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IONIC MECHANISMS INVOLVED IN DIFFERENTIAL CONDUCTION OF ACTION POTENTIALS AT HIGH FREQUENCY IN A BRANCHING AXON

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SUMMARY

1. The ionic mechanisms involved in block of conduction of action potentials following high frequency stimulation were studied in a branching axon of the lobster *Panulirus penicillatus*.

2. A 2–3 mM increase in extracellular K concentration (normal concentration 12 mM) produced block of conduction into both daughter branches.

3. While conduction block induced by high frequency stimulation occurs first into the large daughter branch and only later into the smaller one, propagation into both branches is blocked simultaneously by increased extracellular K concentration.

4. Increasing extracellular K by 2–3 mM resulted in membrane depolarization, reduction in membrane resistance and reduced excitability. The latter two effects were larger than expected from the small depolarization. It appears that increase of extracellular K has direct effects on membrane excitability.

5. It is suggested that block of conduction after high frequency stimulation results from accumulation of K in the extracellular space. However, in order to account for differential conduction block in the two branches one must assume differential buildup of extracellular K concentration around the two branches during high frequency stimulation.

6. Ultrastructural studies using La and horseradish peroxidase as extracellular markers show that the space around the two branches is similar and is open to the extracellular space. Therefore differences in periaxonal volume cannot account for differential buildup of K around the two branches.

7. It is demonstrated that the lobster axon has a Na^+/K^+ electrogenic pump. After blocking this pump with ouabain, stimulation at high frequency resulted in a conduction block in the two branches almost at the same time.

8. Injection of Ca^{2+} intracellularly into the thick branch prevents or delays the appearance of conduction block after high frequency stimulation.

9. A mechanism based on these findings is suggested to explain the differential conduction block seen after high frequency stimulation in a branching axon with almost ideal impedance matching.

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INTRODUCTION

Goldstein & Rall (1974) computed that for a branching axon, the geometrical ratio

$$GR = \frac{M^{3/2} + L^{3/2}}{Ax^{3/2}}$$

(where M, L are the diameters of thick and thin branches, respectively, and Ax is the diameter of the parent branch), determines the safety factor for the propagation of a single action potential from the parent branch into the daughter branches. Where $GR = 1$, the action potential should propagate into the branches with the same safety factor as it propagates along the parent axon. In this case block of conduction after high frequency stimulation should occur at the same time all along the axon and not at the branch point *per se* (Parnas & Segev, 1979).

In contrast to the computations, experiments on a branching excitatory axon ($GR = 1$) which innervates two of the deep abdominal extensor muscles in the lobster (Parnas & Atwood, 1966) showed that at high frequency conduction from the main branch Ax into branch M is rapidly blocked, while it persists in the other branch (Parnas, 1972; Grossman, Spira & Parnas, 1973; Grossman, Parnas & Spira, 1979). Two major questions arise from these findings: (*a*) What causes the conduction block to appear at the branch point *per se* in a branching axon with $GR = 1$ and (*b*) what are the mechanisms allowing for the conduction block to appear first at the thick branch (M) and only later at the thin branch (L)?

During repetitive activation of an axon there is an increase in extracellular K concentration in the periaxonal space (Frankenhaeuser & Hodgkin, 1956). We therefore studied the effects such a change had on membrane excitability, especially since it was found that the increase in extracellular concentration of K and the accompanying depolarization cause conduction block in other axons (Spira, Yarom & Parnas, 1976). At the time of block, branch M is depolarized by only 1–3 mV (Grossman *et al.* 1979). Since such a small depolarization is not sufficient to account for conduction failure, we looked specifically for direct effects of these ionic changes on membrane excitability.

In the present article we show that a 2–3 mM increase in extracellular K concentration (resting concentration 12 mM) is sufficient to reduce membrane excitability such that conduction across the branch point *per se* is blocked. However, in order for the accumulation of K ions in the extracellular space during repetitive firing to be responsible for *differential* conduction block, one must assume a differential increase in extracellular K concentration around the two branches. We suggest that differential activation of recovery mechanisms, in the two branches, accounts for a differential increase in extracellular K concentration and the conduction block observed during high frequency stimulation. We can offer no explanation at this stage, for the finding that conduction fails at the branch point *per se*.

METHODS

The nerve innervating the deep abdominal extensor muscles of the lobster *Panulirus penicillatus* was used. A detailed description of the preparation and experimental setup is given in the preceding article (Grossman *et al.* 1979). Ions were injected intracellularly ionophoretically

using a floating current source (WPI-160). Changes in the concentration of K^+, Ca^{2+}, Mg^{2+} in the bathing fluid were compensated for by Na^+, keeping the osmolarity constant.

Electron microscopy. A modified method of Lane (1972) was used. Immediately after exposing the extensor muscles the nerve bundle was fixed *in situ* for 30 min in 6% glutaraldehyde with 2–3 drops of H_2O_2 in 0·1 M-cacodylate buffer. The pH was adjusted to 7·4 and the osmolarity to 1200 m-osmole with either NaCl or sucrose. After fixation with 1% osmium tetraoxide in cacodylate buffer was followed by dehydration in ethanol series. The nerve was embedded in Epon 812 and thick sections (2–3 μm) stained with 1% toluidine blue were used for light microscopy orientation. Thin sections of 500–700 Å (ultratome LKB III) were stained with uranyl acetate

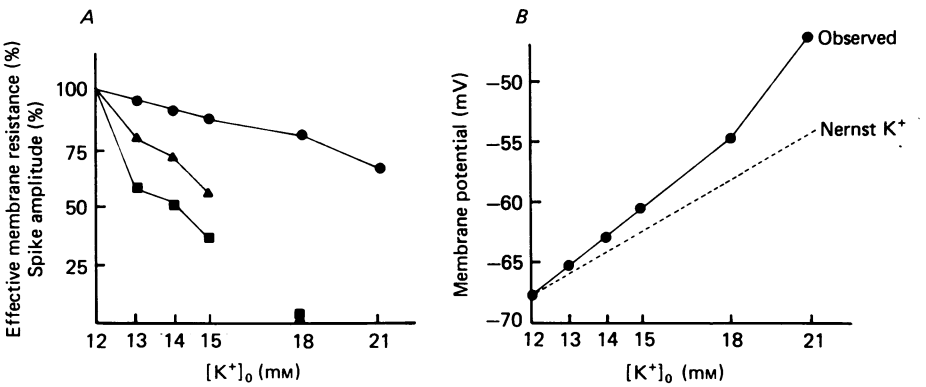

Fig. 1*A*, effect of extracellular K concentration on amplitude of action potential in *Ax* (triangles) and in *M* (squares). The amplitude is given as percentage of the control at 12 mM external K. The change of effective membrane resistance is also given as percentage of control, ●. The K concentration is plotted on a logarithmic scale; ▲, *Ax* spike; ■, *M* spike. *B*, dependence of membrane potential on external K concentration. The expected slope of 58 mV per tenfold change of $[K]_o$ is given as a straight line.

and lead citrate. Horseradish peroxidase (HRP type H Sigma) and La tracing techniques (Graham & Karnovsky, 1966; Machen, Erlij & Wooding, 1972) were used to determine the periaxonal space and diffusion pathways. The preparations were incubated in $La\,Cl_3$ or HRP for 0·25–3 hr. The material was then briefly washed in physiological solution and fixed. The preparations were embedded without staining in Araldite (Ciba). Philips EM300 electron microscope at 40–60 keV were used.

RESULTS

Effects of K ions. The small membrane depolarization (1–3 mV) accompanying high frequency firing of *Ax* is insufficient by itself to reduce membrane excitability and produce conduction block (Grossman *et al.* 1979). Such a depolarization could result from the accumulation of small amounts of K ions in the periaxonal space during repetitive activation of the axon. We therefore checked whether a small increase in K concentration reduces membrane excitability more than expected from the membrane depolarization alone.

In exploratory experiments the minimal extracellular concentration of K that produces a conduction block from *Ax* into *M* was determined. In these experiments intracellular electrodes were placed at *Ax* and *M* (200 μm before and after the branch point), each electrode served for recording and passing of current such

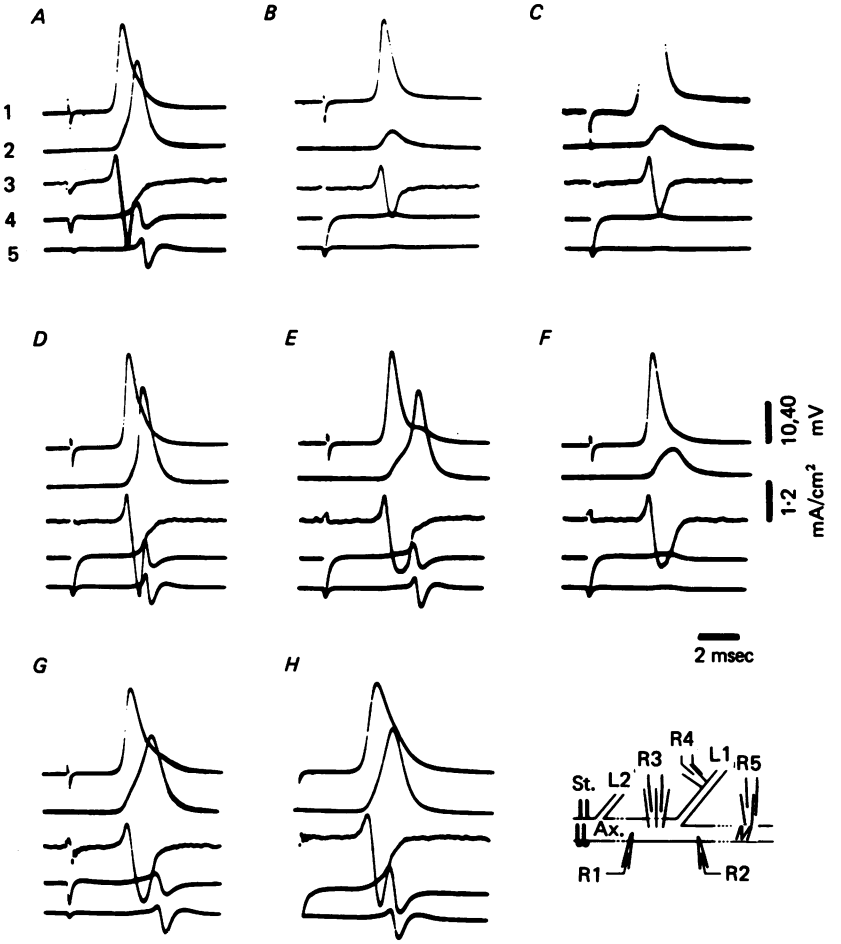

Fig. 2. Effects of increasing the concentration of extracellular K by 2·5 mM on conduction of action potentials from *Ax* to *M*. Experimental setup given in inset. Note that lines 1 and 2 are intracellular records at *Ax* and *M* respectively, line 3 is the membrane current measured by the patch clamp technique at *Ax*, and lines 4 and 5 are extracellular recording at the two branches *L* and *M* respectively. Changes in membrane potential due to the increase in K concentration or current injections are not shown in the Figure. *A*, control. *B*, 10 min after addition of 2·5 mM-K. The membrane was depolarized by 2·5 mV and conduction from *Ax* to both *M* and *L* was blocked. Note the large change in membrane current of *Ax* while there is almost no change in the *Ax* action potential. *C*, intracellular recording at *Ax* was made at a higher gain to enable precise hyperpolarization to the control membrane potential. Current to hyperpolarize the membrane was passed for 30 sec through the *M* micro-electrode. Note that despite the slight increase in *Ax* membrane current (line 3) conduction from *Ax* to *M* and *L* was still blocked. Between *C* and *D* the membrane was hyperpolarized by 12 mV (relative to the resting potential at the control) for 5 sec. *D*, 1 sec after stopping the current injection there was a recovery of invasion of action potential into the two branches and the *Ax* membrane current is as in the control in *A*. *E*, 9 sec after stopping the current injection. Note the slowing in rise time of the *M* action potential. *F*, 11 sec after hyperpolarization was stopped, block of conduction into the two branches. *G*, 15 min of wash with normal Ringer solution, partial recovery. *H*, 3 min more of wash, better recovery.

that the effective membrane resistance could be determined. The axon was stimulated at *Ax* by extracellular electrodes and extracellular electrodes recorded action potentials from *M*. The minimal increase in extracellular K concentration that produced a conduction block from *Ax* into *M* was 2·5 mM. This increase in K concentration produced membrane depolarization (Fig. 1*B*), reduction in effective membrane resistance (about 10%, Fig. 1*A*) and reduction in amplitude of the *Ax* action potential similar to those obtained at the time of conduction block after high frequency stimulation (see also Fig. 2*A*, *B* and Grossman *et al.* 1979).

In more detailed experiments (example Fig. 2) membrane responses were recorded intracellularly at *Ax* and *M* just before and after the branch point (Fig. 2, electrodes R1 and R2 in inset) and the membrane current was measured with the patch clamp technique (see explanation for shape of membrane current in Grossman *et al.* 1979, p. 298) at *Ax* close to the point of bifurcation (Fig. 2, electrode R3). Extracellular electrodes recorded activity at *L* and *M*, 3 mm after the branch point (Fig. 2, electrodes R4 and R5 respectively). About 10 min after the introduction of 14·5 mM-K^+ physiological solution (normal concentration 12 mM) the membrane was depolarized by 2·5 mV (changes in membrane potential not shown). Conduction of single action potentials into *both* the *M* and *L* branches was blocked at the *same time* (Fig. 2*B*, traces 2, 4, 5) and not differentially as seen after high frequency stimulation. Concurrently, the membrane current of *Ax* was greatly reduced (about 50%, Fig. 2*B*, trace 3) with only a small change (8–10%) in the amplitude of prebranch intracellularly recorded *Ax* action potential (Fig. 2*B*, trace 1). At this stage, inward current was injected through the *M* micro-electrode to hyperpolarize the membrane back to its resting level (Fig. 2*C*, trace 1 taken at a high gain). With 30 sec of hyperpolarization, conduction from *Ax* into *M* and *L* was not restored (Fig. 2*C*). Although a slight increase in the *Ax* membrane current was observed, it did not completely recover to the control level. These results confirm again that the small membrane depolarization is not the main cause for the reduction in excitability and that K ions must exert other effects on membrane excitability. Nevertheless, when the membrane was hyperpolarized for 5 sec by 12 mV (relative to the resting potential at the control), *Ax* membrane current increased substantially (and there was a change in its shape) and immediately after the injection of inward current was stopped, conduction of action potentials from *Ax* into *M* and *L* was restored (Fig. 2*D*). The effects of the strong hyperpolarization outlasted the period of current injection and only after 11 sec was conduction blocked again (Fig. 2*E–F*). After 15 min of wash in normal physiological solution, there was a recovery of conduction *both* in the *M* and *L* branches (Fig. 2*G*). (It should be remembered that during recovery from conduction block caused by high frequency stimulation, conduction reappeared first in the *L* branch and only later in the *M* branch (Grossman *et al.* 1979).) With further wash (3 min more), the rise time of the *M* action potential was almost as in the control (Fig. 2*H*). 20 min of wash were required for a complete recovery (not shown).

In the same experiment the effective membrane resistance (R_e) was measured before and after increasing the extracellular K concentration. The values of R_e were obtained by passing a long ramp current pulse and measuring the membrane potential. The slope dV/dI was then plotted against the deviation from the membrane potential at the beginning of the experiment at each point (Spira *et al.* 1976). In the

control (Fig. 3, circles), R_e was constant in the range of +2 to −12 mV deviation from the resting potential. With stronger depolarization a reduction in R_e (rectification) was seen. 10 min after the K concentration was raised to 14·5 mM (2·5 mM increase), the membrane was depolarized by 2·5 mV and showed a reduction of 24 % in R_e (in different fibres the change in R_e was between 10 and 25 %); at that point hyperpolarization did not restore R_e, and the curve now follows a different course

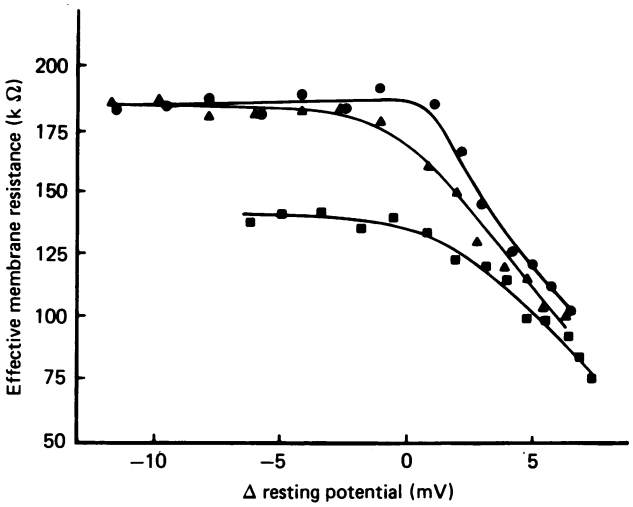

Fig. 3. Effects of external K concentration on effective membrane resistance as a function of membrane potential. The experiment was made on the same axon as in Fig. 2. Effective membrane resistance was measured by passing long (400 msec) ramp current pulses in the hyperpolarizing and depolarizing directions. The membrane resistance was taken as dV/dI at each point of membrane potential. Control: filled circles. Note rectification with 2–3 mV depolarization. Squares, after addition of 2·5 mM-K there is a reduction in effective membrane resistance, which remained low even at hyperpolarization. Triangles: recovery after wash. ●, control; ■, +2·5 mM-K^+; ▲, recovery.

(Fig. 3, squares). After 15 min wash in normal solution, R_e recovered to its original value (Fig. 3, triangles) and dependence on membrane potential. These results indicate that extracellular K affects membrane excitability and conductance more than expected from the membrane depolarization alone.

Additional effects of K can also be inferred from the dependence of the resting potential on extracellular K concentration, given in Fig. 1*B*. The slope is steeper than 58 mV per tenfold change of $[K]_o$ which is expected from the Nernst equation. Indeed, since the membrane of lobster axons is also permeable at rest to Na and chloride ions (Katz & Freeman, 1972; Brinley, 1965) the slope of the curve is expected to be *less* than 58 mV. The slope of the experimental curve is 61·2 mV per tenfold change of $[K]_o$ between 12 and 18 mM-K and it increases to 67·4 mV per tenfold change of $[K]_o$ above 18 mM.

In other experiments the effects of different concentrations of extracellular K (12–21 mM) were measured and we found that always conduction from Ax into M and L was blocked when the extracellular concentration was increased by 3 mM (Fig. 1*A*).

minimal increase necessary to produce block was 2·5 M. At the stage of the block from *Ax* into *M* and *L* (with 3 mM increase) the *M* branch could be directly excited to conduct action potentials up to the branch point. However, at a concentration of 18 mM extracellular K neither *Ax* nor *M* could be excited by direct stimulation. These results show that the safety factor for propagation of a single action potential across the branch point is lower than that for propagation along the axon, even though the geometrical ratio is one.

Ultrastructure. After increasing the extracellular K concentration conduction into both branches was blocked at the same time. With repetitive stimulation, however, conduction into the branches was blocked differentially. If the increased extracellular K concentration is responsible for the block then one must assume differential increase in K concentration around the two branches. A differential buildup of K could result from differences in periaxonal spaces around the branches, the diffusion pathways or recovery processes. We therefore studied the ultrastructural organization of the parent axon and daughter branches.

In lobster axons we observed no additional tissue components, such as fat bodies (Treherne & Moreton, 1970) or extensive connective tissue envelopes (Hatt & Smith, 1975; Smith, 1977), that might form regions restricting diffusion. The enveloping sheath is composed of several layers of interstitial cells containing large nuclei and having long plasmatic processes. Amorphous interstitial material which is found between the glial processes also forms the basal lamina (Pl. 1*A*, *B*, BL). The interstitial material contained numerous fibrils (probably collagen, 50–100 Å in diameter) arranged in an orientation parallel to the longitudinal axis of the axon. One to three glial cells appeared to surround the axon. The membrane of glial cells facing the axon is wavy and interdigitations are found between adjacent glial cells (Pl. 1*B*). The space between adjacent glial cells is about 100–150 Å and no special structure that might restrict diffusion such as tight junctions were seen. In the glial cells, large nuclei, mitochondria and microtubuli were visible. A complex network resembling endoplasmic reticulum seemed to run through the glial cell (Pl. 1*C*, *D*). These networks appeared to be open and connected the basal lamina space (Pl. 1*C*) with the periaxonal space (Pl. 1*D*).

Mitochondria were found all along the parent and daughter branches with no apparent systematic differences in their densities. Neurotubuli (T) and neurofilaments (F) were also observed. A number of large vesicles have a double membrane structure and are probably pockets protruding from the glial cells (De Lorenzo, Dettbarn & Brzin, 1969; Perrachia, 1974). The periaxonal space is about 200 Å in width and is continuous with the interglial space, forming a channel of about 6–10 μm in length (Pl. 2*B*).

The preparation was soaked in solutions containing colloidal La chloride or horseradish peroxidase (HRP). These markers diffused into the periaxonal space, indicating that the periaxonal space is open to the extracellular compartment. Diffusion was through two main pathways: the interglial space (Pl. 2*B*, *C*) and the complex networks penetrating the glial cells (Pl. 2*A* and Holtzman, Freeman & Kashner, 1970). No obvious differences were seen in the ultrastructural organization or length of diffusion pathways in areas close to the bifurcation in the main axon (*Ax*), the wide branch (*M*) or the thin branch (*L*). Therefore, differences in ultra-

structure of the two branches do not suggest an immediate explanation for the differential conduction block.

Electrogenic pump. A factor that might be involved in a differential accumulation of K around two branches of an axon is a different rate of recovery, such as differential activation of an electrogenic Na pump. Activation of an electrogenic Na pump after stimulation at high frequency has been demonstrated by Van Essen (1973) and Jansen & Nicholls (1973) for a branching neurone in the leech, and there is evidence for electrogenic Na pump in *Homarus* axons (Sokolov & Cooke, 1971).

Several lines of evidence suggested the presence of a Na-K pump in the common excitatory axon of *Panulirus*. The hyperpolarization seen after 100 Hz stimulation (Grossman *et al.* 1979) already served as a hint for the presence of such a pump. Lowering of the extracellular K concentration to 1 mM, resulted in 8–10 mV depolarization (eight experiments). The effect was reversible in normal solution. Substituting all Na^+ by Li^+ produced 3–6 mV depolarization (five experiments) together with a reduction of about 10% in effective membrane resistance. The axon remained excitable and showed action potentials which were smaller (by 19%) than the control. It is possible that blocking of the pump with Li^+ (Keynes & Swan, 1959; Thomas, 1972) did not produce a pronounced depolarization because Li^+ is known also to increase the membrane conductance for K (Partridge & Thomas, 1976). Cooling from 16 to 4 °C resulted in an 8 mV depolarization. Intracellular injection of Na ions resulted in a hyperpolarization.

Inhibition of this pump should cause the time to block to decrease and to approach the same value in both branches. Addition of 10^{-3} M-ouabain produced 5–8 mV depolarization accompanied by about 20% reduction in the amplitude of the action potential. This depolarization by itself is not sufficient to block conduction of action potentials from *Ax* into *M* (Grossman *et al.* 1979). In control experiments prolonged depolarization (5–8 mV for 100 sec) produced by outward current injections affected only slightly the duration of conduction at high frequency. Usually the time to block was shortened by only 20% (Grossman *et al.* 1979). In the ouabain treated axons, the time required to produce a conduction block at a given frequency (30–50 Hz) was much shorter (Table 1). As can be seen in Table 1, the time required to stimulate *Ax* in order to block conduction into the thin *L* branch was drastically shorter and the differential development of conduction block in the two branches was almost nonexistent.

Effects of Ca^{2+} ions on conduction. During the period of the action potential, Ca^{2+} ions enter the axon (Baker, Hodgkin & Ridgway, 1971; Stinnakre & Taux, 1973) and after high frequency stimulation, their intracellular concentration reaches levels that reduce outward movement of K ions (Heyer & Lux, 1976*a*, *b*. During high frequency stimulation of the common excitatory axon intracellular Ca^{2+} concentration should reach higher levels in the thin branch *L* than in branch *M*. This difference might be an additional factor for the earlier failure of conduction into branch *M*.

In nine experiments where the extracellular Ca^{2+} was increased to 60 mM the membrane was hyperpolarized by 2–5 mV and the membrane resistance increased by 10–20%, similar to the observations of Wright & Tomita (1965). In addition, the action potential amplitude increased by 2–10 mV and its duration was prolonged

TABLE 1. Effect of 1 mM-ouabain on the time required to produce conduction block after stimulation at high frequency

	Control Time for block (sec)		Ouabain Time for block (sec)		Stimulus frequency
Expt.	*M*	*L*	*M*	*L*	(Hz)
1	120	> 340	4	4	30
2	147	> 240	20	20	30
3	120	> 240	30	17	30
4	29	60	11	11	50
5	5	35	2	2	50
6	5	9	2·5	2·5	50

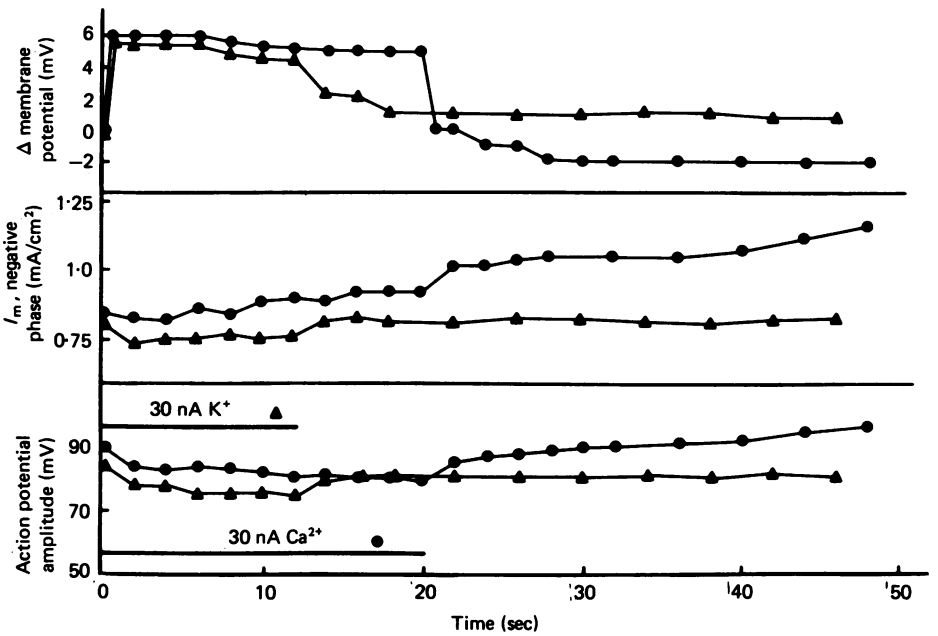

Fig. 4. Effects of intracellular injection of Ca^{2+} and K^+ on amplitude of the action potential, membrane current and membrane potential. Two micro-electrodes, one filled with 0·9 M-$CaCl_2$, the second with 3 M-KCl were inserted into the *Ax* region. A patch clamp electrode was placed in between, 40 μm from each of the micro-electrodes. At first, 30 nA of current for 20 sec were passed through the Ca^{2+} micro-electrode, the indifferent electrode was extracellular. During the current injection there was a depolarization of about 6 mV, and the amplitude of the action potential was slightly reduced. After the current injection was stopped there was a steady increase in the amplitude of the action potential (filled circles for Ca^{2+}). A steady increase in the negative peak of the membrane current was seen during and after Ca^{2+} injection. The membrane was hyperpolarized by about 2 mV. In a control, when K was injected, triangles, the after-effects were missing.

(Adelman & Dalton, 1960). In these experiments, the axon could sustain high frequency stimulation for periods 3–5 times longer than in controls. Similar changes in Na concentration had no effect. We could not study the effects of lowering extracellular Ca^{2+} concentration, as under such conditions the axon deteriorated rapidly.

We also tested the effects of intracellular injections of Ca^{2+} on the conduction of action potentials at high frequency. In the experiment illustrated in Fig. 4, two micro-electrodes were inserted in *Ax* before the branch point. One electrode was filled with 3 mM-KCl, the second with 0·9 M-$CaCl_2$. These electrodes served for K^+ or Ca^{2+} injections. The same electrodes were used in order to pass current and to measure membrane potential. In addition, an extracellular patch clamp electrode was placed between the two micro-electrodes. The distance between the patch clamp and each of the intracellular micro-electrodes was about 40 μm. Extracellular electrodes at *Ax*, *M* and *L* served for stimulation and recording.

During the period of Ca^{2+} injection into the cell (30 nA, 20 sec), the membrane depolarized by about 6 mV and the action potential amplitude was slightly reduced (Fig. 4, circles). However, after Ca^{2+} ionophoresis was stopped, the membrane hyperpolarized beyond the resting potential (-2 mV) and now the amplitude of the action potential and of the membrane current was increased for many seconds (Fig. 4, circles). On the other hand, after K was injected as a control, similar changes in resting potential, action potential amplitude and membrane current were not seen (compare triangles with circles, Fig. 4).

In three other experiments the Ca^{2+} micro-electrode was inserted into the *M* branch. After conduction from *Ax* into *M* was blocked, injection of Ca^{2+} (30 nA, 40 sec) restored conduction for a period of 8–10 sec. With repeated C^{2+} injections, the period of restored conduction could be prolonged to about 20 sec. Thus, we conclude that an increase in intracellular Ca^{2+} concentration prevents or delays conduction block after stimulation at high frequency and promotes recovery in blocked fibres.

DISCUSSION

In the present work, we have studied effects of changes in extracellular and intracellular ion concentrations on conduction of action potentials at high frequency. During the period of the action potential, there is an efflux of K ions to the periaxonal space (Hodgkin & Huxley, 1952; Frankenhaeuser & Hodgkin, 1956) and an influx of Na and Ca into the axoplasm (Hodgkin & Huxley, 1952; Baker *et al.* 1971). The periaxonal space for the common excitatory axon is about 200 Å in width and the structure of the glial cells and diffusion pathways seems to be similar for *Ax*, *M* or *L*. Therefore we can postulate that at least in the beginning of a train of action potentials, K accumulation around the two branches is the same. Assuming the same membrane properties for *Ax*, *M* and *L*, K will then have the same effects in all branches (Parnas & Segev, 1979). In contrast, the difference between surface to volume ratio in the *M* and *L* branches might be the anatomical basis for the differential conduction of action potentials at high frequency in these two branches. Specifically, we suggest that an increase in intracellular concentrations of Na and Ca triggers mechanisms that slow or reverse the processes leading to the development of the block of conduction from *Ax* into *M* and *L*.

K ions. The similarity between the changes seen after repetitive stimulation or application of 2·5–3 mM-K suggests that the main reason for the development of the block is the accumulation of extracellular K during the high frequency activation of the axon. It should be emphasized that in our experiments a small (2·5 mM) increase in K concentration, which is definitely in the physiological range and can be obtained after few impulses at high frequency stimulation (Frankenhaeuser & Hodgkin, 1956) was sufficient to produce the conduction block.

The phenomena seen after block by high frequency stimulation, namely, a 10–20% reduction in effective membrane resistance, about 20% reduction in the amplitude of the *Ax* action potential, about 2·5 mV depolarization and the finding that hyperpolarizing pulses of 12–15 mV could temporarily restore conduction can all be explained by the accumulation of K in the periaxonal space during the repetitive firing. The long rest time (20 min) required for complete recovery after high frequency stimulation also fits well with the wash times required after treatment with the 2·5 mM increased K solution.

The mechanisms behind the effects of a 2·5 mM increase in K concentration are not clear. The membrane of lobster axons behaves as a K electrode only above 25 M external K concentration. Below this concentration the membrane potential is determined, in addition by other ions (Dalton, 1958; Julian, Moore & Goldman, 1962*a*). Therefore, the slope of the curve relating resting potential and extracellular K concentration (below 25 mM) should be *less* than 58 mV per tenfold change in K concentration. In fact, in the common excitatory axon the increase in extracellular K concentration produced membrane depolarization greater than that expected from the Nernst equation (Fig. 1*B*). Such a result is possible if the permeability ratios (P_{Na}/P_K, P_{Ca}/P_K, P_{Cl}/P_K) are not constant but vary with external K concentration. An alternative explanation demands that the contribution of an electrogenic pump to the resting potential varies with external K either by reduced activity or by a variation in the coupling ratio (Thomas, 1972). However, changes in pump activity usually are not followed by changes in membrane resistance (Fig. 1*A*) although such a case has been demonstrated in the leech (Jansen & Nicholls, 1973).

The finding that the increase in extracellular K concentration resulted in reduction of effective membrane resistance points to the increase in conductance for one or more ions. This increase cannot be in G_K alone, since if we assume that all of the change in effective membrane resistance is due to increased G_K the membrane potential should be dependent on extracellular K concentration according to the Nernst equation. We therefore conclude that the reduction in effective membrane resistance and the concomitant membrane depolarization are produced at least in part by an increase in conductance to another ion or ions, whose equilibrium potential is positive with respect to the resting potential.

The possible role of increased extracellular K concentration in producing a conduction block at high frequency in homogeneous and unhomogeneous axons has been discussed in several works. In some of these studies (Hatt & Smith, 1975; Spira *et al.* 1976) the authors argue that K exerts its effects indirectly through membrane depolarization and consequent Na inactivation (Hodgkin & Huxley, 1952). In other works direct effects of K on membrane excitability were shown (Adelman & Palti, 1969*a*, *b*; Adelman, Palti & Senft, 1973) and these effects were modelled

theoretically (Adelman & Fitzhugh, 1975). The results presented in the present paper, using another preparation, show that K has effects on membrane excitability in addition to those expected from membrane depolarization alone. Such direct effects of K on membrane excitability are probably not universal since no specific influence of external K on slow Na inactivation was detected in the giant axon of *Myxicola* (Schauf, Pencek & Davis, 1976). In the work of Adelman & Palti (1969*a*, *b*) in the squid axon 25–100 mM-extracellular K was required in order to affect directly Na inactivation. Adelman & Palti 1969*b*) also showed that by clamping the membrane potential to −120 mV they could overcome the effects of the high extracellular K. In our results, membrane excitability could be restored by 10 15 mV hyperpolarization for the duration of 2–3 sec (Fig. 2). The smaller hyperpolarization required is explained by the much smaller K concentrations used in our study. In the squid (Adelman & Palti, 1969*a*) and lobster Julian *et al.* 1962*a*), it was shown that when the axon's enveloping sheaths remained intact, 8–10 min of incubation were required in order to obtain the effects of K. However, once the K reached the periaxonal space, and the block was releieved by hyperpolarizing current, only a few seconds were necessary after the hyperpolarization was stopped in order for the block to reappear.

In the squid, the amount of K ions flowing through 1 cm^2 of membrane per impulse was estimated as 4×10^{-12} mole (Frankenhaeuser & Hodgkin, 1956; Keynes, 1951). If we assume for the lobster the same value for movement of K ions, and estimate that in the lobster the amount of K pumped out from the periaxonal space is 14×10^{-12} mole/cm^2 sec (Brinley, 1965), then it will take about 250 msec for the added K after a single action potential to be removed from the periaxonal space. Such a low removal rate, theoretically limits the stimulation frequency which will not produce changes in external K to 4 Hz. However, other mechanisms such as diffusion or active uptake by adjacent glial cells (Orkand, Nicholls & Kuffler, 1966; Henn, Haljamae & Hamberger, 1972) might accelerate recovery. Empirically, the frequency at which sufficient potassium accumulates to produce a conduction block is about 30 Hz.

There was one important difference in the way conduction was blocked and restored after stimulation at high frequency and after adding 2·5–3 mM-K^+. With stimulation, the block and recovery appeared at different times in the thick and thin branches, while with K the block and recovery appeared at the *same* time for the two branches. If one assumes the same membrane properties for the two branches, accumulation of potassium should indeed produce the block at the same time in the two branches (Parnas & Segev, 1979). Obviously, the differential block is produced by mechanisms triggered by repetitive stimulation.

Electrogenic pump. Under ouabain, the time required for stimulation at a given frequency to produce a conduction block was considerably shorter than that expected from the membrane depolarization produced by the ouabain treatment. It is reasonable to assume that the important factor accelerating block is the more rapid accumulation of K in the extracellular space after the pump is inhibited. It is important to note in this connection that in those experiments, conduction into the two branches was blocked at almost the same time, similar to the results after application of 2·5–3 mM-K. This suggests that the pump is responsible for the

differential buildup of K concentration around the two branches during repetitive activation.

It is known that intracellular injection of Na into neurones activates electrogenic pumps (Thomas, 1972; Nakajima & Takahashi, 1966). During repetitive firing, Na concentration will increase faster in the thin *L* branch and should activate the pump sooner. Activation of the pump will then have a dual effect. First, it will hyperpolarize the membrane (such a hyperpolarization could not be recorded at this stage from the *L* branch because of its small diameter). Secondly, it will reduce the K concentration around the *L* branch. Both actions will tend to prevent the *L* branch from becoming less excitable. However, with higher frequency or over a longer period of stimulation, the K concentration around branch *L* will eventually reach the level that reduces excitability below that required for conduction of action potentials.

Ca ions. Ca ions have different, and at times antagonistic, effects on membrane excitability and ion conductances when applied extracellularly (Frankenhaeuser & Hodgkin, 1957; Julian, Moore & Goldman, 1962*b*; Adelman & Moore, 1961). In our experiments, increasing extracellular Ca^{2+} concentrations produced a hyperpolarization, an increase in the effective membrane resistance and an increase in the amplitude of the action potentials. Each of these effects improves the safety factor for conduction of action potentials. Intracellular injection of Ca^{2+} was found to alter excitability and potassium conductance (Meech, 1972; Heyer & Lux, 1976*b*). In our experiments intracellular injection of Ca^{2+} improved conduction of action potentials at high frequency.

We can then visualize the appearance of a differential conduction block in the following way. During high frequency activation, K initially accumulates at the same rate around *Ax*, *L* and *M*. Na^+ and Ca^{2+} accumulate intracellularly more in *L* than in *M* and *Ax*. Na^+ then triggers the pump sooner in branch *L*, lowering external K^+ concentration. Intracellular Ca^{2+} in *L* will also increase more rapidly and may reach the levels that improve conduction sooner. The faster buildup of K concentration around branch *M* reduces its excitability and conduction block will occur first into branch *M*. However, according to such reasoning, a larger amount of K, is expected to accumulate around the thickest branch *Ax*. But we showed that while conduction across the branch point is blocked when 3 mM-K^+ are added, 18 mM are required to block conduction along *Ax*. Thus, although we can account for conduction failure and the differential block in the two branches, it is still unclear why block occurs at a specific location, the branch point, in an axon system where the geometrical ratio is 1, and additional mechanisms must be sought.

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EXPLANATION OF PLATES

PLATE 1

A, cross-section of the axon and its sheaths. A, axon; BL, basal lamina; G, glia; N, nucleus; S, sheath.

B, cross-section of a region of two adaxonal glial cells. *A*, axon; F, filaments; T, tubuli; G, glial cell; BL, basal lamina.

C, a section through a glial endoplasmic regiculum. BL, basal lamina; ER, endoplasmic reticulum, arrows point to openings to the basal lamina; G, glial cell; A, axon.

D, same as *C*, showing the opening of the endoplasmic reticulum to the periaxonal space (at left).

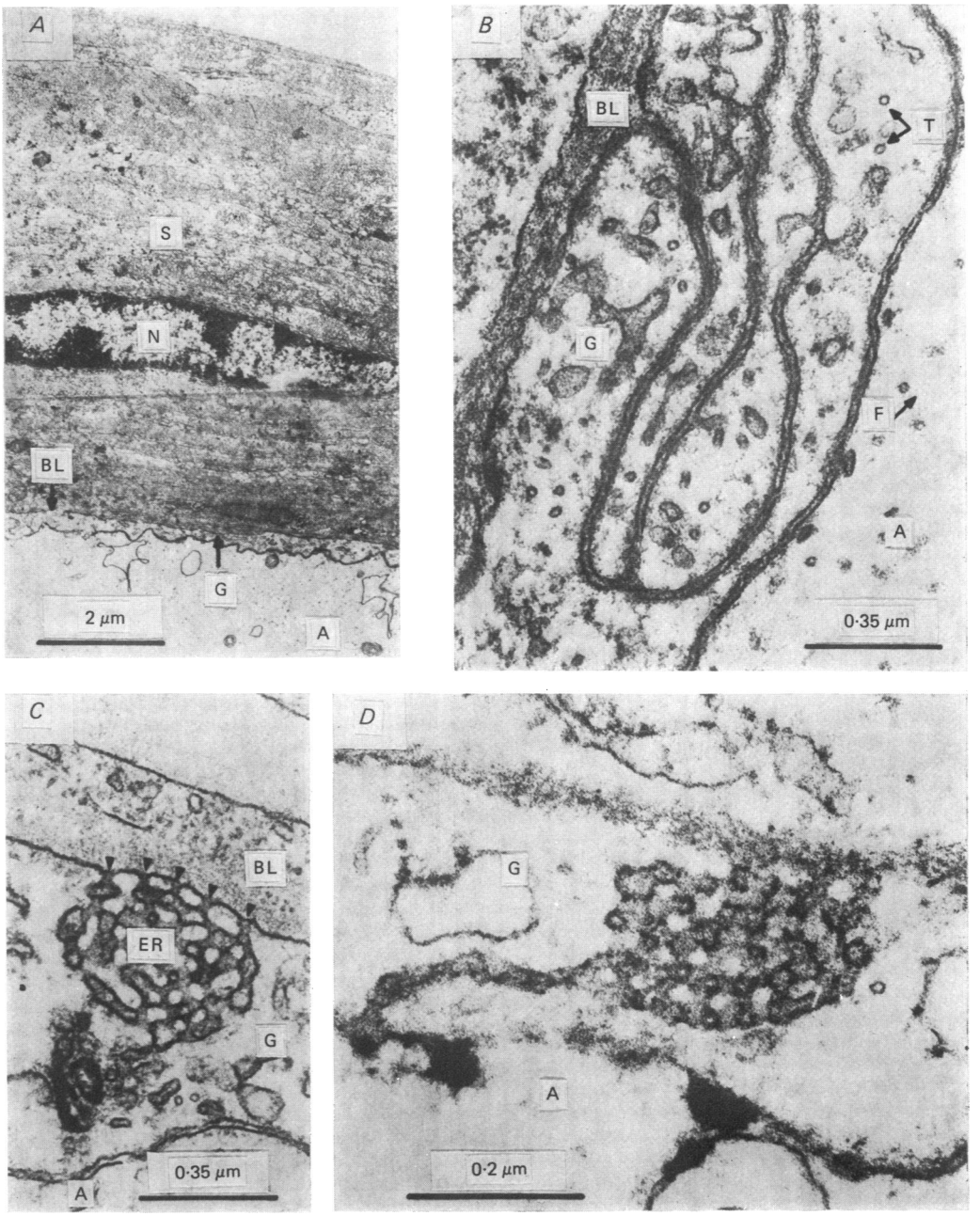
PLATE 2

Diffusion pathways as seen with La and horseradish peroxidase.

A, endoplasmic reticulum crossing a glial cell, showing openings (arrows) to the basal lamina space and the periaxonal space. A, axon; G, glial cell; S, sheath.

B, the space between two glial cells filled with La. A, axon; G, glial cells; S, sheath.

C, HRP staining of the interglial space. From top: S, sheath; G, glial cell; A, axon. Asterisks: pockets protruding probably from glial cells, the glial endoplasmic reticulum is marked by arrows.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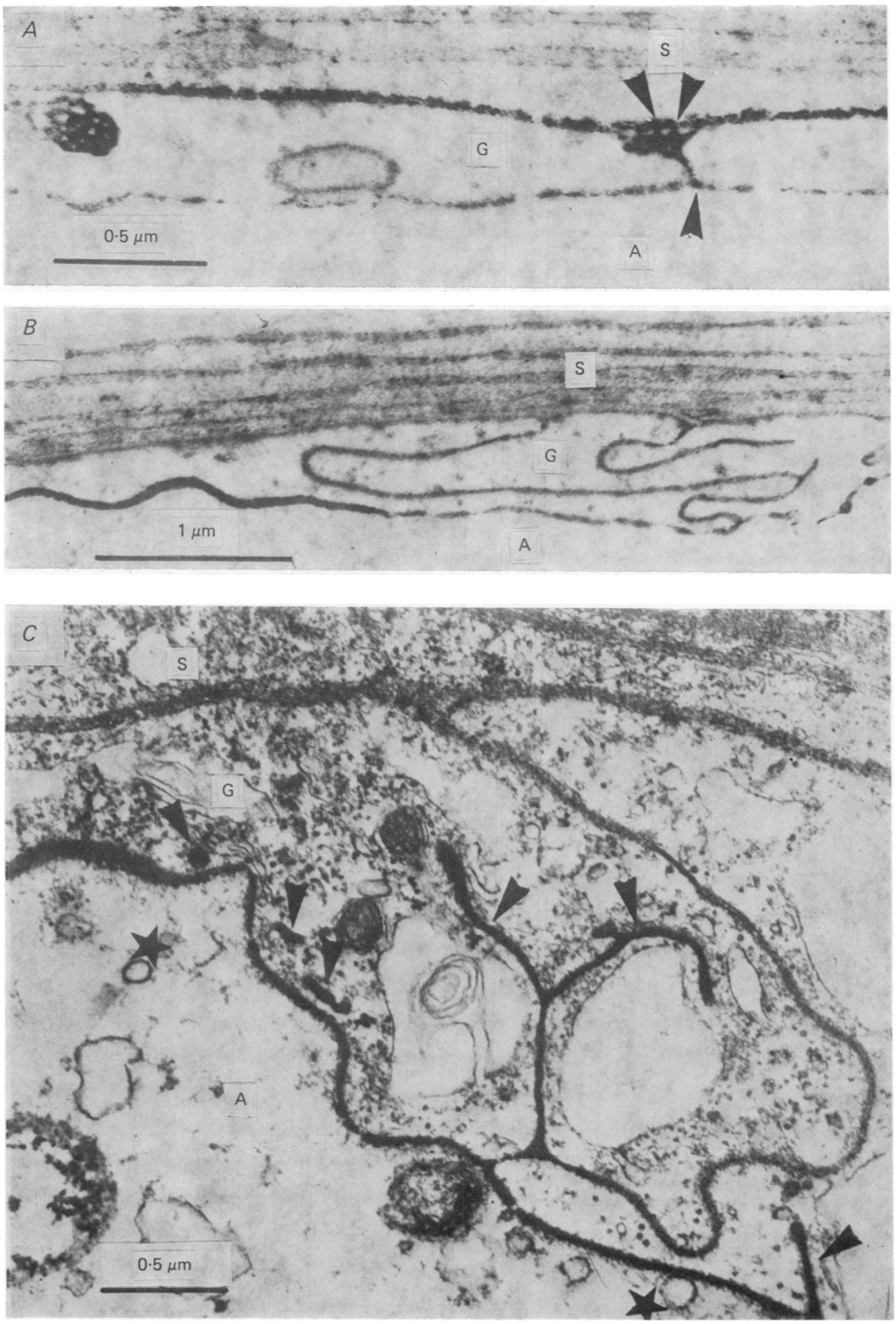

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