Effect of Ethanol on the 
Sodium and Potassium Conductances 
of the Squid Axon Membrane

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Abstract The effects of ethanol on squid giant axons were studied by 
means of the sucrose-gap technique. The membrane action potential height is 
moderately reduced and the duration sometimes shortened by ethanol in sea 
water. Voltage clamp experiments showed that ethanol in sea water reduced 
the maximum membrane conductances for sodium ($g_{Na}'$) and potassium ($g_K'$). 
In experiments with multiple application of ethyl alcohol to the same spot of 
membrane, a reduction of $g_{Na}'$ to 82 per cent and of $g_K'$ to 80 per cent of their 
value in sea water was brought about by 3 per cent ethanol (by volume) while 
6 per cent caused a decrease of $g_{Na}'$ to 59 per cent and of $g_K'$ to 69 per cent. 
Ethanol has no significant effect on the steady-state inactivation of $g_{Na}'$ (as a 
function of conditioning membrane potential) or on such kinetic parameters 
as $\tau_h$ or the time course of turning on $g_{Na}'$ and $g_K'$. It is concluded that ethanol 
mainly reduces $g_{Na}'$ and $g_K'$ in the Hodgkin-Huxley terminology.

Introduction

A few years ago, in preliminary axial–wire voltage clamp experiments on 
squid axons (Moore, 1958), ethanol was found to decrease the membrane conductance for sodium but not to affect that for potassium. It was clear, however, that more extensive data were necessary to describe the manner in which ethanol exerts its effect on the ionic conductances.

Quite recently Julian, Moore, and Goldman (1962 b) have shown the feasibility of a voltage clamp of the lobster giant axon with the exclusive use of external electrodes. It was obvious that this method should be equally applicable to squid axons. There are several substantial advantages of this method over that employing an internal axial wire: (a) It avoids the rather tedious preparation of the axial electrode. (b) It avoids the procedures of the longitudinal insertion of the axial electrode and microelectrode impalement of the membrane. (c) It requires only a very short length of axon ("node") in
good uniform physiological condition. Furthermore, if during experimenta-
tion, a node deteriorates, a fresh spot free of branches or injury can usually
be found by simply moving the axon along through the sucrose gap.

METHODS

Giant axons were isolated from the hindmost stellar nerve of the squid (Loligo pealii),
carefully cleaned, tied off, and placed in a plexiglass chamber. This chamber was an
adaptation of the lobster axon chamber used by Julian, Moore, and Goldman (1962
a) to the larger dimensions of the squid axon. The partitions which separate the cen-
tral pool from the side pools were 0.6 mm wide and 1.4 mm apart. Horizontal holes
of 600 μ diameter were drilled in the partitions meeting vertical sucrose supply holes
of 300 μ diameter. The horizontal holes were gouged out in order to guarantee a
thorough irrigation of the axon by the sucrose solution (Fig. 1, upper part). A three
way stopcock made of plexiglass was placed at the inlet to the central pool as a means
for switching between sea water and test solution fairly rapidly. All solutions flowed
by gravity through a cooling box (with melting ice) and into the chambers. The flow
rate was adjusted by needle valves. The temperature was measured by a constantan-
copper thermocouple which was placed close to the axon in the middle pool.

Electrical contact to each pool was made through saturated KCl-agar bridges to
Ag-AgCl electrodes. They are symbolized by small filled triangles in the lower part
of Fig. 1. Under working conditions two streams of isotonic sucrose solution isolate a
short segment of membrane in sea water (an artificial node) from the ends of the
fiber which are placed in the side pools containing isosmotic KCl solution. The mem-
brane potential of the artificial node is measured between the right hand or potential
pool (connected to high input impedance electrometer amplifier 1 with a closed
loop gain of 5) and the middle pool which is kept virtually at ground potential by
negative feedback of operational amplifier 3. When the switch is closed and the
resistance of the clamp gain potentiometer is decreased, the operational amplifier 2
will inject a current into the left hand pool in a fashion so that $V_m$ matches the com-
mand signals (a constant holding potential and pulses). If the “area pot” setting is
proportional to the membrane area as calculated from microscopic measurements of
fiber diameter and width of the sucrose gap, the output voltage of amplifier 3 is directly
proportional to the membrane current density (Moore, 1963).

The standard procedure for mounting the fiber in the chamber was as follows:
A piece of platinum wire with its one end bent to a small hook was threaded through
both horizontal holes in the chamber partitions. Then all three pools were filled
with sea water without either sucrose solution flowing. The axon was placed in one
side pool and one of the two silk threads with which the ends of the fiber had been
tied off was pulled through the holes with the aid of the wire. The threads were then
seized by two forceps which were connected to a linear motion device. After stretching
the fiber to its intact length by carefully pulling the forceps apart, the whole assembly
was moved parallel to the fiber axis until one end of the axon had passed through
both holes. The sucrose solution was then turned on in both partitions.

By injecting a stimulating current into the “current pool” (via a 40 or 60 megohm
resistor) an action potential of the artificial node in the middle pool was elicited.
FIGURE 1. Experimental arrangement. The upper part represents a cut through the axon chamber parallel to the fiber axis. The lucite partitions are striped, flowing sucrose solution is shown as stippled areas. In the middle pool it leaves only a short segment of the axon, an artificial node, exposed to flowing sea water. The ends of the axon are in side pools containing isosmotic KCl solution.

The lower part of the figure is the electrical equivalent circuit. $R_{ax}$ is the resistance through the axoplasm, $R_s$ the resistance through the sucrose solution. $C_m$, $R_m$, and $E_m$ are the capacity, resistance, and open circuit potential of the nodal membrane respectively. Segments of the axon in KCl solution are shown as short-circuits because of the low membrane resistance and zero membrane potential under these conditions. Electrodes are represented by small filled triangles. $V_m$ is the potential difference between the right hand pool and virtual ground as measured by electrometer amplifier 1. In order to clamp, the switch of the clamp gain control arrangement is closed and the resistance decreased to zero. Operational amplifier 2 will then inject (or withdraw) into the current pool that value of current required to make $V_m$ match with the command signals. Currents through the nodal membrane cause an IR drop across the known feedback resistor $R_f$ of operational amplifier 3. If the area potentiometer is set to a value $β$ calculated from microscopic measurement of the membrane area, the output voltage $I_m$ of amplifier 3 is directly proportional to the membrane current density.

The amplitude of the spike—and especially the difference between threshold potential and peak potential—served as an indicator of the functional state of the membrane area. If this threshold-to-peak potential difference was less than 75 mv, the node was considered unsatisfactory and the fiber was moved on until a "good" patch of membrane was found. Sea water in the side pools was then replaced by isotonic
KCl solution. Rates of flow of the sea water and sucrose streams were adjusted so that the nodal length was approximately one-fourth to one-half of the fiber diameter.

Filtered natural sea water was used throughout. Ethanol solutions were made by mixing absolute ethyl alcohol with sea water. Isotonic sucrose solution contained 725 mM (1962) or 837 mM (1963) of the purest sucrose and had been passed through two ion exchange columns. The isotonic KCl solution consisted of 500 mM KCl and 25 mM CaCl₂. All solutions were passed through a cooling bath en route to the chamber. The solution temperature in the nerve chamber ranged from 4 to 8°C in 1962 and from 10 to 15°C in 1963.

The measured membrane potential, $V_m$, was estimated to be only 1 per cent less than the actual potential across the membrane, $E_m$. From Fig. 1 it can be seen that:

$$V_m = E_m \times \frac{R_s}{R_s + R_{ax}}$$

The external resistance between chambers through the sucrose stream, $R_s$, was measured without an axon in place and found to be 3 megohms. The longitudinal axoplasm resistance varied from 1.5 KΩ to no more than 30 KΩ, depending on the sucrose flow pattern and the time of exposure to sucrose. (The axoplasm resistance increases slowly in the region surrounded by flowing sucrose.) The membrane resistance for a $3 \times 10^{-3}$ cm² nodal area was about 60 KΩ. Therefore, the value of $E_m$ was in turn about 2 per cent lower than $E_m^o$, the open circuit membrane potential.

RESULTS

Resting Potential

With all three pools containing sea water, initiation of the sucrose flow usually resulted in the development of a potential difference of 20 to 50 mv between the middle and the side pool with a time constant in the order of 10 seconds. The potential pool became negative with respect to the central pool at ground potential. It is assumed that this is partly due to a lower resting potential of the short segments of membrane near the tied-off end which is in the side pool. As soon as the sucrose solution exerts its insulating effect this potential difference between an injured and a normal segment of the fiber becomes apparent.

Upon substitution of sea water by isosmotic KCl solution in the side pool a potential difference of $-80$ to $-100$ mv (potential pool with respect to middle pool) was observed. Under the assumptions that isosmotic KCl solution reduced the membrane potential to zero (Curtis and Cole, 1942; Moore, 1959 a) and that the sucrose acts as a perfect insulator, this potential difference represents essentially the membrane potential of the artificial node (inside negative). The membrane potential in the sucrose gap is distinctly higher than that found with internal or microelectrodes (cf. Moore and Cole, 1960). Furthermore it was observed that the membrane potential varied with the gap width; i.e., the narrower the gap, the "higher" the resting potential (more
negative inside). However, in a voltage clamp, it is usually desirable to hyperpolarize the membrane and completely remove the resting "inactivation" of the sodium channel. In the sucrose-gap voltage clamp, little or no hyperpolarizing current was required.

Sea water containing 3 to 10 volume per cent ethanol did not appear to change the resting potential. However, because of the dependence of the membrane potential on the gap width, no unequivocal statement can be made because switching to the test solution frequently resulted in changes of the gap configuration.

![Figure 2](image_url)

**Figure 2.** Effect of ethanol on action potential. Solid line shows action potential in sea water, broken line in 4.5 volume per cent ethanol. Resting potential is -90 mv, zero potential is indicated by short horizontal bar.

**Action Potential**

Action potentials measured with the sucrose-gap method are, in the terminology of Hodgkin, Huxley, and Katz (1952), "membrane action potentials" because the insulating effect of the sucrose solution prevents propagation and the membrane patch is sufficiently short so that one can be assured of isopotentiality of the "nodal" membrane within 5 to 10 per cent at any given time (K. S. Cole, personal communication). The shape of the action potential is comparable to that observed with microelectrodes and no sucrose flow with the exception that it does not show an underswing ("positive afterpotential") in its late falling phase.

The effect of 3 to 10 volume per cent ethanol on the shape of the action potential is relatively small and variable. It decreases the amplitude of the spike by a few millivolts (see Fig. 2) and sometimes shortens its duration (20 per cent at the most). No clear relation between the alcohol concentration and the effect on the action potential shape could be established.

**Membrane Currents during Voltage Clamp**

As a standard procedure, the membrane potential of the node was clamped at a constant holding potential (usually -80 to -100 mv) near its resting value.
and displaced to various levels in a stepwise fashion. The resulting current patterns were recorded in sea water, then in sea water + ethanol, and finally in sea water again.

Fig. 3 summarizes the results of a typical experiment. Current densities are plotted as a function of membrane potentials during the voltage clamp. The curves in the lower half of the graph refer to maxima of the early current transient, those in the upper half refer to steady-state outward currents. All current data are corrected for the leakage current in the manner shown in the inset. They therefore represent essentially the maximum Na and K current.

Figure 3 shows a marked decrease in both the Na and K current during the application of 6 volume per cent of ethanol in sea water. A steady state of the ethanol effect was usually reached within 2 to 5 minutes; the application lasted about 10 to 15 minutes on the average. Further details that can be seen in Fig. 3 are: (a) Maintenance of the general shape of the $I_{Na}$ and $I_K$ curves.
in alcohol, (b) no significant change in the sodium equilibrium potential $E_{Na}$, and (c) full recovery upon return to normal sea water. In some of the experiments we observed an increase (of variable amount) in both $I_{Na}$ and $I_{K}$ in the second sea water run with respect to the first one. This phenomenon can also be seen in Fig. 3.

Membrane Conductances

Since both the peak transient current and the steady-state outward current are linear functions of the membrane potential for $E > -20$ mv, the slopes of the current-voltage curves at these potentials represent the maximum membrane conductance for Na and K ($g_{Na}$ and $g_{K}$ respectively in the nomenclature of Taylor, 1959). Their values in normal sea water (mean of sea water data before and after ethanol treatment, 1962) were 71 mmho/cm² for $g_{Na}$ and 61 mmho/cm² for $g_{K}$.

The equilibrium potential $E_{Na}$ can be determined experimentally as the intercept of the $I_{Na}$ curve with the abscissa as in Fig. 3. Therefore, it is also possible to calculate chord conductances for $E < -20$ mv by the relation $g'_{Na} = I_{Na}/(E - E_{Na})$. A plot of the logarithm of $g'_{Na}$ versus membrane potential is shown in Fig. 4. Ethanol essentially causes a parallel downward shift of all the points on the $g'_{Na}$ curve and, since $g'_{Na}$ is plotted logarithmically, this means a reduction by a constant factor. It should be noted that the slope of the steepest part of the curve (an e-fold change in $g'_{Na}$ by a depolarization of about 5 mv) does not change in the presence of alcohol.

Ethanol was tested in concentrations ranging from 1.5 to 10 per cent (by volume). Because the ethanol effect on the membrane conductances varied considerably from axon to axon and because ethanol appeared to reduce these conductances more markedly in 1962 than it did in 1963, a reliable dose-effect relation could not be established by statistical methods. Nevertheless, it was found that a concentration of:

(a) 1.5 per cent had no effect on the conductances (experiments in 1963 only);

(b) 3 per cent caused a significant reduction of $g'_{Na}$ in 10 out of 12 experiments and of $g'_{K}$ in 9 out of 12 experiments (1962 and 1963 combined), mean reductions of: $g'_{Na}$ to $85 \pm 5$ per cent ($\pm$SE) and $g'_{K}$ to $83 \pm 5$ per cent of their values in sea water;

(c) 4.5 per cent reduced both $g'_{Na}$ and $g'_{K}$ in each of the limited number of runs (4) performed (1962 only, mean reductions of $g'_{Na}$ to $59 \pm 4$ per cent and $g'_{K}$ to $66 \pm 3$ per cent of their values in sea water);

(d) 6 per cent invariably caused a significant reduction of both conductances (combined 1962 and 1963 values: $g'_{Na}$ to $66 \pm 5$ per cent, $g'_{K}$ to $68 \pm 5$ per cent of sea water values);

(e) 10 per cent drastically reduced both $g'_{Na}$ and $g'_{K}$, but usually the mem-
brane did not recover from treatment with this concentration and only 2 complete runs were obtained (1962).

A more conclusive way to establish a dose-effect relation is to apply a number of different concentrations to the same spot of membrane with control sea water runs intervening. However, because of the usual deterioration of axons during long lasting experiments, we succeeded in this test on only 7 occasions. In these experiments, 3 per cent ethanol caused an average reduction of $g_N$ to $82 \pm 6$ per cent (SE) and of $g_K$ to $80 \pm 5$ per cent of their values in sea water. These numbers include one experiment in 4.5 per cent from which the reduction in 3 per cent was interpolated. The application of 6 per cent ethanol reduced $g_N$ and $g_K$ to $59 \pm 5$ per cent and $69 \pm 5$ per cent respectively of their values in sea water.

**Steady-State Inactivation**

In 6 of our experiments the availability of the sodium conductance as a function of the membrane potential prior to the test pulse was determined by the usual method (Hodgkin and Huxley, 1952 a). The amplitude of the test pulse was chosen so as to give a large inward current, and it was preceded by a conditioning pulse of 20 to 30 msec. duration and variable amplitude. The peak $I_{N_a}$ during the test pulse was measured relative to the maximum $I_{N_a}$.
obtainable at strong hyperpolarizations at which inactivation is completely removed. This current ratio was denoted \( h_{\infty} \) because the duration of the prepulse was long enough to allow the membrane properties to reach a steady state. In Fig. 5 the relation between \( h_{\infty} \) and the membrane potential is shown for both sea water and 3 volume per cent ethanol. An empirical equation

\[
\frac{1}{1 + e^{(x-x_0)/k}}
\]

(see Frankenhaeuser and Hodgkin, 1957) was fitted to the measured points. There was no consistent effect of ethanol on either \( E_k \) the potential at which \( h_{\infty} = 0.5 \) or on the quantity \( k \) which determines the slope of the curve. Table I summarizes our results.

**Table I**

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**Kinetic Data**

In order to see whether kinetic changes were responsible for the reduction of sodium current, we extended our measurements to some kinetic parameters.
such as those associated with the time taken for $g_{Na}$ and $g_K$ to turn on and the time constant of recovery of $g_{Na}$ from inactivation.

In order to obtain a simple measurement of the speed with which the sodium conductance turns on during a pulse, the time $t_p$ between the onset of the pulse and the peak of the inward current was determined and plotted as a function of membrane potential. The results of a typical experiment are depicted in Fig. 6. One can see that there is no appreciable difference between the values in sea water and in sea water–ethanol mixture. The scatter of the points for $E \leq 40 \text{mv}$ is due to the rather flat time course of the inward current in this potential region which makes the determination of $t_p$ somewhat arbitrary.

![Figure 6. Effect of ethanol on time to peak of $I_{Na}$. Time $t_p$ in msec., defined as shown by inset, plotted versus membrane potential during pulse. Points in sea water before and after application of ethanol are shown as open and filled circles respectively, points in 4.5 volume per cent ethanol as triangles.](image)

The time course of recovery from inactivation was determined with two equally strong pulses of an amplitude chosen to give a large $I_{Na}$, after the manner of Hodgkin and Huxley (1952 a). The duration of the first pulse was long enough for the inward current to reach or pass its peak. The second pulse was longer and was initiated at various times following the cessation of the first one. The peak inward current during the second pulse increased with increasing intervals between pulses until it reached a steady-state because sodium inactivation is removed when the potential is held at the resting or hyperpolarized level between the pulses. The logarithm of the difference between the sodium current during the second pulse and its steady-state value was then plotted as a function of time (delay of the second pulse). From this
slope the time constant \( \tau_h \) for the holding potential was calculated. In the few experiments done, no change by ethanol greater than the error of the method could be detected.

Finally, we studied the effect of ethanol on the time course of the turn on of potassium current at the sodium equilibrium potential. The logarithm of the difference between the steady-state potassium (outward minus leakage) current and its value at earlier times was plotted against time. The slope of the linear part of this plot was not significantly affected by ethanol.

**DISCUSSION**

*Sucrose Gap and Voltage Clamp*

Before discussing the effect of ethanol on the membrane conductances we should compare our normal sea water results with the sucrose-gap method to those obtained by the conventional methods. To illustrate the performance of our method of voltage clamping, Fig. 7 shows the time course of both the membrane potential and the membrane current during a pulse. The rise time of the potential step (from 0.1 to 0.9 of the final value) is less than 25 \( \mu \text{sec} \) with a maximum overshoot of 20 per cent lasting 100 \( \mu \text{sec} \). This is short enough for observation of initial or "leakage" currents for temperatures in the vicinity of 6°C. The current pattern resembles very closely in shape and amplitude that which is observed with internal wire electrodes (cf. Hodgkin, Huxley, and Katz, 1952; Moore and Cole, 1963). The only difference seems to be that in our experiments the initial or leakage current step \( I_L \), is larger than what one finds with the conventional methods. We took leakage conductance \( g_L \) as given by the expression \( g_L = \frac{I_L}{E-E_H} \), where \( E_H \) is the holding potential. \( g_L \) was found to be in the order of 10 mmho/cm², which is about twice as much as usually observed with internal electrodes. How-
ever, the ionic current-potential curves (corrected for $I_x$) in the fashion of Fig. 3 are essentially the same as determined by the axial wire set-up (cf. Moore, 1959 b; Taylor, 1959).

The action potentials observed in the unclamped condition with the sucrose-gap method differ from those usually seen with internal electrodes because the sucrose solution always hyperpolarizes the nodal membrane at the narrow gap width required for a good clamp. The absence of an undershoot of the action potential under this condition seems to be a direct consequence of the elevated membrane potential. Normallv, i.e. without sucrose hyperpolarization, the absolute value of the resting potential is less than the absolute value of the potassium equilibrium potential, $E_K$. On the downstroke of a spike, $g_{Na}$ has already passed through its maximum and returned to a low value while $g_X$ is still increasing and causes the membrane potential to move towards $E_K$. Under normal conditions this yields to an undershoot (after-hyperpolarization, "positive after-potential"). In the case of a spike originating from a hyperpolarized potential brought about by other means than a drastic shift of $E_K$ (which is highly improbable in this situation), a persistent increase of $g_K$ towards the end of an action potential can only result in an after-depolarization (a delayed return to the original level or a "negative after-potential").

The behavior of the after-potential in our experiments suggests that the observed hyperpolarization represents a genuine change of the potential across the excitable membrane and not a measuring artifact. The experimental basis for this notion was supplied by Julian, Moore, and Goldman (1962 a) on lobster axons which also show a strong hyperpolarization in the sucrose gap. These authors measured simultaneously the node membrane potential with external electrodes and with an impaled microelectrode and found the same values with the two methods.

The mechanism of this hyperpolarization is not fully understood at the present time. Stämpfli (1963) proposes a local current flow through the node driven by a diffusion potential at the sucrose-saline boundary. Although this local current has not yet been proved experimentally, there is indirect evidence which seems to confirm this concept. Stämpfli (1963) could show that the hyperpolarization is absent or the diffusion potential is abolished by interposing a vaseline seal between the two solutions.

In this connection we performed a number of experiments in which we set the membrane potential of an artificial node at several levels by an appropriate adjustment of the gap width and a constant current. For a given steady membrane potential, the spike configurations, and especially the sign and magnitude of the after-potentials, were identical and independent of the method by which the membrane potential was adjusted. The fact that hyperpolarization increased as the gap became narrower is compatible with Stämpfli's idea be-
cause a decrease in membrane area means an increase in the nodal current density and therefore a larger voltage change. The response of the membrane potential to nodal area changes was at least as fast as our chart recorder. This speed of response is in better agreement with the local current hypothesis than alternative mechanisms as primary sources of the hyperpolarization; e.g., changes of the internal composition. Long term changes of the internal ion concentrations, however, appear to occur as a result of a long lasting current flow and a possible washout of the internal ions by flowing sucrose solution. In fact, during extended experiments we found an increase of the longitudinal resistance of the axon in the region of the fast flowing sucrose solution.

**Ethanol Effect**

In the voltage clamp experiments reported here, ethanol was found to reduce the membrane conductance for both sodium and potassium ions. No marked effect of ethanol on the kinetic parameters could be detected and the inactivation of $g_{Na}$ was found unaffected; i.e., $h_{m}$ was the same function of membrane potential. Therefore we interpret our results in terms of the Hodgkin-Huxley theory (1952) as mainly a decrease of $g_{Na}$ and $g_{K}$; i.e., in the maximum value which the ionic conductances can attain. Ethanol was found to cause about equal depressions of the sodium conductance and of the potassium conductance.

This is in contrast to earlier observations with an axial-wire voltage clamp used by one of us (Moore, 1958) in which ethanol caused a large decrease of $g_{Na}$ without affecting the value of $g_{K}$. A part of this difference appeared to be due to the fact that, in the earlier experiments, the squid axon membrane was clamped at its resting potential. It was slightly depolarized by ethanol and the membrane conductance for Na seemed to be more affected by ethanol because of the partial inactivation.

Quite recently, Binstock and Armstrong (1963) reported that various alcohols including ethanol (at low thermodynamic activities) affect mainly the inward current ($I_{Na}$). In these axial wire–voltage clamp experiments, however, the test pulse was preceded by a hyperpolarizing pulse so that the inactivation of $g_{Na}$ cannot explain the discrepancy. Hagiwara and Tasaki (personal communication) found that ethanol depresses the early inward current more than the steady-state outward current at room temperature, but at low temperatures the steady-state current is also markedly decreased by ethanol. In this connection, the findings of Spyropoulos (1957) are of interest, namely that action potentials of squid fibers, which were considerably reduced by ethanol at room temperature, regained their full amplitude when the temperature of the bathing solution was lowered.

These results suggested another possible explanation of the discrepancy be-
between the observations of Binstock and Armstrong (1963), who worked at 7–12°C, and ours at 4–8°C in 1962. However, in 1963 we performed most of our experiments at a temperature range from 10 to 15°C without finding a more pronounced effect of ethanol on $g_N$ than on $g_K$. Only at room temperature did ethanol appear to reduce $g_N$ more than $g_K$. This observation was made in a number of separate experiments of the Hagiwara and Tasaki type in which ethanol was tested at two different temperatures on the same spot of membrane. The data from these experiments are not included in the mean values given in Results.

Therefore the question as to why the squid membrane in a sucrose gap reacts differently to ethanol remains unanswered. This is the more surprising as our voltage clamp results in normal sea water were virtually identical with those obtained with internal electrodes. We thought that perhaps the hyperpolarizing currents in sucrose may have been responsible for this. We have performed a few experiments with lobster axons to see whether the potassium conductance was less affected by ethanol when the sucrose hyperpolarization was counteracted by the voltage clamp. The potassium currents were still markedly reduced even when the membrane was held at $-60$ mv between pulses. It appears then that the presence of sucrose solution renders the potassium conductivity more susceptible to ethanol.

In a number of preparations ethanol shows a depolarizing effect, as in frog striated muscle (Knutsson, 1961), heart muscle (Gimeno, Gimeno, and Webb, 1962), mammalian nerve (Wright, 1947), and frog nerve (Gallego, 1948; Posternak and Mangold, 1949), in which a restoration of the reduced action potential could be achieved by anodal polarization. With the exception of frog muscle, depolarization was reported to be accompanied by a decreased action potential or conduction block. A decline of action potentials without explicit mention of a depolarization has also been described for lobster motor axons (Adelman, 1959).

**COMPUTATIONS**

In order to correlate the observed ethanol-induced changes in the ionic conductances in the voltage clamp with the minimal change in action potential shapes, a number of action potentials were computed from the equations of Hodgkin and Huxley (1952 b). A Runge-Kutta program was written in Fortran for use on the Duke University's IBM 7072 digital computer. We compared spikes computed for normal values for $g_N$ and $g_K$ with action potentials computed for one-half normal values for $g_N$ and $g_K$. Sucrose hyperpolarization was simulated by (a) setting the leakage potential $V_L$ at $+20$ mv; and (b) passing enough steady current ($10 \mu A/cm^2$) to cause the membrane to seek a new steady-state potential of about $+20$ mv. In each of these cases, shown in Fig. 8 only small or subtle differences appeared in the
shapes of the action potentials. As already noted, the experimentally observed action potentials in sea water and ethanol show little or no differences.

In contrast, if the sodium conductance $g_{Na}$ alone is reduced to one-half the normal value, there is a distinct and clear reduction in the duration of the spike as well as in its height (Fig. 8 B). Such a change in shape was in fact observed in the previously mentioned early preliminary experiments with an axial wire (Moore, 1958) in which the sodium conductivity alone was found to be reduced.

Thus we have a good correlation between action potential shapes and voltage clamp currents in both sets of experiments. Differences then appear to be independent of the voltage clamp methodology. These results then support
the notion mentioned previously that it is the presence of sucrose which is responsible for the differences in the observations on the potassium conductivity.

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