a local distention of the pores to all ions as is evidenced by the increase in slope of the leakage current *vs.* voltage curve; *i.e.*, an increased leakage conductance.

The action of a non-ionic detergent may be considered to be somewhat different from that of the ionic detergents. Probably the non-ionic detergent adheres only by its hydrophobic group to the lipoid component of the axon. Therefore, the adsorption may be considered to be perpendicular to the axon membrane. If this is the case, then the area occupied by one non-ionic detergent molecule will be much smaller than that of an ionic detergent

molecule. In order to have a screening effect, a much more concentrated solution would be necessary.

The ionic detergent is a powerful depolarizing agent for squid axon membrane. Its action appears to be similar to that of local anesthetics, *e.g.* 0.1 per cent cocaine or 0.1 per cent procaine (Shanes, Freygang, Grundfest, and Amatniek, 1959; Taylor, 1959) in the sense that both detergents and local anesthetics decrease the peak inward sodium current and steady outward potassium current by means of decreases in g'_{Na} and g'_K. The threshold for excitation is increased by both agents. However, the ionic detergents, especially the cationic ones, are powerful depolarizers, while local anesthetics do not change the resting potential appreciably.

The anionic detergents which we have tested have either a sulfate or a sulfonate group, which suggests the possibility that their action results from removing Ca^{++} from the axon membrane. This seems unlikely, inasmuch as Frankenhaeuser and Hodgkin (1957) and Adelman and Moore (1961) have reported a shift to the left in a hyperpolarizing direction of the sodium conductance *vs.* potential curve in sea water of low Ca concentration which is opposite to the shift observed in anionic detergents. In addition, there was practically no change in the resting potential in this low Ca solution, in contrast to the changes in resting potential produced by anionic detergents. Therefore, the temporary increase of resting and action potentials at the early stage of an application of anionic detergent is probably not related to a Ca-depleting action. When the axon is left in anionic detergent solution for a while, the depolarizing action of the detergent takes place in an irreversible manner and finally causes a marked reduction in the sodium and potassium conductances. These effects are virtually identical to those produced by cationic detergents.

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REFERENCES

1. ADELMAN, W. J., and MOORE, J. W., *J. Gen. Physiol.*, 1961, **45**, 93.
2. ADELMAN, W. J., and TAYLOR, R. E., *Nature*, 1961, **190**, 883.
3. COLE, K. S., *Arch. scient. physiol.*, 1949, **3**, 253.
4. COLE, K. S., and MOORE, J. W., *J. Gen. Physiol.*, 1960 *a*, **43**, 971.

5. COLE, K. S., and MOORE, J. W., *J. Gen. Physiol.*, 1960 *b*, **44**, 123.
6. FRANKENHAEUSER, B., and HODGKIN, A. L., *J. Physiol.*, 1957, **131**, 218.
7. HODGKIN, A. L., and HUXLEY, A. F., *J. Physiol.*, 1952 *a*, **116**, 449.
8. HODGKIN, A. L., and HUXLEY, A. F., *J. Physiol.*, 1952 *b*, **116**, 473.
9. HODGKIN, A. L., and HUXLEY, A. F., *J. Physiol.*, 1952 *c*, **116**, 497.
10. HODGKIN, A. L., HUXLEY, A. F., and KATZ, B., *J. Physiol.*, 1952, **116**, 424.
11. HÖBER, R., ANDERSH, M., HÖBER, J., and NEBEL, B., *J. Cell. and Comp. Physiol.*, 1939, **13**, 195.
12. KISHIMOTO, U., *Ann. Rep. Scient. Works, Fac. Sc., Osaka Univ.*, 1959, **7**, 115.
13. MARMONT, G., *J. Cell. and Comp. Physiol.*, 1949, **34**, 351.
14. MOORE, J. W., and ADELMAN, W. J., *J. Gen. Physiol.*, 1961, **45**, 77.
15. PONDER, E., and PONDER, R. V., *J. Gen. Physiol.*, 1954, **37**, 441.
16. SCHULMAN, J. H., and RIDEAL, E. K., *Proc. Roy. Soc. London, Series B*, 1937 *a*, **122**, 29.
17. SCHULMAN, J. H., and RIDEAL, E. K., *Proc. Roy. Soc. London, Series B*, 1937 *b*, **122**, 46.
18. SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H., and AMATNIEK, E., *J. Gen. Physiol.*, 1959, **42**, 793.
19. TAYLOR, R. E., *Am. J. Physiol.*, 1959, **196**, 1071.
20. WASANO, T., OGATA, M., and GOTO, M., *Japan. J. Physiol.*, 1956, **6**, 137.