Analysis of Spike Electrogenesis and Depolarizing K Inactivation in Electroplaques of *Electrophorus electricus*, L.

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ABSTRACT Voltage clamp analyses, combined with pharmacological tools demonstrate the independence of reactive Na and K channels in electrically excitable membrane of eel electroplaques. Spike electrogenesis is due to Na activation and is eliminated by tetrodotoxin or mussel poison, or by substituting choline, K, Cs, or Rb for Na in the medium. The K channels remain reactive, but K activation is always absent, the electroplaques responding only with K inactivation. This is indicated by an increased resistance when the membrane is depolarized by more than about 30 mv. The resting resistance (1 to 5 ohm cm^2) is dependent upon the ionic conditions, but when K inactivation occurs the resistance becomes about 10 ohm cm^2 in all conditions. K inactivation does not change the EMF significantly. The transition from low to high resistance may give rise to a negative-slope voltage current characteristic, and to regenerative inactivation responses under current clamp. The further demonstration that pharmacological K inactivation (by Cs or Rb) leaves Na activation and spike electrogenesis unaffected emphasizes the independence of the reactive processes and suggests different chemical compositions for the membrane structures through which they operate.

An observation by Altamirano (1955) implied that the intracellularly recorded spikes of eel electroplaques differ from the conductile responses of most other cells. The resistance of the electrogenically reactive caudal membrane of the electroplaques increased two- to threefold over the resting value when this membrane was depolarized by applied currents which exceeded a certain threshold. The increase was evident during the falling phase of the spikes which were elicited by the applied currents and it persisted as long as the current pulses were applied (*ca.* 20 msec.). The resistance increase also

occurred on applying currents to cells which had been exposed to a medium enriched in KCl, when spike electrogenesis had been thereby eliminated (Altamirano and Coates, 1957). It was suggested (Grundfest, 1957 *a*, 1960, 1961) that the increase in resistance indicated the occurrence of depolarizing potassium inactivation, the block of K-permselective channels in the membrane by depolarizing stimuli. Data on cardiac muscle (Weidmann, 1951, 1956) and on frog axons (Tasaki and Freygang, 1955) which indicated the presence of K inactivation in the spike electrogenesis of these cells were also noted at that time.

K inactivation is to be regarded as a response of the membrane which is analogous to the process of depolarizing Na inactivation that is incorporated into the ionic theory of spike electrogenesis (Hodgkin and Huxley, 1952). However, Na conductance is usually low in the resting cells, indicating that the reactive Na-permselective channels are closed at rest. Na inactivation which blocks these channels from reacting to stimuli thus does not decrease the resting conductance. The K-permselective channels usually form the pathway of highest conductance in the resting membrane and if any of the reactive K-permselective channels are open at rest, K inactivation will cause an increase of the membrane resistance above the resting level.

Studies on a number of cells have confirmed the existence of depolarizing inactivation processes for ions other than Na and also of analogous inactivations which are initiated by hyperpolarizing currents (Grundfest, 1961, 1962, 1963, 1965). When inactivation processes cause the membrane resistance to increase, the non-linearity of the current-voltage (I-E) relation may exhibit a negative slope region in which an increase of voltage causes a decrease in the current, as the resistance changes from its lower to the higher value. Accordingly, inactivation processes can give rise to regenerative, triggered responses which resemble spikes in many ways. Among the several varieties of "anomalous" responses of electrically excitable membranes some have been described which are due to inactivation processes.

Quantitative studies on hyperpolarizing responses of lobster muscle fibers (Reuben *et al.*, 1961) and gymnotid electroplaques (Bennett and Grundfest, 1962 *a*, 1962 *b*, 1965) confirm the above analysis. Similar studies have also been carried out in this laboratory on depolarizing inactivation of electroplaques of some weakly electric gymnotid fishes (Bennett and Grundfest, 1962 *a*, 1962 *b*, 1965; Goldman and Grundfest, unpublished data) and puffer supramedullary neurons (Nakajima and Kusano, 1963, and data to be published). However, the analysis of the responses of eel electroplaques provides further data on the process of depolarizing K inactivation because, as the present work demonstrates, the process of K activation is absent from the spike electrogenesis of these cells. This paper details the results of work in which single electroplaques were used. The study included voltage clamp

analysis of the processes of spike electrogenesis, and of their modification under various experimental conditions. Preliminary reports of the work have been published (Nakamura *et al.*, 1964 *a*, 1964 *b*).

METHODS

Preparations Particularly large electric eels, 1.5 to 2 m long, were used for the experiments so as to obtain large electroplaques. The single cells were from the caudal portion of the Sachs organ and were dissected (Schoffeniels, 1961) from slices which were removed from the tail as needed (Altamirano *et al.*, 1953). Ten or 20 slices could be obtained from a single eel, each providing up to 5 or 6 isolated and viable cells. The normal bathing medium for the tissue was a saline one which is similar in ionic content to the blood (Hargreaves and Frota-Moreira, 1949).[1] However, in some experiments all the NaCl was replaced isosmotically with choline chloride, KCl, CsCl, RbCl, K-acetate, or K-pyroglutamate.

After dissection from the slice the electroplaque was mounted in a two compartment lucite chamber (Fig. 1, left) similar to that described by Schoffeniels (1961), except that the electroplaque was placed horizontally, with its caudal, innervated, and electrogenically reactive membrane uppermost. The horizontal position made it easier to introduce microelectrodes under visual observation. The cell was held between an array of thin mylar plates which were furnished with windows of appropriate size. When the plate array was clamped between the two main portions of the chamber, the electroplaque formed the only pathway for the exchange of material between the upper and lower compartments, each of which was filled with solution. The medium contained in either compartment could be changed independently. The experiments were performed at room temperatures, *ca.* 22°C.

Voltage Clamping The application of clamping currents through an intracellular electrode (Hodgkin *et al.*, 1952; Moore and Cole, 1963) was impractical in the present case because of the morphological and electrophysiological characteristics of the cells (*cf.* Grundfest, 1957 *a*). The electroplaques are roughly rectangular wafers. Their two major surfaces are up to 20 to 25 mm long and up to 2 mm wide. The thickness of the wafer varies in different regions from as little as about 10 or 20 μ up to about 300 μ, the thickest being the sites of long papillae which are given off at the rostral surface. The membrane resistance is very low, 3 to 6 ohm cm^2 in the caudal, reactive surface and about 0.1 ohm cm^2, or perhaps less, in the electrogenically inert rostral surface. The length constant is well below 1 mm. On the basis of measurements of the total current output (Cox *et al.*, 1946), the peak current density during a response was estimated to be of the order of 50 ma/cm^2. In fact, the inward current observed in the present work ranged between 35 and 91 ma/cm^2 (Table I).

By virtue of the same morphological and physiological characteristics of the electroplaques, however, the clamping current could be applied through external electrodes, one each in the upper and lower compartments of the chamber (Fig. 1, right). Large currents could be passed in either direction across the low resistance,

[1] In millimoles per liter, 169 NaCl; 5 KCl; 3 $CaCl_2$; 1.5 $MgCl_2 \cdot 6H_2O$; 1.2 Na_2HPO_4; ca. 0.3 NaH_2PO_4, adjusted for pH 7.0.

electrogenically inert rostral surface without effect on this membrane. Thus a current flowing in the rostro-caudal direction behaved essentially as an outward current depolarizing the electrogenically reactive caudal membrane, while a current in the opposite direction was inward and hyperpolarizing for the caudal surface.

The characteristics of the electroplaques make rather stringent demands upon the clamping circuitry. Because of the low resistance and high capacitance of the mem-

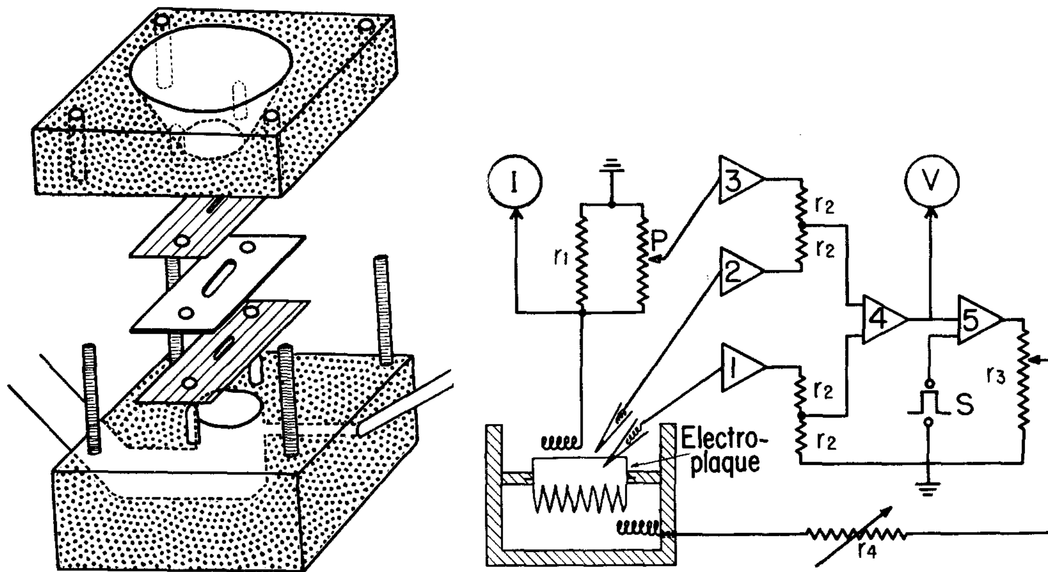

FIGURE 1. Diagrams of the two compartment chamber (left) and of the voltage-clamping circuit (right). The two main sections of the chamber are of lucite, fitted with accurately machined locating pins and clamping bolts. Three mylar diaphragms are shown. The electroplaque was positioned on one and the assembly of diaphragms was clamped between the two parts of the chamber. The major functions of the components of the clamping circuit are described in the text. The current-limiting resistors r_3 and r_4, 50 kohms and 5 kohms respectively, were reduced to zero as the circuit was balanced for the initial condition of zero current.

brane (*ca.* 15 to 50 μf/cm^2; Keynes and Martins-Ferreira, 1953; and the present work), we were not always successful in eliminating an early oscillatory capacitative current (Fig. 3). However, although the capacitative current was large, its duration was brief so that the artifact did not result in a serious error.

The current of the differential output amplifier (Fig. 1, No. 5; Tektronix model 54/53D-132) was limited to about 10 ma. The whole caudal surface of an electroplaque has an area of about 0.2 cm^2 and to clamp it at the peak of inward current would have required up to about 20 ma. It was accordingly technically simpler to limit the area of the electroplaque which was exposed as the "window" between the two compartments of the chamber. All the windows used in the experiments had one of three openings with areas of 0.022, 0.044, and 0.065 cm^2, respectively. It was

estimated that spread of excitation laterally into the unclamped portions of the cell may have introduced an error which was no greater than 10 per cent.

The membrane potential across the caudal surface was monitored through a pair of microelectrodes which straddled the caudal membrane (Fig. 1). These electrodes were connected to the inputs of the neutralized capacity amplifiers 1 and 2 (Amatniek, 1958). The voltage drop across the series resistance of the fluid which separated the two tips could be appreciable, since the current density was high and the resistance of the membrane was low. This IR drop was compensated by a circuit similar to that of Hodgkin *et al.*, (1952). Full compensation was only rarely possible, since the circuit then tended to become oscillatory. The summed output was fed to a differential amplifier (No. 4) from which the monitor of the control voltage (V) was taken to one trace of a cathode ray oscillograph, as well as feeding one input of the differential output amplifier (No. 5). The second input to this amplifier received the command signal (S). The current (I) was measured as the voltage drop across r_1.

The present work deals only with the properties of the electrically excitable membrane. Electrical stimuli, however, can also excite the nerve fibers which remain on the caudal surface of the cell even after the dissection, and this can give rise to an excitatory postsynaptic potential and to an indirect spike (Altamirano, Coates, and Grundfest, 1955). The neurally evoked responses were therefore blocked by applying *d*-tubocurarine in many of the experiments. The doses employed (0.1 to 50 μg/ml) were not large enough to affect the electrically excitable membrane (Altamirano *et al.*, 1955). The *d*-tubocurarine was usually applied only to the caudal surface.

RESULTS

About 100 electroplaques were studied in the course of the work and in some cases up to 5 or 6 experiments were performed on a single cell. Various electrophysiological data with current and voltage clamp methods taken from 13 experiments are summarized in Table I. The resting potentials were between -64 and -96 mv, the average (-84.9) lying within the range reported by other workers (Altamirano, 1955; Altamirano, Coates, and Grundfest, 1955; Keynes and Martins-Ferreira, 1953). The time constant of the membrane ranged between 57 and 180 μsec., the average value (114 μsec.) being somewhat higher than that (75 μsec.) reported by Keynes and Martins-Ferreira (1953). The mean critical firing level was a depolarization by about 27 mv. Stronger pulses which depolarized the caudal membrane further caused spikes to rise earlier, and with a vanishingly brief latency (Fig. 2), but strong currents applied in the opposite direction did not elicit the directly evoked responses (Altamirano, Coates, and Grundfest, 1955).

The effects of depolarizing currents of various strengths on the membrane potential and resistance are shown in Fig. 2. The resting membrane resistance in this experiment was less than 4.5 ohm cm^2. In response to a brief, slightly suprathreshold current the electroplaque developed a spike lasting about 1.2 msec. and 144 mv in amplitude (A). When spikes were evoked by

TABLE I

DATA OBTAINED WITH CURRENT CLAMP AND VOLTAGE CLAMP MEASUREMENTS ON A REPRESENTATIVE SAMPLE OF ELECTROPLAQUES. ALL MEASUREMENTS MADE IN CONTROL SALINE MEDIUM

τ = time constant of the membrane. RP = inside negative resting potential. Spike duration measured at 90 per cent repolarization. Threshold = critical firing level for spike electrogenesis. G_L = membrane conductance for small displacements of the potential from the resting value. G_K = membrane conductance calculated from the limiting slope for large depolarizations. G_{Na} = membrane conductance during inward current. I_{Na} = peak inward (sodium) current, corrected for leak current. E_{Na} = inside positivity for reversal of sodium current.

				Current clamp spike				Voltage clamp							
Cell No.	Window area	τ	RP	Amplitude	Duration	Threshold	Rate of rise	G_L	G_K	G_K/G_L	G_{Na}	$G_{Na}G_L$	I_{Na} peak at		E_{Na}
	cm² × 10⁻³	μsec	mv	mv	msec	mv	v/sec	mho/cm²			mho/cm²		ma/cm²	mv	mv
26	33	—	88	112	2.5	32	336	0.69	0.32	0.46	0.62	0.9	−64	−36	77
27	33	140	82	128	3.8	28	306	0.43	0.17	0.39	0.94	2.2	−85	−15	75
28	33	57	72	124	3.3	28	228	0.25	0.11	0.44	0.37	1.5	−37	3	101
34	65	73	88	152	2.9	18	619	0.59	0.12	0.20	0.82	1.4	−91	−40	84
35	65	103	96	134	2.9	20	360	0.36	0.12	0.33	0.90	2.5	−67	−45	37
36	65	120	88	128	2.9	26	520	0.29	0.13	0.45	1.25	4.3	−52	−22	26
38	65	116	96	145	2.9	28	290								
41	65	180	76	138	2.5	28	352	0.67	0.09	0.13	0.86	1.3	−86	−26	73
42	33	103	96	146	3.0	26	428								
47	33	120	84	142	3.1	30	330	0.55	0.08	0.15	0.94	1.7	−74	−17	75
50	33	—	88	152	3.3	24	440	0.17	0.08	0.47	0.49	2.9	−45	−24	85
51	22	150	64	148	1.9	32	480	0.19	0.10	0.53	0.36	1.9	−35	−18	82
53	22	88	86	135	1.5	20	304	0.23	0.16	0.69	0.72	3.1	−67	−12	84

current pulses of increasing strengths and each lasting 7 msec. (*B-E*), the peak voltages increased as shown on the graph by open circles and the dotted line. The slope of this line represents a resistance of about 1.6 ohm cm², indicative of the increased conductance which causes spike electrogenesis. However, the falling phases of the spikes now terminated on a plateau which grew higher with higher currents (filled circles and heavy line). The slope of the line indicates a dynamic resistance of about 7.5 ohm cm². Thus, these data confirm the conclusion (Altamirano, 1955) that depolarization causes an increase in the membrane resistance of the electroplaques.

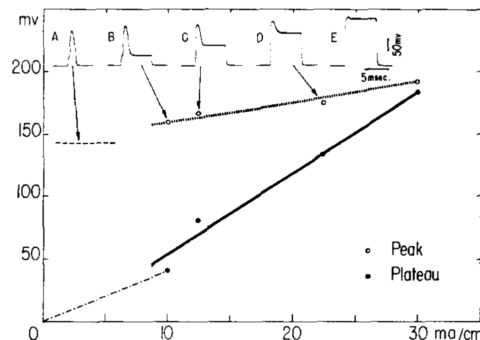

FIGURE 2. Changes in membrane resistance during passage of depolarizing currents *A*, a spike was elicited by a pulse lasting 0.1 msec. The peak (144 mv) is shown by the horizontal broken line in the graph below. *B-E*, longer lasting pulses elicited the spikes. The peak voltages are shown by the open circles and dotted line and the terminal plateau by the filled circles and heavy line. The resting resistance of the membrane (dot-dash line) was approximately 4.5 ohm cm². Further description in text.

Voltage clamp data provide unequivocal evidence that depolarization eventually causes the membrane resistance to rise above the resting value (Figs. 3 and 4). Record *A* of Fig. 3 shows the spike of the electroplaque evoked by a depolarizing stimulus lasting about 0.7 msec. It had an overshoot of 49 mv and a total amplitude of 135 mv. In records *B* and *C* are shown the changes in current under voltage clamp conditions when the caudal membrane was hyperpolarized to two different levels. Effects of depolarizing the membrane are shown in records *D* to *I*. Since the cell had been curarized to eliminate neural responses, the second component of inward current in *F* was probably caused by local differences in responsiveness of the membrane (*cf.* Altamirano, Coates, and Grundfest, 1955). With a still larger depolarization (*G, H*) only an early large inward current was observed. It has a peak value of about 60 ma/cm² (uncorrected for "leak" current) in this cell (Fig. 4) and lasted somewhat more than 1 msec. (Fig. 3*G*). As in the case of squid axons (Hodgkin and Huxley, 1952) the inward current diminished (*H*) and disappeared (*I*) with further depolarization. A striking result of the voltage

clamp data on the eel electroplaques is the absence of the delayed increase in outward current which has been ascribed to K activation (Hodgkin and Huxley, 1952). In fact, there was a little change in the late outward current for rather large changes in membrane voltage (Fig. 3*E-I*).

The complete data of this experiment are plotted in Fig. 4 in the conventional voltage clamp presentation with membrane potential on the abscissa and current as the ordinate. Hyperpolarization of the caudal membrane by more than 125 mv from its resting level (−87 mv) resulted in a

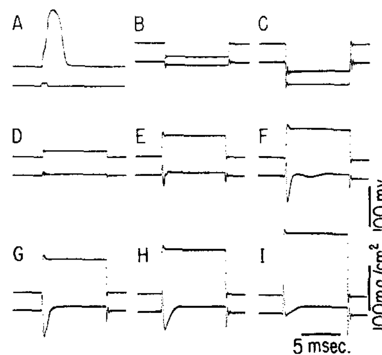

FIGURE 3. Voltage clamp measurements in an eel electroplaque. Initial resting potential −87 mv. *A*, the spike response of the cell to a brief depolarizing stimulus prior to the voltage clamp measurements had an overshoot of 49 mv. *B*, *C*, inward currents in response to clamping the membrane at two levels of hyperpolarization. Note the brief high frequency oscillation at the beginning and end of the pulses. Note that the voltage trace crosses the current trace in *C*. *D*, outward current during a weak depolarization. *E*, a small brief early inward current appeared with a stronger depolarization. It increased markedly with still further depolarizations (*F*, *G*). The late inward current in *F* probably represents delayed excitation of a portion of the membrane. *H*, with still stronger depolarization the early inward current began to diminish and was almost abolished at a depolarization to +50 mv. Note that the plateau of the later phase of outward current increased less than double for the threefold increase in membrane potential between *E* and *I*.

linear (ohmic) increase of the inward current. In this range therefore there was no hyperpolarizing activation or inactivation (Grundfest, 1961). The slope of the line drawn through the resting potential indicates a membrane resistance of 4.2 ohm cm^2, in good agreement with the values of earlier workers (Keynes and Martins-Ferreira, 1953; Altamirano, 1955; Altamirano and Coates, 1957). With larger hyperpolarization there was a time variant decrease in current, an indication of hyperpolarizing inactivation, which gives rise to hyperpolarizing responses during the application of constant current stimuli (Grundfest, 1961). The hyperpolarizing responses of eel electroplaques will be described elsewhere.

When the membrane potential was changed in a positive direction by 30 mv a flow of inward current was triggered, as in other spike-generating cells, except that the current density at the peak was very high. The potential at which the initial current ceased to be inward was +62 mv. The peak slope conductance in the negative slope region was about 2.5 mho/cm². The

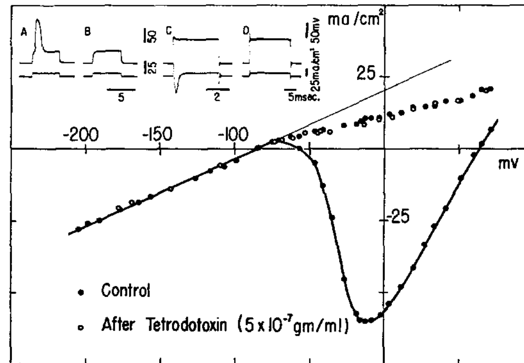

FIGURE 4. Voltage clamp measurements showing absence of delayed activation and the occurrence of depolarizing inactivation. Same experiment as in Fig. 3. Filled circles represent the full data which are sampled in the records of Fig. 3. The initial inward current (not corrected for the leak current) commenced at a depolarization of about 25 mv and increased with increasing depolarization to a peak of about 60 ma/cm². The late outward current, after the inward current had subsided, was smaller than would be expected for an ohmic resistor (thin line) indicating that the depolarization was eliciting an inactivation process. Note the absence of the delayed activation which is seen in squid giant axons and other cells. After this series of measurements the cell was poisoned with tetrodotoxin. The response causing the inward current component was eliminated, but the inactivation process remained unaffected (open circles). The insets show some additional records from this experiment. *A*, before tetrodotoxin a current pulse evoked a spike and a subsequent depolarization as in Fig. 2. *B*, after tetrodotoxin the same current no longer elicited a spike, but the level of the depolarization at the plateau was unchanged. *C*, *D*, voltage clamp records respectively before and after treating the electroplaque with tetrodotoxin. The inward current was abolished in the poisoned cell, but the amplitude of the outward current was not affected. Note that the amplitude rapidly reached its steady level, indicating that K inactivation is rapid.

conductance in the positive slope region was 0.93 mho/cm², as compared with about 0.23 mho/cm² for the resting cell.

The steady state currents measured at 5.5 msec. after the beginning of the voltage pulses, or well after the inward current component had subsided, did not show the marked increase which is associated with K activation (Hodgkin and Huxley, 1952). Instead of increasing the outward current, depolarizations of 30 mv and more caused the current flow to decrease below the value of the "leakage current" expected for an ohmic resistor. The chord conductance (0.13 mho/cm²) fell to half the resting value.

The initial inward flow of current was eliminated by introducing the puffer fish poison, tetrodotoxin,[2] into the upper compartment of the chamber (Fig. 4). Neither the resting conductance nor the decrease in outward current on depolarization was affected (open circles). The mussel poison[3] (Schantz, 1960) acts in the same way as does tetrodotoxin, and at about the same level of concentrations (10^{-7} to 10^{-6} gm/ml). That the initial inward current which is blocked by tetrodotoxin or the mussel poison is due to influx of Na in eel electroplaques is demonstrated by the fact that the inward current was also eliminated when choline chloride was substituted for the NaCl of the

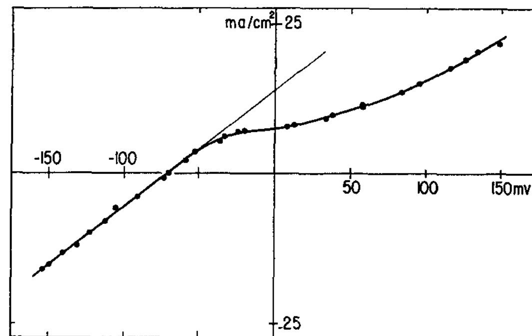

FIGURE 5. Elimination of the inward current component by removal of Na from the medium. The resting potential was −71 mv and the NaCl was replaced with equimolar choline chloride. The resting membrane resistance of the cell was 5.2 ohm cm² (thin line). Depolarizing clamping voltages caused relative diminution of the current. The chord resistance at zero membrane voltage was 9.8 ohm cm².

external medium (Fig. 5). Only the saline of the upper chamber was displaced by the choline saline in this experiment. An indication of the ion involved in the inactivation process during depolarization is given by the data of Fig. 6. The limiting slope of the voltage-current relation in the tetrodotoxin-poisoned cell extrapolated for zero current to the initial resting potential. Thus, it appears likely that the depolarization caused potassium inactivation. Other experiments which confirm this conclusion are described below.

In the cells in which the inward current was eliminated by mussel poison, by tetrodotoxin, or by removal of Na, the "delayed" effect of the depolarization on the steady state current persisted. Thus, the membrane component which is involved in the K inactivation process is pharmacologically distinguishable from that which is normally involved in Na activation.

Figs. 4 to 6 show further that elimination of the initial inward current

[2] Tetrodotoxin was obtained from the Sankyo Co., Tokyo.

[3] We are indebted to Dr. E. J. Schantz (United States Army Biological Laboratories) for the pure sample of mussel poison.

leaves only a single non-linearity in the voltage-current relation obtained with voltage clamp data. It may therefore be expected that the reciprocal ("current clamp") measurements with current as the independent variable would also yield the same relation. The data in the lower graph of Fig. 6 fulfill this expectation. They were obtained on the same cell as were the voltage clamp measurements of the upper graph, but they represent current clamp measurements. The resting membrane resistance was 5.9 ohm cm² and on applying depolarizing currents it shifted to a maximum of 9.3 ohm cm². The limiting slope of the current-voltage relation for strong depolarizations also extrapolated to the resting potential at zero current.

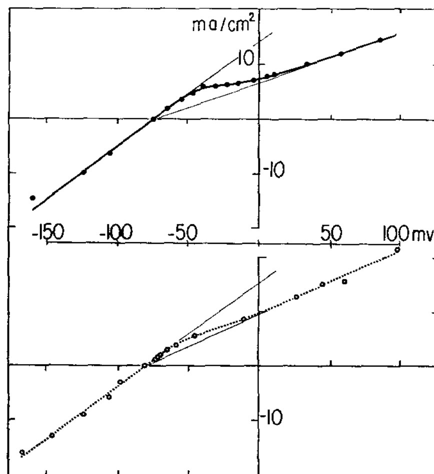

FIGURE 6. Current-voltage relations in an electroplaque poisoned with tetrodotoxin. *Above,* voltage clamp measurements. *Below,* current clamp measurements. The resting potential was −77 mv. The resting resistance was 5.9 ohm cm². On depolarization the resistance increased to 12.2 ohm cm² in the voltage clamp measurements and to 9.3 ohm cm² in the current clamp data. Note that the limiting slopes of both high resistance branches extrapolate to the resting potential as the origin.

An increase in resistance when outward currents were applied was observed also in electroplaques which had been depolarized by exposure to high KCl (Altamirano and Coates, 1957). Thus, depolarizing inactivation may be expected to persist even in the presence of high K. Fig. 7 which shows voltage clamp data on a cell that was exposed to a Na-free isosmotic KCl medium confirms this. In the KCl medium the resting membrane resistance was 1.3 ohm cm². During depolarizing currents it increased to about 5 ohm cm². The voltage current characteristic exhibits a clear cut negative slope region.

A second feature illustrated in Fig. 7 is that the transition from high to low conductance occurred on depolarizing the cell by about 20 to 30 mv, as in the electroplaques of Figs. 4 to 6. However, the resting membrane potentials in the latter experiments ranged from about −90 to −70 mv, whereas in the experiment of Fig. 7 it was −27 mv. Thus, the inactivation is not wholly dependent on the absolute membrane potential but results from a change in the potential relative to the steady state condition. A third aspect seen in the data of Fig. 7 is that the line describing the limiting slope of the low conductance condition extrapolates back for zero current very close to

the resting value of the membrane potential. This finding indicates that the fourfold change in conductance involves only or mainly the K component.

All these effects are also observed when the medium is enriched with a K salt of an impermeant anion. In Fig. 8 are voltage clamp data on an electro-

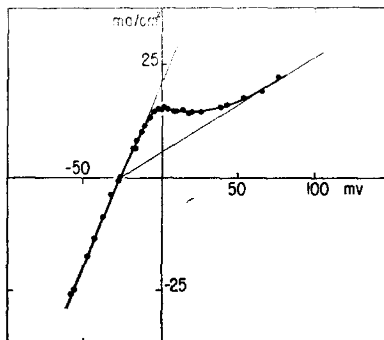

FIGURE 7. Voltage current measurements in an electroplaque which was bathed in isosmotic KCl. The resting potential decreased to −27 mv. The resting resistance also decreased because of the high K medium, becoming 1.3 ohm cm². Further depolarization by 20 mv and more caused an increase in the membrane resistance to a peak value of 5 ohm cm², for depolarizations to +50 mv or more. Note that the line of this limiting slope extrapolates back to the resting potential. Note also the marked negative slope characteristic in the transition from the low to the high resistance values

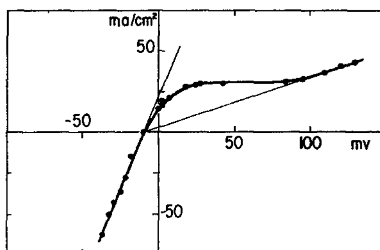

FIGURE 8. Voltage clamp data on a cell bathed in isotonic K acetate The resting potential was −9.6 mv and the resting resistance, measured from the data in the hyperpolarizing quadrant, was 0.45 ohm cm². With further depolarization of the membrane the resistance increased to 3.3 ohm cm². Note that the limiting slope for the high resistance state extrapolates to the resting potential.

plaque which had been depolarized in an isotonic K-acetate medium. The membrane was more strongly depolarized in this cell than in that of Fig. 7 and the resting resistance was also lower. The membrane conductance (*ca.* 1.9 mho/cm²) began to decrease with further depolarization. A negative slope characteristic developed with the membrane inside-positive by about 20 mv and the limiting slope of the low conductance branch also extrapolated to the initial zero current point.

In the experiment of Fig. 9 are shown the voltage clamp and current clamp data for a cell which was bathed in isotonic K-pyroglutamate. The resistance determined in the high K medium from the current clamp measurements (open circles) appeared to be lower (0.44 ohm cm²) than that (1.08 ohm cm²) determined from the voltage clamp data (filled circles). The difference may reflect changes of membrane properties introduced by the exposure of

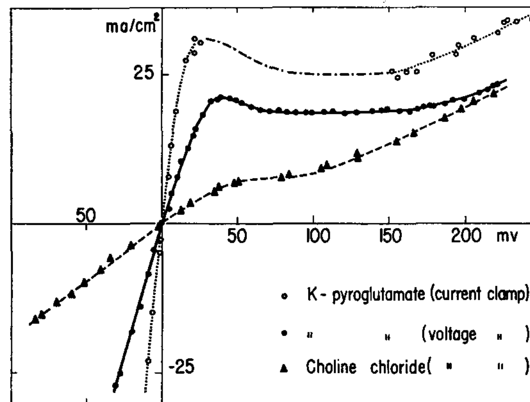

FIGURE 9. Voltage current curves under different conditions. *Open circles*, current clamp measurements on an electroplaque, bathed in isotonic K-pyroglutamate. *Filled circles*, voltage clamp measurements on the same electroplaque. The resting potential was −14 mv in both. The resting resistances were 0.44 ohm cm² and 1.08 ohm cm² respectively. In the current clamp experiments the negative slope region was a forbidden zone, the potential shifting spontaneously from about +25 mv to about +160 mv as the resistance increased to 8 ohm cm² while the current remained constant. The negative slope characteristic is also shown in the voltage clamp data when the membrane resistance increased to 9.6 ohm cm². The triangles and broken curve show the experiment of Fig. 5, in which the cell was bathed in isotonic choline chloride, but with the origin at the resting potential (−77 mv) shifted to the right by 63 mv. Note that the depolarizing inactivation begins at nearly the same place on the graph for all 3 experiments. Also the limiting slopes for the 2 sets of voltage clamp measurements are nearly the same.

the membrane to very heavy currents. For comparison the data of Fig. 5 are also included, replotted so that the initial zero current points of all 3 experiments coincide.

All 3 sets of measurements gave similar values for the membrane resistance in its high resistance state, which ranged between 8 and 10 ohm cm². A peak of current outflow occurred for depolarizations of 30 to 50 mv from the respective resting potential levels, and the peaks were particularly marked for the electroplaque which had been exposed to the high K medium, so that the curves for still stronger depolarizations exhibited a clear cut negative slope characteristic. Indeed, under constant current conditions the negative slope region was a forbidden zone. No measurements could be made in this

part since the membrane potential shifted abruptly toward the more positive value characteristic of the high resistance state.

The instability which is shown in the current clamp data of Fig. 9 in the range of the negative slope characteristic is further illustrated with the

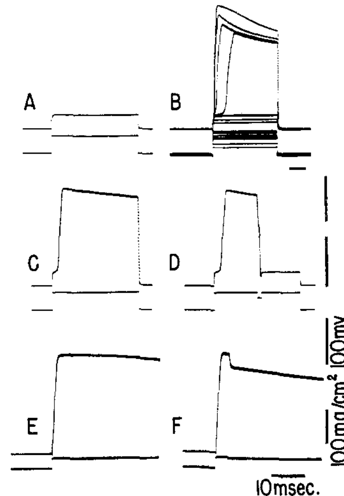

FIGURE 10. Depolarizing inactivation responses of two eel electroplaques. *A-D*, cell in isosmotic KCl. Resting potential −10 mv. The resistance for a depolarization below about 34 mv (*A*) was 1.0 ohm cm², and increased to about 6 ohm cm² with stronger depolarizing current (*B*). The change, registering as an increase in membrane potential, occurred slowly with a just threshold current, and more rapidly with stronger currents. The peaks of the inactivation responses increased with increasing currents. Note that the potential tended to decay approximately exponentially during the course of the 40 msec. pulses. *C*, a subthreshold current which lasted 25 msec. (as in *A*) was augmented briefly by a superimposed additional pulse of current lasting 1 msec. This was sufficient to trigger the depolarizing inactivation response. *D*, same as in *C*, except that another 1 msec. pulse, but of inward current, was also applied halfway during the longer lasting outward current. The response initiated by the first brief pulse was now abolished. *E-F*, another electroplaque bathed in isotonic K-pyroglutamate. Resting potential −14 mv. The applied pulse initiated a depolarizing inactivation response which fell slightly during the duration of the sweep (*E*). A sudden diminution of the pulse caused a rapid decrease in the membrane voltage (*F*). The decay of the voltage was also accelerated. The 10 msec. time scale for *B* is shown in the lower right of the record. Current and voltage calibrations for *A-D* are to the right of record *D*.

records of Fig. 10, taken from 2 different experiments. The cells were in an isosmotic KCl (*A–D*) or K-pyroglutamate (*E*, *F*) medium. When the depolarizing current changed the membrane potential by 34 mv there occurred a further change in membrane potential. This change developed more rapidly with stronger applied current (*B*). It was triggered in all-or-none fashion by superimposing a brief pulse on a "subthreshold" depolarizing

current (*C*) and was abolished by a subsequent brief hyperpolarizing pulse (*D*). The peak voltage was dependent upon the level of the depolarizing current (*E*, *F*). Although the high resistance state of the strongly depolarized membrane persisted as long as the current was applied (40 msec. in record *B*), there appeared to be a secondary effect which resulted in a slow decrease in the potential. This phenomenon requires further investigation.

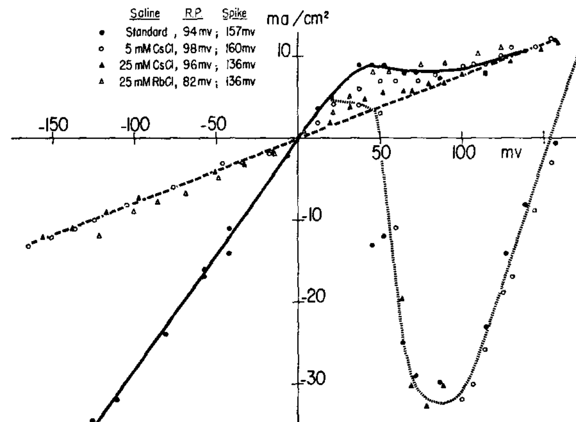

FIGURE 11. Pharmacological K inactivation induced by Cs or Rb. Voltage clamp data on a single electroplaque. The data are plotted with the initial membrane potentials coincident. Filled circles are for the cell in the standard medium. Open circles represent measurements made 5 to 10 minutes after the saline solution was changed to one in which 5 mM/liter CsCl replaced the KCl. The resting potential and spike amplitude (shown in the inset) were unchanged, as was also the initial component of inward current flow. The resistance in the hyperpolarizing quadrant rose to the same high value as that which developed during depolarizing K inactivation. The negative slope characteristic in the depolarizing quadrant became less prominent. It was almost abolished in the presence of 25 mM/liter CsCl (filled triangles). Substitution of 25 mM/liter RbCl for the CsCl did not affect the resistance in the hyperpolarizing quadrant, but the negative slope characteristic became more prominent again. The initial inward current which was diminished probably due to the depolarization caused by the RbCl, is not shown.

The foregoing data have demonstrated that the negative slope characteristic is due to a depolarizing inactivation response of the K conductance system. It may be expected, therefore, that pharmacological K inactivation (Werman and Grundfest, 1961; Grundfest, 1961, 1965) would raise the membrane resistance for inward as well as outward currents. If the pharmacological inactivation were complete, the I-E relation would become linear and the resistance would be uniform and high for all levels of measurement (Grundfest, 1961). In a search for various agents that might act selectively on the K conductance system, it was found that substitution of the K in the normal medium with Cs or Rb produced the effects shown in Fig. 11. The resistance measured with inward currents was immediately elevated to the

same value as that which prevails during depolarizing K inactivation. The resting membrane potential was almost unaffected. The Na conductance system also was unaffected and the spikes evoked by brief depolarizing currents were essentially unchanged. As measured with depolarizing voltage pulses, the K conductance system was at first also little affected by the sub-

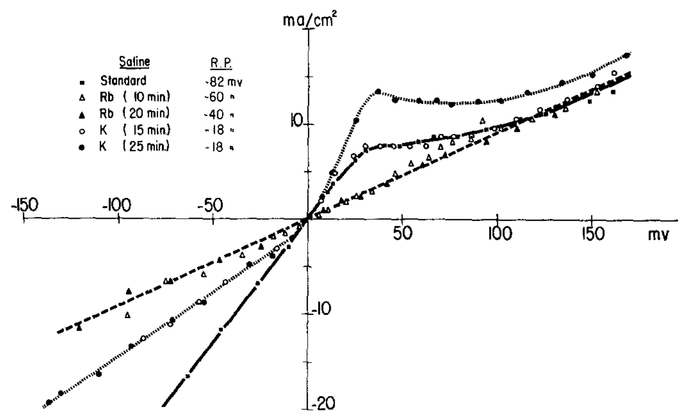

FIGURE 12. Reversibility of pharmacological inactivation. Voltage clamp data on a single electroplaque, the initial membrane potentials (shown in the inset table) being made the origin. The initial inward current component which developed in the control measurements (not shown) was eliminated on substituting a saline solution containing 174 mM/liter RbCl. Within 10 minutes after the change the I-E relation became linear and was not affected by further exposure to the RbCl medium. The slope of the I-E relation was the same as that in the depolarizing quadrant when K inactivation had increased the resistance in the control measurements. The electroplaque depolarized markedly on exposure to an isosmotic saline solution containing 174 mM/liter KCl. The membrane resistance fell, but in the hyperpolarizing quadrant it remained higher than in the initial condition, and much higher than when cells were exposed to high K media only (Figs. 7 to 9). In the depolarizing quadrant the I-E relation at first coincided with that of the control condition (open circles), but on continued exposure to the high KCl medium the cell developed the marked negative slope characteristic which was seen in Figs. 7 to 9. Note that in this experiment also, the shift from high to low conductance appears to be triggered by a change in voltage which is approximately constant, irrespective of the initial resting potential.

stitution of 5 mM CsCl for the KCl. After prolonged exposures to Cs (or Rb), and more readily to higher levels of these cations, the I-E relation became linear over the whole range of measurements, the resistance becoming uniformly high. The Na conductance system was affected relatively little, except through the effect that the cells were becoming depolarized or Na was diminished. On replacing the 25 mM CsCl with RbCl the resistance in the hyperpolarizing quadrant was not changed, but in the depolarizing quadrant the negative slope characteristic was again in evidence. The effect is presumably due to differences in the kinetics of the actions of these cations which

are presently under study. The ultimate effect of Rb is likewise to produce pharmacological K inactivation for outward as well as inward currents and consequent linearization of the I-E relation (Fig. 12).

The experiment of Fig. 12, in which the Na as well as the K had been replaced with Rb, also presents some data on the reversibility of the pharmacological inactivation. Replacement of the Rb with K caused a rapid depolarization to a steady level of −18 mv. The resistance in the hyperpolarizing quadrant fell, but even after 25 minutes' exposure to 174 mM/liter KCl the resistance was still high relative to the initial level, and much higher than the resistance of cells which were exposed only to high K (Figs. 7 to 9). In the depolarizing quadrant, the conductance increased for low values of depolarization and decreased again for large depolarizations. After 15 minutes in the KCl medium the curve was identical with that of the control, except that the initial membrane potential was markedly different. The negative slope characteristic became more prominent after longer exposure to the high KCl, because the conductance for low depolarizations was increasing. However, the change in membrane potential at which the negative slope became evident was about the same as in the control.

DISCUSSION

The Membrane Processes during the Spike Electrogenesis Among other things, the foregoing data provide evidence for the view (Keynes and Martins-Ferreira, 1953) that the spike electrogenesis of eel electroplaques, like that of squid axons (Hodgkin and Katz, 1949), is dependent on the presence of Na. When the latter is removed from the medium, or when the cell is poisoned with tetrodotoxin or the mussel poison, the inward current which causes the depolarizing electrogenesis is abolished (Figs. 3 to 6). However, the phase of delayed increased conductance due to K activation (Hodgkin and Huxley, 1952) which is prominent in spike electrogenesis of squid axons and other cells is absent in eel electroplaques.

Since the repolarizing electrogenesis of K activation is absent, the spikes of eel electroplaques are terminated by Na inactivation only. The fact that the latter process does occur is indicated not only by the subsidence of the inward current during sustained depolarization (Fig. 3), but also by the absence of an inward current when the cells are subjected to depolarization in media with high K (Figs. 7 to 9 and 12), when spike electrogenesis is abolished (Fig. 10; *cf.* also Altamirano and Coates, 1957). Spike electrogenesis is also abolished in eel electroplaques as a consequence of the depolarization which is caused when acetylcholine or other agents activate the excitatory synapses (Altamirano *et al.*, 1955). The Na inactivation process obviously has a rapid time course during spike electrogenesis, since the inward current phase in voltage clamp experiments ends within 0.5 to 1.0 msec. (Fig. 3). The detailed kinetics of Na inactivation will be the subject of another report.

The membrane of the electroplaques undergoes a secondary change to increased resistance in the steady state, no matter whether there is spike electrogenesis (Figs. 2 to 4 and 11), or whether the latter is eliminated by a variety of means (Figs. 3 to 10 and 12). This increase is independent of the nature of the anion (Figs. 7 to 10). The increase occurs rapidly, appearing early during the falling phase of the spike (Fig. 2), and when spike electrogenesis is eliminated the rapidity of the increase in resistance is beyond the time resolution of the voltage clamp (Fig. 4, record *D*). The increase indicates the occurrence of depolarizing K inactivation (Grundfest, 1957 *a*, 1960, 1961). K inactivation appears to be a rather general phenomenon (Grundfest, 1961, 1965), although it is usually masked by occurrence of K activation. The absence of the latter process in eel electroplaques discloses the depolarizing K inactivation most clearly and directly. However, the inactivation process, and an analogous one of hyperpolarizing inactivation have also been observed in many other cells (Grundfest, 1961, 1963, 1965).

Depolarizing K inactivation occurs in skeletal muscle fibers of crayfish (Reuben and Gainer, 1962) and frog (Nakajima *et al.*, 1962). Anomalous rectification (Katz, 1949; Adrian and Freygang, 1962; Adrian, 1964) and "upside down" responses of frog muscle fibers involve depolarizing K inactivation, with hyperpolarizing K activation also playing a role in the former (Grundfest, 1961, 1965). The presence of depolarizing inactivation as a component in spike electrogenesis of vertebrate cardiac muscle (Grundfest, 1957 *a*, 1957 *b*) has been confirmed by a number of investigators (Carmeliet, 1961; Hall *et al.*, 1963; Deck *et al.*, 1964; Deck and Trautwein, 1964; Hecht and Hutter, 1965). For example, the records of Fig. 10*B* of the present paper are comparable to those of Fig. 4 in Deck, Kern, and Trautwein (1964) and the presence of K inactivation is also shown clearly in the voltage clamp data of Fig. 2 in Deck and Trautwein (1964). Voltage clamp analyses have also disclosed the presence of K inactivation in supramedullary neurons of puffer (Nakajima and Kusano, 1963, and data to be published) and squid giant axons (Ehrenstein and Gilbert, 1964). As already noted, both depolarizing and hyperpolarizing K activation which occur in electroplaques of various weakly electric gymnotids have been analyzed with voltage clamp techniques (Bennett and Grundfest, 1962 *a*, 1962 *b*, 1965).

Some Inferences Regarding the Structure of the Electrically Excitable Components of the Membrane The present data provide some rather decisive clues as to the structure of the permselective and reactive membrane which have considerable bearing on theoretical aspects of bioelectrogenesis and on the character of models that are designed to explain membrane properties. A basic postulate of the Hodgkin-Huxley theory (1952) is the existence of separate conductance branches for the reactive systems involving Na and K. However, as has been emphasized by Finkelstein and Mauro (1963), much

of the theoretical treatment of membrane properties (*e.g.*, Goldman, 1943; Hodgkin and Huxley, 1952; Hodgkin and Katz, 1949; Teorell, 1953, 1962) implicitly or explicitly assumes that the membrane is a homogeneous system.

Evidence for the pharmacological specificity and independent occurrence of Na and K activation (Grundfest, 1961, 1963) has recently been strongly reenforced with the demonstration by voltage clamp measurements that tetrodotoxin and the mussel poison act solely on the Na conductance system of certain electrically excitable membranes. Block of Na activation by tetrodotoxin without affecting K activation in frog muscle fibers was indicated by the work of Narahashi *et al.* (1960) and Nakajima *et al.* (1962). The latter workers also found that depolarizing K inactivation of the muscle fibers is likewise unaffected by the toxin. Voltage clamp data on lobster axons (Narahashi *et al.*, 1964) and on squid giant axons (Nakamura *et al.*, 1964 *b*, 1965) and eel electroplaques (Nakamura *et al.*, 1964 *a*, 1964 *b*; and the present work) establish the selectivity of the action of tetrodotoxin and of mussel poison on the Na component alone.[4]

The membrane sites which are involved in the Na activation process of conductile membrane thus appear to have a different chemical configuration from those which are responsible for changes in K conductance. They must therefore also be presumed to be spatially different. This difference implies a heterogeneity in the cell membrane which may give rise to a number of consequences that are envisaged by electrochemical theory (*cf.* Höber, 1945; Carr and Sollner, 1964; Finkelstein and Mauro, 1963). The evidence for the existence of independently reactive Na- and K-permselective channels is also considerably strengthened by the finding that pharmacological K inactivation can eliminate the reactive K conductance system, without affecting the Na conductance component (Fig. 11).

The occurrence of K inactivation in eel electroplaques independently of the fact that K activation is absent gives rise to a further question, whether the activation and inactivation processes may also involve different sites. This question cannot be answered definitively by available data and is at present under further study. However, it would seem reasonable to assume that both processes operate within the same sites. Eel electroplaques have a very low membrane resistance and this may be conceived of as due to high permeability for K. In other words, the K channels are essentially in their "activated" (open) state under resting conditions and the sole or main change

[4] Neither agent, even in concentrations of 10^{-5} to 10^{-4} gm/ml affects the electrically inexcitable membrane components of receptor neurons, or of synapses (Grundfest, 1964). Furthermore, they do not block spikes which can be produced in the normally gradedly responsive muscle fibers of arthropods (Ozeki and Grundfest, 1965). These poisons thus act specifically upon normally spike-generating conductile membrane and only on the component of the latter which is involved in Na activation. Procaine, which has different actions in different types of cells, also blocks only the Na activation mechanism in eel electroplaques (unpublished data), whereas in the normally gradedly responsive arthropod muscle fibers procaine induces spikes (Grundfest, 1961).

is to the "inactivated" (blocked) state, when the channels become unreactive to stimulation.

While the kinetics of the K inactivation process have not yet been examined in detail, the available data indicate some striking features. Although the process is a response to depolarization, long lasting depolarization of the membrane by raising the K of the medium does not appear to initiate the inactivation process (Figs. 7 to 10, and 12), whereas Na inactivation does occur (Fig. 10). The K inactivation in strongly depolarized cells is initiated by electrical stimuli when the membrane becomes depolarized by about 30 mv (Figs. 7 to 10). This degree of change is also the threshold for inactivation in cells which have normal resting potentials (Figs. 4 to 6). A comparison is shown in Fig. 9 in which a common origin is given to data from a cell with a resting potential of −71 mv and from another in which the resting potential was about −14 mv. A similar comparison is made on a single cell in Fig. 12, when the membrane potential changed from −82 to −18 mv.

The implications of Figs. 11 and 12 are particularly noteworthy. By substituting Cs or Rb for K in the medium the resistance was raised to the same extent as it was by depolarizing K inactivation. However, in the presence of Cs or Rb the increase obtained for inward currents as well and, in fact, developed more quickly than for outward currents. Eventually the current-voltage relation became linear; *i.e.*, the membrane was no longer electrically excitable (Grundfest, 1961). However, the pharmacological K inactivation only affected the K channels and the Na channels remained responsive to stimuli (Fig. 11).

The increase in resistance due to depolarizing K inactivation is substantial, particularly in the electroplaques which are exposed to high K. It must be assumed therefore that most of the channels which are specifically permeable to K at rest can also react to the depolarizing stimuli. Since the resistance attained in the presence of Cs or Rb was also identical with that produced by depolarizing K inactivation (Figs. 11 and 12), it was the reactive, K-permselective channels which were eliminated by the Cs and Rb.

The channels which constitute the leak resistance cannot be specified definitively. However, it is noteworthy that the membrane resistance in the "high" state was nearly the same (*ca.* 10 ohm cm^2), no matter whether the cells were in a highly conducting (high K, Rb, or Cs) medium or not, as is also apparent (Grundfest, 1961) in the data of Altamirano and Coates (1957). This fact suggests that the leak resistance is in channels which are not readily permeable for the cations. However, it was also independent of the nature of the anions and other data (unpublished) indicate that the membrane is relatively impermeable for Cl. The leak resistance indicates that the membrane resistivity is of the order of 10^7 ohm cm. Thus, it is not beyond possibility that the leak current is carried by hydrated H^+ and/or OH^-.

Studies on the long term events following application of depolarizing currents to eel electroplaques have not yet been made. There is an indication (Fig. 10*B*) that the resistance decreases slowly, and probably exponentially, perhaps as a result of ionic changes which may occur during sustained flow of large currents. However, in neither the voltage clamp nor the current clamp data is there an indication that the low conductance state due to K inactivation is altered by increasing the potential or current still further, at least for levels at which the membrane is inside-positive by more than 200 mv. The electroplaques of *G. carapo*, *Hypopomus*, and *Steatogenys* also exhibit only the inactivation process (Bennett and Grundfest, 1965). However, in those of *Eigenmannia* strong depolarizing currents cause a secondary increase in conductance and their inactivation response is oscillatory during a prolonged outward current (Goldman and Grundfest, unpublished data).

While inactivation responses occur in electroplaques which are at their resting potential (Fig. 2), they become much more striking when the cells are strongly depolarized by exposure to high K. This effect results not from the absence of the spike electrogenesis, but from the larger disparity between the limiting values of the membrane resistance of the two states when the cells are depolarized. The increased disparity is due mainly to the changes in the resistance of the resting membrane in the presence of high K. Thus, the ratio of the high to low resistance values approaches 10 or 12 in the cells which have been depolarized with K (Figs. 7 to 10), while it is only 2 to 3 in cells within the normal range of resting potential (Figs. 2 to 6).

If the increased conductance in the K-treated cells were due to an increase in the number of available K channels, the change could be regarded as K activation. Since this process is absent as a response to depolarizing currents it seems likely that the increased conductance in the already open K channels results, at least in part, from the increased conductivity of the medium in the K-permeable channels when K replaces Na. The latter ion appears to be effectively impermeant in the resting cells, since the resting resistance is not markedly affected by the substitution of choline for Na (Fig. 5). However, we cannot rule out the possibility that K exerts a specific action to decrease the membrane resistance, perhaps through effects induced by a change in the ionic profile within the membrane. An indication of a possible effect is the persistence of high conductance to outward currents after the conductance for inward currents is diminished by low concentrations of Rb or Ca (Fig. 11).

The effects on membrane conductance of substituting various anions for Cl have not yet been examined systematically. It appears likely, however, (Figs. 7 to 9) that the nature of the anion may have little or no effect on the limiting slopes of the high and low conductance states, at least when the electroplaques are bathed in a strongly K-enriched medium. Nevertheless, it

must be noted that the over-all conductance of a system which contains several parallel elements with different characteristics may be quite complex. For example, the resistance characteristics at different membrane potentials of mammalian cardiac fibers are different when the muscle is bathed in a K-free medium depending on whether the anion is Cl or acetylglycinate. However, the resistance characteristic becomes almost independent of the anion when the muscle is exposed to 50 mM K and is thereby depolarized to about the same degree (Carmeliet, 1961).

The resistance increase during depolarizing inactivation is due to a change in the permeability only for K, and there is no change in the EMF of the system, like that which occurs at the expense of the potential energy of the cell during Na activation. Thus, in the sense that both are due to changes in permeability of the reactive membrane components, activation and inactivation responses have some degree of resemblance (Grundfest, 1961, 1965; Finkelstein, 1964). However, there is a basic difference in that spike production is a self-sustaining ("autogenetic") response, no matter whether it is due to Na activation, or to the movement of another ion in the appropriate sense, as when K spikes, Cl spikes, or Ca spikes are generated (Grundfest, 1961, 1963, 1965). In contrast, inactivation responses do not draw upon the potential energy of the cell, require the supply of extrinsic energy, and are terminated when the stimuli are ended (Figs. 2 and 10). In the sense that the transition from the high conductance to the low conductance state, or *vice versa* is regenerative (Fig. 10) inactivation responses are all-or-none, like the spike. However, the amplitude of the change in membrane potential is not independent of the stimulus, but is strictly related to the amplitude of the current which is supplied and maintained during the response (Figs. 2 and 10).

The Negative Slope Characteristic A negative slope characteristic is the common property of a large number of devices which exhibit triggered metastable states or oscillatory phenomena. Some of these devices, of quite different intrinsic components and mechanisms, have been proposed as models for the responses of electrically excitable membrane. The rather simple properties of the inactivation response of eel electroplaques permit the formulation of certain restrictions upon the varieties of models which are relevant to the problem, particularly in the context that excitable membranes operate through a mechanism, changes in permeability for specific ions, which is not as yet known in non-living systems.

Models which depend upon certain time-variant energy storage elements (capacity, inductance, thermal reservoirs) are immediately ruled out of consideration. The system responsible for inactivation responses is a voltage-determined increase in resistance due to a decreased permeability of the cell

membrane for only one ion species. Thus, as the voltage is increased in voltage clamp measurements there is normally a tendency toward an increased current. However, as the current-carrying channels become inactivated, the resistance also increases with the increasing voltage and in some cases the rate of the latter change is the more rapid. Thus, the net current flow decreases markedly and the I-E relation then develops a negative slope. Under current clamp conditions the resistance increase results in an increased IR drop across the membrane. The interaction between the increase in membrane potential and the increase in membrane resistance then is a regenerative effect that causes the all-or-none inactivation response. It seems likely that the relative kinetics of the ohmic, positive slope effect (a rise in current with voltage), and of the negative slope effect (the reactive increase in membrane resistance), determine the form of the transition region of the voltage current characteristic. The reactive increase of resistance, a non-linear phenomenon, like the resistance increase in conductance during spike electrogenesis, may be regarded as an amplification.

Hyperpolarizing responses which are also due to a regenerative increase in resistance (Grundfest, 1961; Reuben *et al.*, 1961; Stämpfli, 1963), likewise, exhibit a negative slope characteristic (Moore, 1959; Bennett and Grundfest, 1962 *a*, 1962 *b*, 1965). In frog nodes the slope of the transition from the low to the high resistance state is modified by changing the pH of the medium (Müller-Mohnssen and Balk, 1964) or the Ca level (Müller-Mohnssen and Balk, 1965), from a steep one of high amplification to a shallow trough or none at all.

The more or less sigmoid link between the two limiting slopes of the voltage current characteristic may be viewed in probabilistic terms (Grundfest, 1957 *a*, 1957 *b*), as the integral of a population of sites which are permeable for K and which react by inactivation at different values of depolarization. The voltage clamp data indicate a spread of thresholds in the electroplaques ranging between 50 and 100 mv in different experiments. A somewhat similar spread of threshold is evidenced in voltage clamp data on hyperpolarizing and depolarizing inactivation of gymnotid electroplaques (Bennett and Grundfest, 1965). It has also been suggested (Grundfest, 1957 *a*, 1957 *b*) that the elementary Na channels which are involved in the electrogenesis of spikes and graded responses are likewise distributed as a population with different activation thresholds. Regenerative involvement of the whole population of "electrogenic units" (Grundfest, 1957 *a*, 1957 *b*) is delayed when a spike is evoked by liminal stimuli in space-clamped squid axons (Hodgkin *et al.*, 1952). Inactivation responses, whether to depolarizing or hyperpolarizing currents, also develop after a delay when the stimuli are liminal (*cf.* Fig. 10*B* of the present paper; Fig. 4 of Deck *et al.*, 1964; Stämpfli, 1958; Reuben *et al.*, 1961; Grundfest, 1961; Bennett and Grundfest, 1965).

Na Influx during Activity The peak net inward current attained a value of about 60 ma/cm^2 on the average. The influx which lasted about 1 msec. thus represented about 3×10^{-5} coulombs of inward flow, or an influx of 3×10^{-10} M of Na/cm^2/impulse. This is about 100 times greater than the Na influx during the spike of squid giant axons (*cf.* Hodgkin, 1964, Table 3). The "equilibrium" potential for Na (E_{Na}) is about 70 mv and from the Nernst relation indicates an intracellular level of 8 to 10 mM Na/liter. If the average thickness is assumed to be about 100 μ the volume of a very large electroplaque is only about 2×10^{-6} liter, and contains about 2×10^{-8} M of Na. The influx of Na is about 10^{-12} M/impulse. Thus, a single spike must cause a considerably larger change in the intracellular level of Na than occurs in squid axons (Hodgkin, 1951). In fact, since some regions of the cell are only about 10 to 20 μ thick, very large local differences in concentration of Na may be expected to occur.

Electroplaques can respond to repetitive direct stimulation at 10 to 25/second for quite long times and may discharge continuously at 25 to 50/second under neural control while the fish is cruising or hunting food. It thus appears necessary to postulate a very active "pump" mechanism for restoring the initial level of Na. K inactivation may itself be a sign of the demands of the pump system. Elimination of the K efflux during the spike would help to maintain inside positivity during which time internal K would be conserved by block of the K channels and the cation efflux would be directed into the remaining, presumably more or less non-selective channels of both major surfaces.

Possible Function of K Inactivation The desideratum of electric organ functioning is maximum electrical output. Repolarizing electrogenesis leads to diminution of the effects of the inward current and depolarizing voltage change of the spike. Absence of K activation thus conserves energy that would be lost in that degenerative process. The nearly general occurrence of K activation in cells which have been studied thus far is in keeping with the importance of pulse shaping and of speedy restoration of membrane potential for the needs of conductile activity. These are unnecessary, however, for the electroplaques. The occurrence of depolarizing K inactivation helps to prolong the spike of eel electroplaques and may therefore increase the effectiveness of the discharge of the electric organ.

With respect to the characteristics of the organ discharges the weakly electric repetitively discharging *Sternopygus* and *Eigenmannia* are more closely related to *Electrophorus* than are the diphasically discharging gymnotids. During the discharge of *Sternopygus* and *Eigenmannia* electroplaques the membrane resistance undergoes a remarkable sequence of changes (Bennett and Grundfest, 1959; Bennett, 1961; Goldman and Grundfest, unpublished data). In the normally occurring repetitive discharges the membrane con-

ductance is increased during the e.p.s.p. and the rising phase of the spike which the e.p.s.p. evokes. Toward the peak of the spike the spike conductance diminished to 2 or 3 times below the resting value. Thus far, therefore, the events resemble somewhat those occurring during spike electrogenesis in *Electrophorus* electroplaques. However, as the cells of the weakly electric forms depolarize, the conductance increases again, apparently by onset of K activation, since the change speeds repolarization. The electroplaques presumably have a rapidly developing and rapidly reversible depolarizing K inactivation, which overcomes the K activation process that would begin to develop during the rising phase of the spike. The activation, however, manifests itself when the membrane potential falls. The sequence may be regarded as a specific adaptation to the needs of the repetitively discharging cells. It combines the advantage of K inactivation as an economy in the ionic generator with the desirable feature of a maximum voltage output in the organ as a power source for electrosensory receptors. The subsequent onset of repolarizing electrogenesis speeds resetting the electroplaques for another discharge. Very rapid onset and abolition of depolarizing K inactivation are observed in electroplaques of other gymnotids (Bennett and Grundfest, 1962 *a*, 1962 *b*, 1965).

A cycle also involving K inactivation and subsequent activation, though on a vastly different time scale, is observed in vertebrate cardiac muscle (Weidmann, 1951, 1956). Depolarizing inactivation probably has no functional significance in frog muscle fibers, since it manifests itself only in a variety of experimental media that are rather different from the normal environment of the cells (Nakajima *et al.*, 1962). However, when it does occur it may develop a negative slope in the conductance characteristic (Adrian and Freygang, 1962; Adrian, 1964), and various degrees of membrane "instability" (Adrian, 1960; Nakajima *et al.*, 1962). Oscillatory phenomena are also observed in the prolonged responses of lobster muscle fibers (Grundfest, 1961; Reuben *et al.*, 1960), but the system is rather complex since the prolonged responses are compounded of both K inactivation and prolonged activation of depolarizing electrogenesis.

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