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Contributions of Various Ions to the Resting and Action Potentials of Crayfish Medial Giant Axons

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Summary. The membrane of crayfish medial giant axons is permeable at rest to ions in the rank K > Na > Ca > Cl. With K present, variation of the other ions has little or no effect, but with K absent the axon hyperpolarizes when Na is reduced or eliminated by replacement with Tris (slope ca. 30 mV/decade Na_0). The hyperpolarization is independent of the presence of Cl or its absence (substitution with methanesulfonate or isethionate). The resistance increases progressively as Na is removed. These changes persist after the spike is blocked with tetrodotoxin. An increase in Ca causes depolarization (slope ca. 20 mV/decade) provided K, Na and Cl are all absent, but in the presence of Cl there is little or no change in membrane potential on increasing Ca to 150 mM. The depolarization induced by Ca is associated with an increased resistance. Spike electrogenesis involves Ca activation as well as Na activation, but the after-depolarization at the end of the spike is due to a conductance increase for Ca. Two alternative equivalent circuits for the resting and active membrane are discussed.

The spikes of crayfish giant axons, which have amplitudes of 100 to 125 mV starting from a resting potential of −80 to −90 mV, terminate with marked after-depolarization (Watanabe & Grundfest, 1961). The first part of this paper presents an analysis of the ionic basis of the after-depolarization in the circumesophageal region of the medial giant axons. In the course of the study it was found that, when the bathing medium is free of *K*, the resting potential is determined in part by the concentration gradients of other ions. These findings and their analysis form the second part of the paper.

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** Requests for reprints.

Materials and Methods

All the crayfish studied in the present work were *Procambarus*, obtained from California. The thoracic nerve cord and the circumesophageal connectives were removed from the animal. The nerve cord was divided into its right and left branches under a binocular microscope, and the sheaths around the half of the nerve cord and its circumesophageal extension were removed. The length of the total preparation was about 3.5 cm. The medial giant axon in the connective was cleaned for a length of about 8 mm so as to permit quick exchange of bathing solutions. The diameters of the axons ranged between 120 and 150 μ.

The axon was impaled with one or two 3 M KCl-filled microelectrodes. The resistance of the latter ranged between 15 and 30 MΩ. One electrode was used for recording the membrane potential differentially against a KCl-agar/Ag-AgCl bridge; the second was used in the experiments in which it was desired to apply currents intracellularly. The axon was also stimulated extracellularly through a pair of fine platinum wires applied to the nerve cord. The electrophysiological equipment was standard for the laboratory. All the experiments were done at room temperature (19–23 °C).

The standard bathing saline was a modification of Van Harreveld's (1936) solution. It contained (in mM) 205 NaCl, 5.4 KCl, and 13.5 $CaCl_2$, with pH adjusted to 7.3 with Tris buffer (Girardier, Reuben, Brandt & Grundfest, 1963; Reuben, Girardier & Grundfest, 1964). In the experiments in which the cations were varied, the total osmolarity was adjusted to 464 mM with Tris. In certain experiments, Cl was replaced with methanesulphonate (MS) and/or isethionate (Ise). Tetrodotoxin (TTX; Sankyo Company, Tokyo) was used in some experiments.

Results

Electrical Characteristics of Resting Axons

Cable properties were determined on seven fibers bathed in the standard saline (Table 1). In this series as well as in all other axons under similar

Table 1. *Electrical properties of medial giant axons*

No.	diam. (μ)	E_M (mV)	λ (mm)	R_{eff} ($\times 10^3$ Ω)	r_i ($\times 10^3$ Ω/cm)	R_i (Ω cm)	r_m ($\times 10^3$ Ω cm)	R_m ($\times$ Ω cm^2)
11	150	83	2.0	40	410	80	16.0	750
13	140	86	1.7	48	570	105	16.3	734
14	160	83	1.6	40	500	100	12.8	643
17	150	85	1.9	55	570	91	20.0	940
19	160	82	2.0	54	540	108	21.6	1080
20	170	84	2.6	54	420	92	27.0	1210
21	145	85	1.6	50	620	103	15.8	726
Mean	153	84	1.8	49	520	97	18.5	870
S.E.		±1.0						±144

λ, length constant; r_i, internal resistance; R_i axoplasmic resistivity; r_m membrane resistance; R_m specific membrane resistance.

ionic conditions, the resting potential varied very little. Motor axons of lobster walking leg also exhibit little variation in resting potential (ca. -80 mV; Freeman, Reuben & Grundfest, *unpublished*). The length constant (λ) ranged between 1.6 and 2.6 mm and the membrane resistance between 640 and 1200 Ω cm^2.

For the 24 axons of Figs. 1–5, the mean resting potential in the control saline was -84 ± 1 mV, and R_{eff} was $52 \pm 11.3 \times 10^3$ Ω. When the axons were bathed in a K-free saline, they hyperpolarized to -99 ± 1.9 mV and R_{eff} increased to $67 \pm 14.1 \times 10^3$ Ω. The axons remained hyperpolarized in the K-free medium for at least several hours. The fiber of Fig. 1 hyperpolarized from -83 to -100 mV within 10 min after the K-free saline was introduced, and the resting potential remained at this value 1 hr later in the K-free medium.

The Action Potential

Spikes and After-Depolarization. For the series of experiments shown in Figs. 1–5, spikes were elicited by stimulating the nerve cord and were recorded intracellularly in the circumesophageal region. The mean spike amplitude was 110 mV when the axons were bathed in the control saline. This is somewhat smaller than the amplitude of the spikes in the lateral giant axons (Watanabe & Grundfest, 1961). The overshoot (ca. 24 mV in the axon of Fig. 1) was essentially unchanged when the axon hyperpolarized on removal of K.

The spike has a small and brief (<100 μsec) foot on its rising phase, and the falling phase is almost as steep as the rise. Thus, the brief duration of the spike can be approximated by a pair of straight lines projecting onto the resting potential as the base (Gasser & Grundfest, 1939). The maximum deviation from the straight line on the falling phase was taken as the peak of the after-depolarization. The small amplitude of the potential (peak ca.

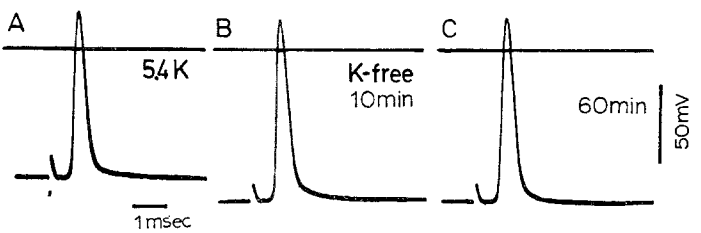

Fig. 1 A–C. Propagated action potentials in the standard saline (A) and at 10 min (B) and 60 min (C) following removal of K. Upper trace is the zero reference. The membrane hyperpolarized by 17 mV on removing K, but the overshoot (24 mV) did not change significantly. The after-depolarization was somewhat more prominent in 0-K

4–5 mV) makes it difficult to estimate its duration, beyond about 3 msec. The after-depolarization is more pronounced and longer lasting in the septate axons (Watanabe & Grundfest, 1961; Kusano & Grundfest, 1965), in the stretch receptor axons (Obara & Grundfest, 1968), and in the motor axons of lobster walking legs (Freeman, Reuben & Grundfest, *unpublished*).

Conductance Change during the After-Depolarization. Two microelectrodes were used for these experiments. The graphs of Fig. 2 show the steady-state characteristics for an axon bathed in 5.4 mM K_0 (open circles) and in 0-K (filled circles). The triangles show the corresponding measurements for the peaks of the after-depolarizations. The five superimposed records (*A* and *B*) show the method for making these measurements. The upper traces monitor the pulses of successively increasing, intracellularly applied

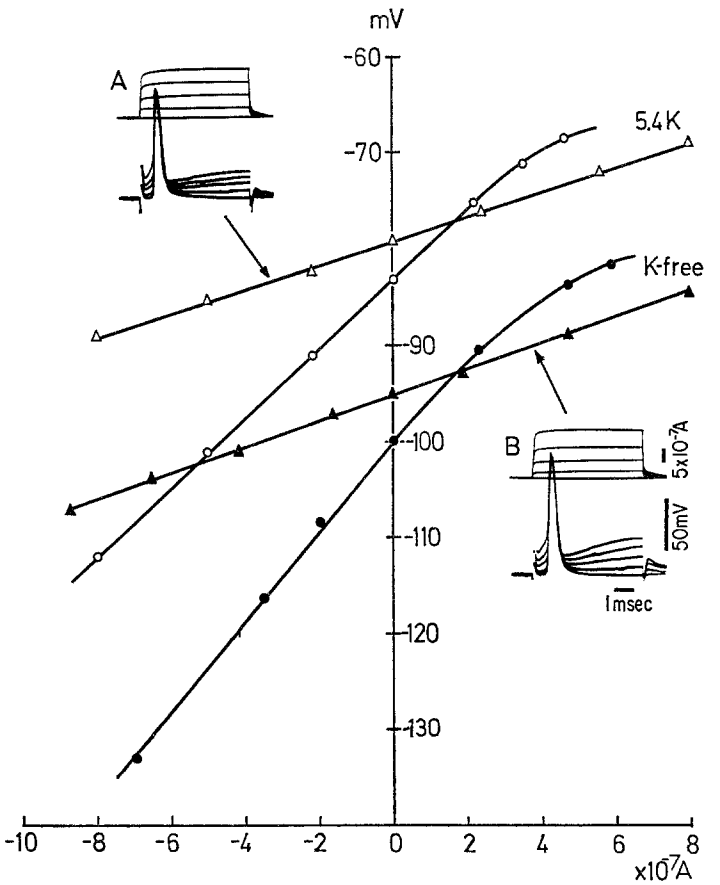

Fig. 2A and B. $I-E$ relations for the membrane at rest (○) and at the peak of the after conductance that is associated with the after-depolarization (△). Open symbols in 5.4 mM K; filled symbols, in the K-free saline. Inset records (A and B) show the method of the measurements which is described in the text

outward currents. In this experiment the pulses lasted 6 msec; in other experiments durations of up to 15 msec were used. The lower traces show the voltages recorded during the applied currents. An externally evoked spike was timed to invade the recording region after the beginning of each pulse. The spikes were affected very little by the applied currents, denoting that the conductance is high during this electrogenesis. The membrane potential just prior to onset of the spike had reached about the same values as were attained at the ends of the 6 msec pulses, indicating that the time constant of the axonal membrane is about 1 msec. The increase in conductance that is responsible for the after-depolarization lasted some 4 to 5 msec. The level at which after-depolarization deviates from the spike is affected by the applied current, and these values (triangles) which represent the peak conductance of the after-depolarization form the less steep lines of the graph.

The input resistance of the axon of Fig. 2 at rest was 36×10^3 Ω in the standard saline and increased to 48×10^3 Ω in the K-free medium. The $I-E$ characteristic for the after-depolarization shows that the resistance had decreased to 12×10^3 Ω in the control saline and that it increased very little, to 13×10^3 Ω, in the K-free saline. With no current applied, the peak after-depolarization was 4 mV in the presence of 5.4 mM K and 5 mV in the K-free medium. The characteristics for the resting membrane and for the after-depolarization cross at membrane potentials which are slightly more positive than these values. Because it is a singular point, the crossover provides a convenient index value for the peak after-depolarization.

Effects of Varying K_0. The records of Fig. 3A show the changes in the resting potential and in the responses to stimulation in one axon when K_0 was varied from 0 to 16.2 mM. The spike amplitude decreased as the membrane depolarized on increasing K_0; with $K_0 > 16.2$ mM, propagation was blocked. The records of Fig. 3B on the same axon show superimposed traces of spikes that arrived at the recording site just after outward currents lasting 10 msec were applied. The graph of Fig. 3 summarizes the data obtained in this way on eight axons. The resting potentials in the different concentrations of K_0 are averaged values (filled circles, continuous line) as are the overshoots (open circles and continuous line). In the low range of K_0, the relation between membrane potential and log K is not linear (Strickholm & Wallin, 1967). In the experiment of Fig. 3, a 10-fold increase in K_0, from about 2 mM to about 20 mM, depolarized the cell by about 30 mV. The peak after-depolarizations, obtained as described in connection with Fig. 2, are plotted for the individual axons. In general, the slope of the

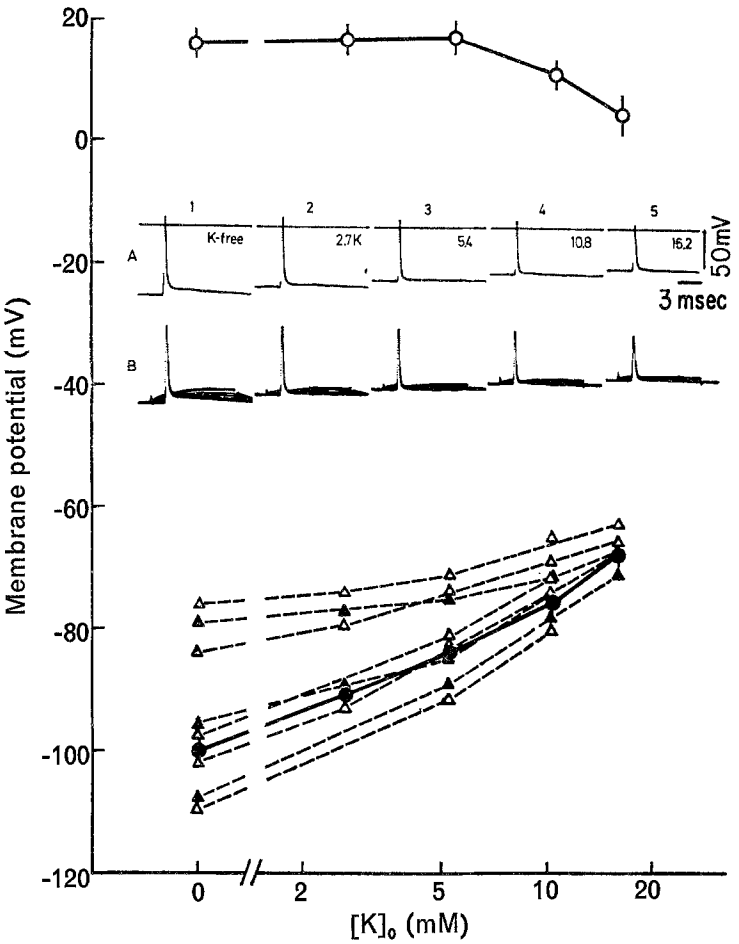

Fig. 3. Changes induced by varying K_0. A_{1-5}, records obtained in the various solutions. B_{1-5}, presentations like those of Fig. 2A and B for the various concentrations of K_0. o, overshoots as a function of K_0. •, corresponding changes in resting potential. Values are averages of eight experiments. Vertical lines show the standard deviation in these and subsequent graphs. △, changes in the peak after-depolarization plotted separately for each experiment

change in the "reversal potential" of the after-depolarization was similar to that of the change in resting potential. The peak of the after-depolarization deviated from the resting potential most markedly and the duration was also greatest when the axons were hyperpolarized in 0-K. In 16.2 mM K_0, the after-depolarization became negligible. These data do not rule out the possibility that the emf of the K battery may be involved in the electrogenesis of the after-depolarization, but the ydo indicate that the contribution probably does not predominate in the electrogenesis.

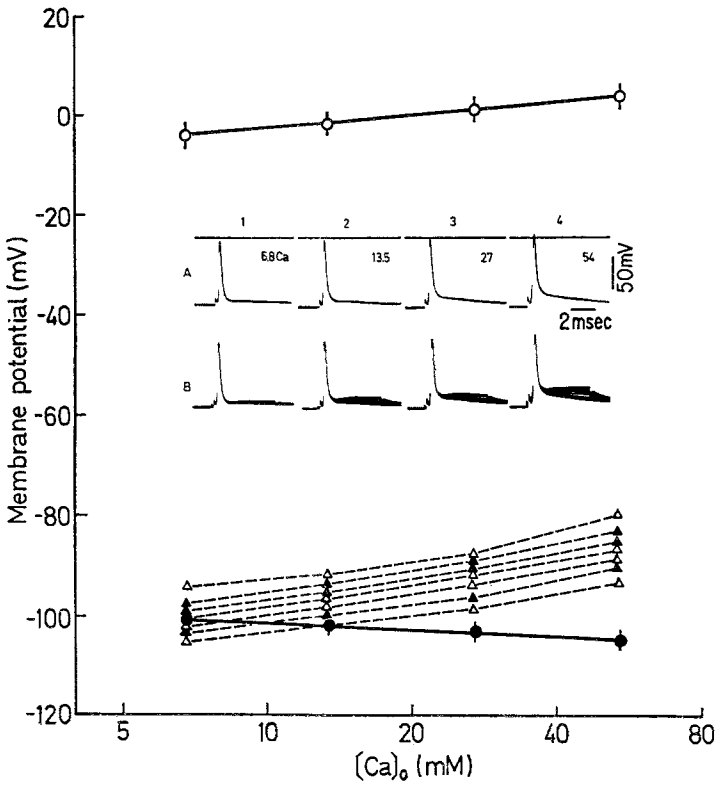

Fig. 4. Changes induced by varying Ca_0. The bathing medium was K-free and contained 150 mM NaCl throughout. Osmolarity was adjusted with Tris-Cl as described in the text. The presentations are similar to those of Fig. 3. Resting potentials and spike peaks are averages of seven experiments

Effect of Changing Ca_0. A very different relation is obtained when Ca_0 is varied. For these experiments (Fig. 4), NaCl was reduced to 150 mM and KCl was eliminated completely. The osmotic deficit was made up by adding Tris-Cl. As Ca_0 was increased, Tris was reduced correspondingly. Thus the measurements of Fig. 4 were done with Na_0 constant and in zero K. The records of Fig. 4A and B are similar to the corresponding data of Fig. 3A and B, but in this case with $CaCl_2$ varied between 6.8 and 54 mM. The resting potentials and spike amplitudes plotted on the graph are averaged values for seven experiments; the peak amplitudes of the after-depolarizations are plotted for each axon as functions of Ca_0. The membrane hyperpolarized by about 8 mV as Ca_0 was increased from 6.8 to 54 mM. The spikes, which were low in amplitude because of the reduced Na_0, developed a small overshoot when Ca_0 was increased to 54 mM. The slope of the increase was 8 mV/decade Ca_0. As Ca_0 was increased, the peak

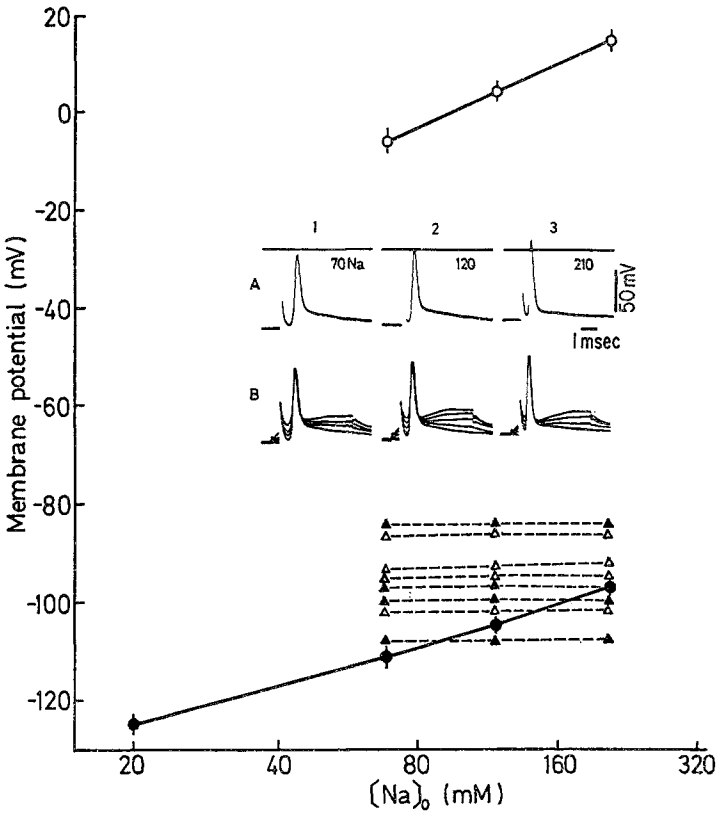

Fig. 5. Changes induced by varying Na_0. Presentations as in Figs. 3 and 4. Resting potentials and spike amplitudes are averages of eight experiments. The bathing solution was K-free and contained 13.5 mM $CaCl_2$. Osmolarity adjusted with Tris-Cl

after-depolarization increased with a slope of about 16 mV/decade Ca_0 and the duration increased markedly (Fig. 4A). There was no significant change in resistance for the after-depolarization. Thus, the data of Fig. 4 indicate strongly that the after-depolarization is due mainly to the emf of the Ca-battery, which is inside-positive, on the evidence of its contribution to the spike electrogenesis.

Effect of Changing Na_0. Experiments similar to those of Figs. 3 and 4 were done with different concentrations of NaCl in the bathing solution. The solutions were K-free and contained 13.5 mM $CaCl_2$. The records of Fig. 5A and B show the effects of 70, 120 and 210 mM NaCl. Lower values of Na_0 did not permit electrogenesis of a propagating spike. However, as shown in the graph, the membrane hyperpolarized to about -125 mV when Na_0 was reduced to 20 mM. The slope of the change in resting potential was

29 mV/decade Na_0 while the peak of the spike increased with a slope of 45 mV/decade Na_0. The peak after-depolarization remained practically independent of Na_0. Thus it appears very likely that the Na conductance is not high during the after-depolarization, whereas the spike electrogenesis is due mainly to an increase in Na conductance. The marked dependence of the resting potential on Na_0 was a surprising finding. Since the data of Fig. 5 were obtained in axons bathed in K-free solutions, it seemed likely that it is this condition that increases the relative permeability to Na and the resting membrane to behave as a Na-sensitive electrode system. An investigation of this possibility led to the data of the following section.

Ionic Batteries of the Resting Potential in K-Free Media

The Na-Battery. The conclusion that the Na-battery contributes signally to the resting potential in K-free solutions was confirmed in experiments such as that of Fig. 6A. In the presence of 5.4 mM K, reduction of Na from 200 to 20 mM induced only a small hyperpolarization (from −83 to −88 mV), and that hyperpolarization was abolished on restoring the higher concentration of Na. When the axon was then exposed to a K-free solution which contained 200 mM Na, it hyperpolarized rapidly to about −100 mV. Now, when Na was reduced to 20 mM, the axon hyperpolarized further to almost −125 mV. This was reversed on returning the axon to the 200 mM Na. Ca was kept constant in this experiment at 13.5 mM.

The effect on the resting potential of reducing Na was independent of whether the anion was permeant (Cl) or impermeant (MS), as seen in Fig. 6B. All the solutions used in this experiment were K-free and contained 13.5 mM Ca. The initial resting potential therefore was nearly −100 mV in the presence of 200 mM NaCl. Marked hyperpolarization occurred when NaCl was reduced to 70 mM, and there was further hyperpolarization on reducing NaCl to 20 mM. The axon was then exposed to 200 mM NaMS, which caused a rapid depolarization to about the same level as in 200 mM NaCl. Subsequent reductions of NaMS to 70 and 20 mM caused hyperpolarizations of about the same magnitude as when NaCl was reduced to these levels. Thus, the contribution of the Na-battery to the resting potential in the K-free saline is essentially independent of the degree of permeance of the anions in the bathing medium.

The fact that the strong effect of Na_0 on the resting potential occurs when the solution is K-free is emphasized in the graphs of Fig. 6C. Na_0 was varied in the presence of 10.8 or 5.4 mM K_0 and in the absence of K_0. The open circles are averaged values for seven experiments in Cl. The filled

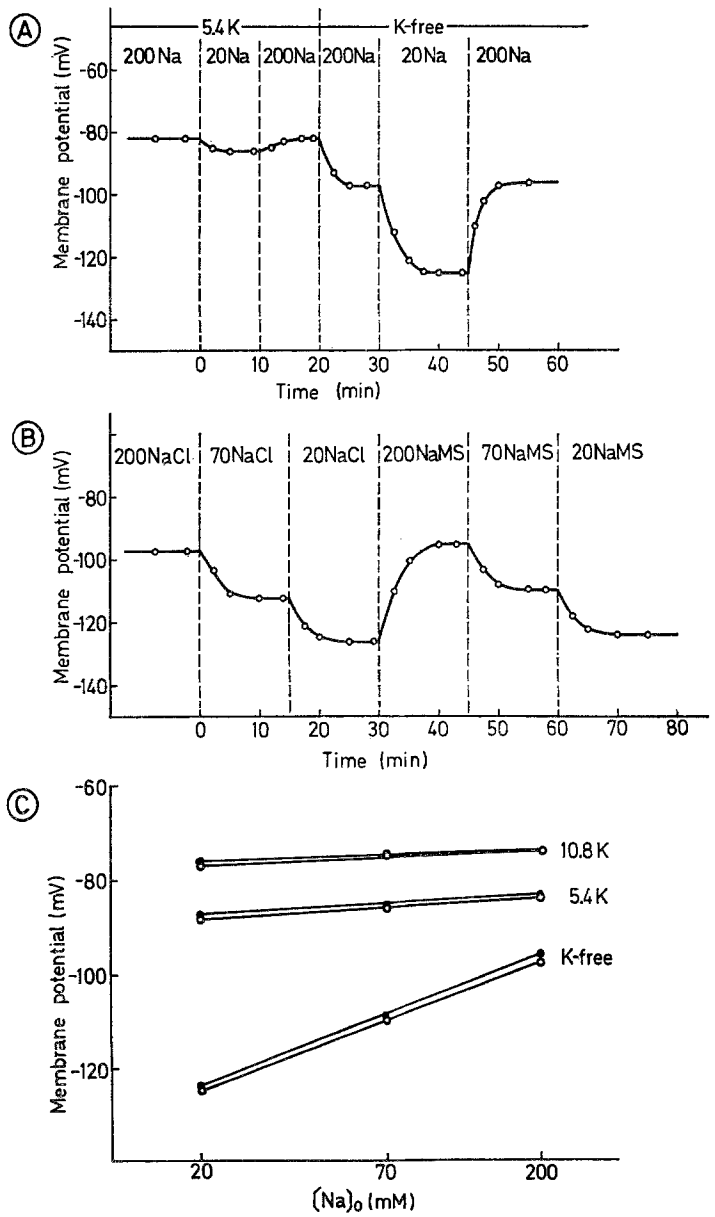

Fig. 6A–C. Resting potential as a function of Na_0 under various experimental conditions. A and B: Time course of changes in resting potential when Na was changed as described in the lines above the graphs. The salines all contained 13.5 mM Ca. A, the marked effect of changing from 5.4 mM K_0 to 0-K on the response of the membrane to reducing Na_0. B, there was no effect on changing from Cl to MS in the K-free saline. C, the relation between E_M and Na_0 in the presence of K (5.4 and 10.8 mM) and in the K-free saline. Each point is the average of seven or eight experiments. ○, in the presence of Cl; •, in the Cl-free solutions. Ca was constant at 13.5 mM

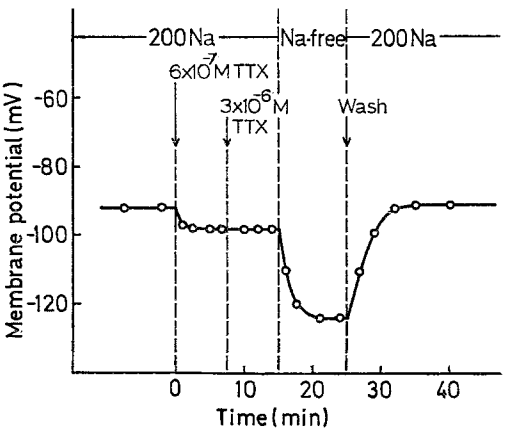

Fig. 7. Hyperpolarization induced by reducing or removing Na_0 is not blocked by TTX. K-free saline, 13.5 mM $CaCl_2$. Addition of 6×10^{-7} M TTX, which is more than sufficient to block spike electrogenesis, caused 6 mV hyperpolarization. There was no further change on raising TTX to 3×10^{-6} M. The axon hyperpolarized to about -125 mV on removing Na in the presence of TTX. The resting potential returned to its original value in the control saline

circles are similar averages when MS was the anion. The resting potential changed by 32 mV/decade Na_0 in the K-free salines, but when K_0 was 5.4 or 10.8 mM the effect of changing Na_0 was small, 2 and 4 mV/decade Na_0, respectively. As already shown in Fig. 6B, the nature of the anion was not an important factor. The data of Fig. 6C indicate further that the sensitivity of the membrane as a K-electrode increased when Na_0 was reduced. In the presence of 200 mM Na, the slope of the E_M-log K_0 relation was about 30 mV; it increased to about 50 mV when Na_0 was reduced to 20 mM.

Effect of TTX. TTX blocks spike electrogenesis in crayfish axons (Ozeki, Freeman & Grundfest, 1966; Obara & Grundfest, 1968) presumably by eliminating Na activation as in axons of lobster (Narahashi, Moore & Scott, 1964) and of squid (Nakamura, Nakajima & Grundfest, 1965*a*). TTX causes a small hyperpolarization (ca. 6 mV in Fig. 7) when applied in the presence of 200 mM Na. However, the large hyperpolarization that is induced on eliminating or reducing Na_0 in the K-free saline is not blocked by TTX.

Effect of Changing Ca. All the foregoing experiments except that of Fig. 4 were done with the Ca_0 maintained constant at 13.5 mM. The experiments illustrated in Figs. 8 and 9 investigated the permeability of the membrane to Ca. It was found that in the K-free media the Ca battery also

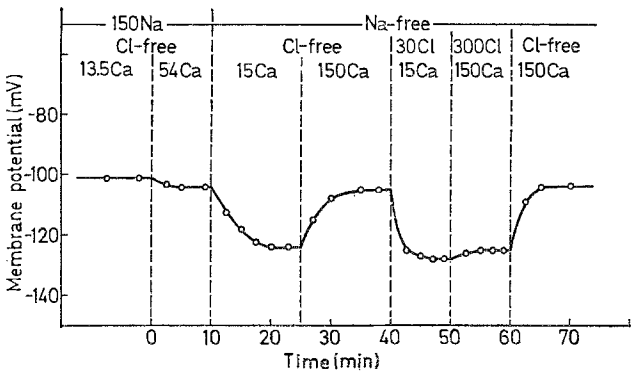

Fig. 8. Effects of changing Ca_0 under several experimental conditions. K-free solutions. The axon was equilibrated in 150 mM Na-Ms (+Tris-MS) and 13.5 mM Ca-Ise. Increasing Ca (as Ise) to 54 mM caused only a small hyperpolarization. The bathing medium was then made Na-free. In the presence of 15 mM Ca, the axon hyperpolarized but it depolarized on increasing Ca to 150 mM. In the next two panels, the Ca was present as $CaCl_2$ and when it was increased from 15 to 150 mM there was only a small depolarization from a level of about −130 mV. On removing the Cl, the axon again depolarized

contributes markedly to the resting potential, but only in the absence of Na and Cl. In the experiment of Fig. 8, Na_0 (as NaMS) had been reduced to 150 mM for the reasons stated in connection with Fig. 4, and the osmotic deficit was made up with Tris-MS. The medium was K-free and initially contained 13.5 mM Ca-Ise. On increasing Ca to 54 mM, the axon hyperpolarized from −102 to −104 mV. All the Na was now removed, with a return to 15 mM Ca_0. The axon hyperpolarized to −124 mV. When Ca-Ise was now increased to 150 mM, there was depolarization back to −105 mV. When the Ca was again reduced to 15 mM, but this time as $CaCl_2$, the axon hyperpolarized again, slightly more in fact than in 15 mM Ca-Ise. An increase of $CaCl_2$ to 150 mM caused only a slight decrease in the resting potential, but return to 150 mM Ca-Ise again caused depolarization, to about −105 mV.

The contributions of the Na, Ca and Cl batteries in setting the resting potential are emphasized in the four graphs of Fig. 9 with averaged data obtained in six to nine experiments of each type. When Na (150 mM) was present (circles), an increase in Ca caused only a slight hyperpolarization and the effect was independent of the nature of the anion (Cl, open circles; MS and Ise, filled circles). In the absence of Na, an increase in Ca_0 caused depolarization (triangles), but the degree of this depended on the nature of the anion. When Cl was present (open symbols), the depolarization was only 4.5 mV/decade Ca_0, but with impermeant anions present in the medium (filled triangles) the axons depolarized 20 mV/decade Ca_0.

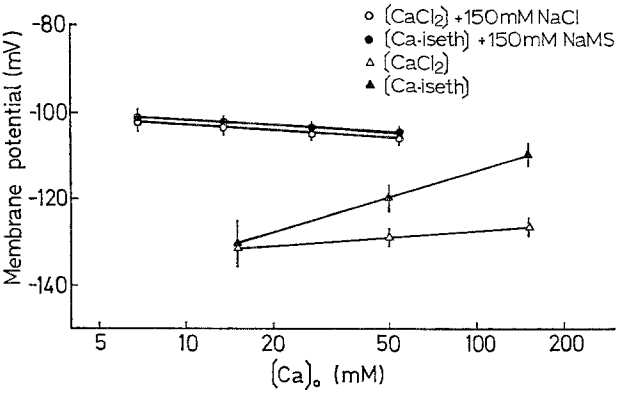

Fig. 9. Variation of the resting potential with Ca_0 under the several conditions shown in Fig. 8. All points are averages of eight experiments. The salines were K-free. ○, in 150 mM Na_0 and Cl as the anion. ●, in 150 mM Na_0 but without Cl. △, Na_0 absent but Ca varied as $CaCl_2$. ▲, both Na and Cl absent

Table 2. *Changes in E_M and R_{eff} induced by varying Na_0. (K-free saline containing 13.5 mM Ca)*

	Na (mM)			
	210	70	20	0
A. Cl present				
E_M(mV)($n=3$)	98± 1	112± 1	126± 1	134 (1 axon)
R_{eff}($\times 10^3$ Ω)	52± 6.7	65±10.4	72±12.7	90 (1 axon)
B. Cl-free saline				
E_M(mV)($n=6$)	97± 2.1	111± 3.5	125± 4	135 (3 axons)
R_{eff}($\times 10^3$ Ω)	75±17.3	88±21.2	95±22.7	94 (3 axons)

Effective Resistance in Different Media. The resistance (R_{eff}) increased by about 30% in the absence of K (Fig. 2). It increased further when Na was reduced, rising by 30 to 50% in 20 mM Na (Table 2). The $I-E$ characteristics for different levels of Na were essentially linear and overlapping, so that for a given value of membrane potential there could be several values of resistance. Thus, although removal of Na caused a hyperpolarization of some 30 mV (Figs. 6 & 10), the increase in R_{eff} was independent of the change in resting potential. R_{eff} was higher in Cl-free salines although removal of Cl had no effect on the steady-state value of E_M (Table 2).

When Ca was raised to 150 mM in the absence of K, Na and Cl, the resistance increased further (by about 20%), despite the fact that the axons now depolarized by about 20 mV from their strongly hyperpolarized state (Figs. 9 & 11, and Table 3). The characteristic remained linear (Fig. 11).

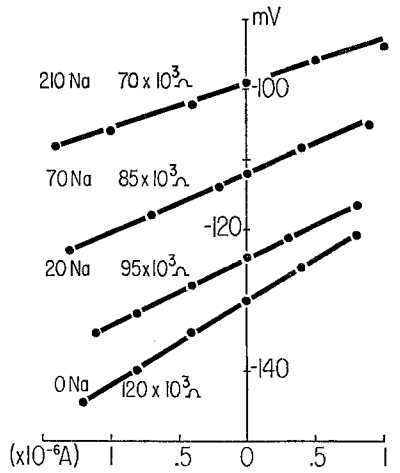

Fig. 10. Characteristics of an axon (no. 137, included in Table 2) as a function of different concentrations of Na. The initial bathing solution contained 210 mM Na, 13.5 mM Ca, and no K or Cl. NaMS was then replaced with Tris-MS to reduce Na to 70 mM, 20 mM and 0. Each successive reduction of Na induced further hyperpolarization, and R_{eff} increased from 70×10^3 to 120×10^3 Ω

Table 3. *Effects of varying Ca under various ionic conditions. K-free salines*

Ca (mM)	150 mM Na				0 Na			
	Cl present		0 Cl		Cl present		0 Cl	
	E_M(mV) ($n=8$)	R_{eff}($\times 10^3$ Ω) ($n=3$)	E_M ($n=8$)	R_{eff} ($n=4$)	E_M ($n=8$)	R_{eff} ($n=4$)	E_M ($n=8$)	R_{eff} ($n=6$)
6.8	102 ±2.3	75 ±9.6	101 ±2.2	86 ±16.6				
13.5	104 ±2.3	80 ±9.5	102 ±2.0	92 ±10.1				
15.0					131 ±2.1	79 ±8.2	130 ±3.3	93 ±10.8
27	105 ±2.3	88 ±12.4	104 ±2.0	102 ±12.3				
50					129 ±2.5	91 ±5.3	120 ±2.8	108 ±20.2
54	107 ±2.9	103 ±14.4	106 ±1.9	114 ±13.5				
150					126 ±2.0	104 ±5.6	110 ±1.9	122 ±21.6

Thus, although the depolarization on increasing Ca indicates the change in the emf of the Ca battery, Ca also has another action. The decrease in conductance on increasing Ca reflects the "stabilizing" effect of Ca on the membrane (Shanes, 1958). Presumably, the removal of K and Na also changes

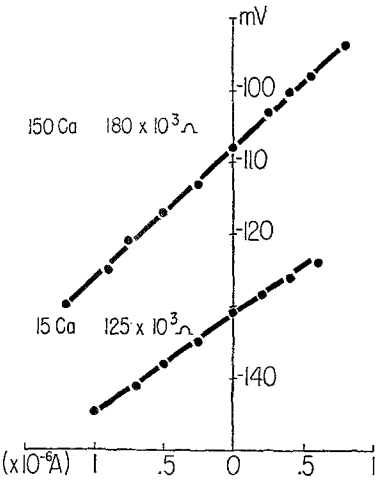

Fig. 11. Characteristics of axon no. 107 (included in Table 3) as a function of Ca concentration. In the control (0-K, 13.5 mM Ca and 210 mM NaMS present), the resting potential was −98 mV and R_{eff} was 90×10^3 Ω. Upon replacing all Na with Tris, the axon hyperpolarized to −131 mV and R_{eff} increased to 125×10^3 Ω. Ca was then increased from 15 to 150 mM. Although the axon depolarized to −108 mV, R_{eff} increased to 180×10^3 Ω

the properties of the cell membrane to increase its resistance, since the characteristics in Fig. 10, like those in Fig. 11, are linear over a considerable range of depolarization as well as hyperpolarization in the vicinity of the various resting potentials. Whereas E_m changed little when Ca_0 was increased in the presence of Na and/or Cl, R_{eff} increased progressively as Ca was increased (Table 3). It is also noteworthy that R_{eff} was increased by removal of Cl at all levels of Ca_0 and also independently of the resting potential.

Discussion

The evidence of Figs. 1–5 points clearly to the occurrence of three activation processes, for Na, K, and Ca, respectively. These are represented in the equivalent circuits of Fig. 12 as conductance channels with arrows. The increased permeabilities that are induced by depolarizing stimuli are probably in the order $P_{Na} \gtrsim P_K \gg P_{Ca}$. The emf's of the Na and Ca batteries are inside-positive whereas that of the K battery is close to the resting potential.

As in other axons, the primary event in the spike electrogenesis is a TTX-sensitive Na activation. Although the inside-positive Ca battery can also contribute to the overshoot of the spike (Fig. 4), propagating spikes do not occur in the axons when Na_0 is reduced to below 70 mM or when Na

activation is blocked by TTX[1]. Presumably, the conductance increase for the Ca battery is too small to provide the current that is needed to overcome cable losses and generate an all-or-none impulse. However, the conductance increase for Ca persists longer than does Na activation, since the after-depolarization is a function of Ca_0. There is a clear-cut increase in after-conductance even when the axon is hyperpolarized by an inward current and by removal of K_0 (Fig. 2). This indicates that the hyperpolarization does not quench the Ca activation that causes the increased conductance.

The K activation which causes the after-depolarization of the squid axon as the membrane potential shifts toward the inside-negative emf of the K battery (Hodgkin & Huxley, 1952) is quenched by the hyperpolarization. The terminal portion of the "undershoot" of the spike is associated with an increased impedance during which the membrane resistance increase to about three times above the resting value (Shanes, Grundfest & Freygang, 1953). Quenching of K activation is also indicated at the termination of the diphasic spikes of *Tenebrio* and *Ascaris* muscles and of the negative spikes in the latter form (Grundfest, 1967). The hyperpolarization that results from K activation in frog slow muscle fibers does not, however, quench the conductance increase (Grundfest, 1966, Fig. 12).

The curvature of the steady-state $I-E$ characteristic when the membrane is depolarized by some 10 to 15 mV (Fig. 2) indicates that K activation occurs during the spike. Judging from the brief duration of the spike and its almost symmetrical rising and falling phases, K activation develops a large increase in conductance early during the spike. Similarly brief and nearly symmetrical spikes also occur in the lateral, septate giant axons (Watanabe & Grundfest, 1961; Kusano & Grundfest, 1965), and in the axons but not the soma of the stretch receptors (Obara & Grundfest, 1968). The emf of the K battery (E_K, Fig. 12) probably does not differ greatly from the resting potential, since the crayfish axon spikes do not exhibit an undershoot like that of squid axons.

The Cl battery apparently plays no role in spike electrogenesis. Its emf is very strongly inside-negative, since the presence of Cl in the bathing medium counterbalances the depolarization induced by increasing Ca and tends to maintain the resting potential in 0-K 0-Na in the vicinity of -130 mV (Fig. 9). Thus, if depolarizing Cl activation had occurred, there would have been a drastic effect on the $I-E$ characteristics of Fig. 2, similar to but more extreme than the change observed during the Cl activation of skate elec-

1 *Footnote added in proof:* Non-propagating spikes are obtained in the presence of Ba, Ca, or Sr (and TTX), when Na and K are absent (Suzuki & Grundfest, unpublished).

troplaques (Cohen, Bennett & Grundfest, 1960, and *unpublished*; *cf.* Fig. 13 in Grundfest, 1966).

Whereas only the three cations play a role in generating the action potential of the crayfish medial axon, the ionic battery for Cl as well as those for the cations may contribute to the resting potential (Figs. 3–9). However, the contributions of these ionic batteries differ considerably. When the axon was bathed in the control saline (5.4 mM K_0) or with double this concentration present, there was little change in the resting potential as Na_0 was varied (Fig. 6A & C), whereas there was a depolarization of about 30 mV as K_0 was varied between 2 and 20 mM with Na present (Fig. 3). Thus, the permeability to K appears to be the major determinant of the resting potential. With Na_0 absent or reduced to 20 mM, the E_M-log K_0 relation in the range of 0 to 10.8 mM K_0 increased to about 50 mV (Fig. 6C). Thus, the permeability of the membrane to Na is the most important perturbing factor in causing its deviation from an ideal K electrode. The permeability of the membrane to Ca and Cl may contribute, but to a smaller degree. A further complexity may result from change in the relative permeabilities of the membrane to the various ions. Strickholm and Wallin (1967) explained the deviation in the E_M-log K_0 relation by postulating that the ratio $P_{Na}:P_K$ varied as K_0 was varied. An increased permeability to Na, when Ca was eliminated, was observed in crayfish muscle fibers (Reuben, Brandt, Girardier & Grundfest, 1967) and in squid axons (Tasaki, Takenaka & Yamagishi, 1968).

When the axon was bathed in a K-free saline, the membrane also behaved as a moderately selective Na electrode, since the axons hyperpolarized by about 30 mV when Na_0 was reduced from 200 to 20 mM (Figs. 5 & 6). The deviation from the theoretical (Nernst) relation of ca. 58 mV again indicates that other ionic batteries participate in setting the resting potential. The Cl battery can play only a negligible role in relation to the Na battery, since the slope of the E_M-log Na_0 relation was not changed significantly by replacing Cl_0 with an impermeant anion (Fig. 6C). There was only a small change in the resting potential, and to hyperpolarization, on increasing Ca_0 in the presence of 150 mM Na_0 (Fig. 9). Thus, it seems unlikely that the permeabilities of these ions could produce the large deviation from an ideal Na electrode.

Presumably, therefore, it is the emf of the K battery that is the major factor. Leakage of K from the interior into the space around the axon (Frankenhaeuser & Hodgkin, 1957) probably sets up a gradient of K_i/K_0 that limits the emf of the K battery to between -120 and -130 mV (Figs. 7–10). Although the permeability to K becomes low (Fig. 2), it can

affect the Na-electrode characteristic as the presence of Na affects the K-electrode characteristic.

The resting membrane is also permeable to Ca and Cl. However, just as the permeability for Na becomes evident when there is no K in the bathing solution, so is the permeability to Ca evidenced when both Na and K are absent. This permeability is masked also in the presence of Cl, since the depolarization that was induced on raising Ca in the absence of Cl was virtually abolished when Cl was raised to 100 mM (Fig. 9, triangles). With 15 mM Ca present, the resting potential (ca. −130 mV) was essentially independent of the presence or absence of Cl. In this case, too, the maximum hyperpolarization was probably set by the gradient K_i/K_0 due to the leakage of K from the cell into the extracellular spaces. The gradient would also account for the deviation of the Ca-electrode characteristic from the ideal value (ca. 29 mV/decade Ca) when Cl was absent. When Ca was increased as the Cl salt, E_{Ca} and E_{Cl} both increased in opposite directions. However, the inside-negative E_{Cl} should have increased about four times faster than E_{Ca} since two monovalent anions were added for every divalent cation. The fact that E_M remained essentially constant on increasing $CaCl_2$ signifies that the two ionic batteries contributed approximately equally to the resting potential. These contributions are functions of the transport numbers, τ_{Ca} and τ_{Cl}, as well as of the increments in the emf's ΔE_{Ca} and ΔE_{Cl}. The four-fold larger values of ΔE_{Cl} must be approximately compensated by a τ_{Ca} that is about four times larger than τ_{Cl}.

Although the effects on E_M of removing Cl (Figs. 6 & 9, and Tables 2 & 3) indicate that the permeability of the membrane to Cl is considerably lower than that to Ca and still lower than that to Na or K, removal of Cl causes a marked increase in R_{eff}, which is independent of the resting potential (Tables 2 & 3). This finding indicates that removal of Cl may have specific effects on the membrane resistance analogous to the effects of increasing Ca.

The two alternative equivalent circuits of Fig. 12 are both compatible with the present data. The diagram of Fig. 12A supposes that the resting potential is determined by four voltage-insensitive and therefore invariant (electrogenically unreactive, Grundfest, 1966) conductances for the respective ionic batteries, K, Na, Ca and Cl. These conductances rank in the order K > Na > Ca > Cl. We have firm evidence only that the Na channels for the spike electrogenesis must differ from those involved in the resting potential, since it is only the former that are blocked by TTX, whereas the hyperpolarization induced by reducing Na_0 in a K-free saline is not affected (Fig. 7). We have not yet analyzed the smaller hyperpolarization that is induced by TTX in the presence of Na. It is of about the same magnitude

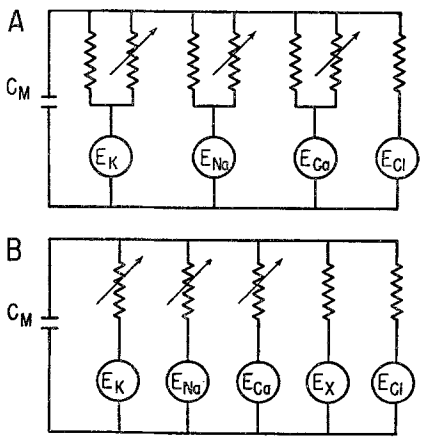

Fig. 12A and B. Two possible equivalent circuits for the crayfish medial giant axon. Spike electrogenesis involves electrically excitable conductance pathways for Na, K and Ca, shown as resistances with arrows. A: The unreactive ("leak") channels for different cationic batteries are separate and in parallel with the respective reactive channels. B: The "leak" conductance path is common to all cations. A separate unreactive conductance pathway for Cl is also included. Further description in text

as the hyperpolarization that is produced when Na_0 is reduced from 200 to 20 mM in 5.4 K_0 (Fig. 6). TTX causes a hyperpolarization of about 5 mV in squid giant axons (Freeman, 1970, 1971) and in lobster axons (Freeman, *personal communication*). It is possible that some of the electrogenically unreactive Na channel structures have the same chemical properties as do the electrically excitable, or, alternatively, that some of the latter, TTX-sensitive channels are open in the resting state. Further experiments will be required to clarify this matter.

Evidence for a separate system of invariant K channels is clear-cut for eel electroplaques (Nakamura, Nakajima & Grundfest, 1965*b*; Morlock, Benamy & Grundfest, 1968; Ruiz-Manresa, Ruarte, Schwartz & Grundfest, 1970; Ruiz-Manresa, 1970). In numerous other cells the resting characteristic is not altered by tetraethylammonium or similar agents that block K activation (Hagiwara & Saito, 1959; Hille, 1967). Thus, it appears likely that the K channels for the resting state of the membrane differ from those that can be opened (or closed) by electrical stimuli. The inclusion of a dual channel system for Ca is largely hypothetical at this time. However, the depolarization that is induced by increasing Ca (Figs. 8, 9 & 11) is accompanied by a decrease in conductance (Fig. 11 and Table 2), whereas the after-depolarization following the spike is accompanied by an increase in conductance which indicates Ca activation (Fig. 2).

In the alternative circuit of Fig. 12B, the three cation species are assumed to be capable of moving through the same electrically inexcitable, unreactive

regions of the membrane, but with different mobilities. In other words, these regions are effectively homogeneous and negatively charged, conforming to this extent to the requirements of the Nernst-Planck regime of a homogeneous membrane (Finkelstein & Mauro, 1963). The emf (E_x) depends on the mobilities of the respective cations and their electrochemical driving forces. It is also possible that Cl moves through the negatively charged regions. As the co-ion of the negative fixed charges, the mobility of Cl should be low. It is low, in fact, about four times less than the mobility of the positively charged divalent Ca.

The single invariant cationic conductance of Fig. 12B is equivalent to the "leak" conductance of the squid giant axon (Hodgkin & Huxley, 1952), since the latter is impermeable to Cl (Grundfest, Kao & Altamirano, 1954; Freeman, Reuben, Brandt & Grundfest, 1966). E_x is not the emf of any one cationic battery, and it is noteworthy in this connection that the nature of E_L in the squid equivalent circuit is still unclear. We have no information regarding the distribution of the various ions in our preparations, so that we cannot specify the respective ionic emf's of Fig. 12. Wallin (1967) has concluded that neither K nor Cl is in electrochemical equilibrium in the medial giant axon.

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