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# EFFECTS OF ALTERED EXTRACELLULAR AND INTRACELLULAR CALCIUM CONCENTRATION ON HYPERPOLARIZING RESPONSES OF THE HAMSTER EGG

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SUMMARY

1. Upon fertilization the hamster egg shows transient, periodic hyperpolarizing responses (h.r.s) due to a Ca-activated K conductance; these are superimposed on a gradual, hyperpolarizing shift of the resting potential (h.s.) (Miyazaki & Igusa, 1981*a*, 1982*a*). The h.r.s and h.s. were further analysed by changing external divalent cations or by injection of $Ca^{2+}$ into the egg, to study the mechanisms of the increase in the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$).

2. The series of h.r.s was abolished by the removal of external $Ca^{2+}$. The frequency of the h.r. was decreased by lowering the $[Ca^{2+}]_o$ or by adding $Mn^{2+}$or $Co^{2+}$, and it was increased by raising the $[Ca^{2+}]_o$ in a time- and concentration-dependent manner. The h.r. frequency was decreased on sustained depolarization with steady current, and increased on hyperpolarization.

3. In contrast to the h.r. frequency, the amplitude, conductance increase and reversal potential of each h.r. were little affected by $[Ca^{2+}]_o$, $Mn^{2+}$ or $Co^{2+}$.

4. The h.s. was decreased by lowering the $[Ca^{2+}]_o$, by adding $Mn^{2+}$ or $Co^{2+}$, or by injection of EGTA. The h.s. may reflect continuous Ca influx stimulating a Ca-activated K conductance ($G_K$).

5. In unfertilized eggs a regenerative h.r. was induced by Ca injection with an apparent threshold. The relationship between $G_K$ and the injected $Ca^{2+}$ showed a steep jump at the critical current, associated with a four-fold increase in $G_K$. The regenerative h.r. was followed by a refractory period of 1–2 min.

6. In inseminated eggs the periodic sperm-mediated h.r.s (s.–h.r.s) were interrupted by interposed h.r.(s) induced by Ca injection(s): the periodicityof s.–h.r.s was reset by Ca-induced h.r.

7. In inseminated eggs the regenerative h.r. was induced by Ca injection with a much smaller pulse than necessary in unfertilized eggs. The refractory period was shortened to 40–50 sec, comparable to the period of s.–h.r.s.

8. In inseminated eggs periodic h.r.s similar to s.–h.r.s were produced by continuous, repetitive injections of $Ca^{2+}$ with constant pulses. The frequency of these h.r.s was dependent on the injection current.

9. It is concluded that each h.r. indicates an enhancement of the increase in $[Ca^{2+}]_i$,

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probably the result of Ca-induced Ca release from intracellular stores. A possible mechanism for periodic increase in $[Ca^{2+}]_i$ reflected in s.–h.r.s is proposed, based on a linkage of the continuous Ca influx to Ca release.

## INTRODUCTION

At the early stage of fertilization, a dramatic rise in the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) has been shown to occur in eggs of the sea urchin (Steinhardt, Zucker & Schatten, 1977) and medaka fish (Gilkey, Jaffe, Ridgway & Reynolds, 1978). The inseminated egg also shows a transient depolarization of the membrane potential called the activation (or fertilization) potential (Hagiwara & Jaffe, 1979). Golden hamster eggs have been found to show recurring, transient hyperpolarizations in response to fertilization (Miyazaki & Igusa, 1981*a*). The hyperpolarizing responses (h.r.s) appear in an all-or-none manner. They occur continuously at fairly constant intervals of 40–50 sec, especially when multiple sperm enter the egg.The first three to four h.r.s reach −80 to −75 mV from the resting potential of *ca.* −30 mV, and later h.r.s reach −65 mV, superimposed on a gradual shift of the resting potential (h.s.) to −40 mV (Miyazaki & Igusa, 1981*a*). Each h.r. is found to be caused by an increase in the membrane permeability exclusively to $K^+$ ions and it is based on a Ca-activated K current (Miyazaki & Igusa, 1981*b*, 1982*a*), which has been found in a variety of cells (Meech, 1978). These observations have led to the conclusion that $[Ca^{2+}]_i$ increases periodically during fertilization of the hamster egg.

Cytoplasmic free $Ca^{2+}$ regulates muscle contraction (Ebashi & Endo, 1968). In non-muscle cells, the intracellular $Ca^{2+}$ also mediates a number of cellular processes (reviewed by Rasmussen & Goodman, 1977) such as membrane permeabilities to inorganic ions (Meech, 1978; Hagiwara & Byerly, 1981), exocytotic secretion (Douglas, 1968) and cell motility (Hitchcock, 1977). Therefore, the analysis of the periodic increase of $[Ca^{2+}]_i$ in the hamster egg may be considered to be of general importance as a study of the movement of Ca ions in non-muscle cells. The present paper is concerned with the analysis of the periodic h.r.s to deduce the mechanism involved in the periodic increase of $[Ca^{2+}]_i$, based on the following questions: (1) whether the increase is due to $Ca^{2+}$ influx across the plasma membrane or due to Ca release from intracellular binding sites or stores; (2) whether a regenerative and periodic increase in $[Ca^{2+}]_i$ is induced by intracellular injection of $Ca^{2+}$.

Preliminary accounts of this work have appeared elsewhere (Miyazaki & Igusa, 1982*b*; Igusa & Miyazaki, 1982).

## METHODS

*Eggs.* Adult females of the golden hamster were treated with intraperitoneal injection of 20 i.u. pregnant mare serum (PMS, Sankyo), followed 48 hr later by 20 i.u. human chorionic gonadtropin (HCG, Teikokuzohki) (Yanagimachi, 1969). The superovulated females were stunned by a blow to the head, and mature eggs were collected from the oviducts between 14 and 15·5 hr after the HCG injection. The eggs were freed from cumulus cells by treatment with 0·05% hyaluronidase (P-L Biochem.) in culture medium or standard saline (see below) for 30–60 sec at 23–26 °C, and then by repeated aspiration with a glass capillary. The surrounding zona pellucida was dissolved by applying 0·07% trypsin (GIBCO) for 3 min. The eggs were washed with culture medium or standard saline several times after each treatment. The zona-free eggs allow the sperm to reach the egg plasma membrane soon after insemination, and promote better micro-electrode impalements. The eggs were transferred to a 0·4 ml. drop of medium in a plastic Petri dish and covered with

paraffin oil. The dish was treated with poly-L-lysine (Yavin & Yavin, 1974) to make eggs stick to the bottom more easily. The isolated eggs often underwent spontaneous activation during incubation for more than 1·5 hr, releasing the second polar body. The spontaneously activated eggs were discarded. The mean diameter of the egg is about 72 $\mu$m.

*Sperm and insemination.* Spermatozoa obtained from the cauda epididymis were suspended in modified Tyrode solution containing 10% bovine adrenal gland extract, and 20–30 $\mu$l. of the suspension was added to 0·4 ml. of Tyrode solution containing 20% human serum (Yanagimachi, 1970). The sperm were incubated for more than 4 hr at 37 °C (gas phase, 5% $CO_2$ in air). These procedures are necessary to make sperm undergo 'capacitation' to be capable of interacting with eggs (Austin, 1952). Experiments were carried out at 30–32 °C on a heated microscope stage. After penetration of the egg with a micro-electrode, 5–20 $\mu$l. of sperm suspension was added to the medium surrounding the egg, using a microsyringe. Final sperm concentration was $1–5 \times 10^4$ cells/ml. Severe polyspermy occurs in the zona-free egg. The present experiments were performed during fertilization associated with more than several sperm entries into each egg. The sperm head and entire tail are incorporated into the egg cytoplasm in 1 hr after the attachment to the egg surface (*in vivo* experiments, Sugawara, Totsukawa & Masaki, 1980).

*Electrical recordings.* A glass micro-electrode filled with 4 M-K acetate (or 3 M-KCl in some cases) was used for recording the membrane potential. The resistance of the electrodes ranged between 40 and 100 MΩ. Membrane potentials were recorded on a pen recorder and an oscilloscope. To monitor a change in the membrane conductance, constant current pulses were applied through the intracellular electrode by means of a bridge circuit. To avoid bridge unbalance, a second electrode was sometimes used for injecting d.c. currents. Since the current–voltage relation ($I–V$ curve) is linear at potentials between −10 and −150 mV (Miyazaki & Igusa, 1982*a*), changes in the amplitude of the potential step in response to the current pulse indicate those of the membrane conductance.

*Ca injection.* Ca ions were injected iontophoretically into the egg by passing currents from a second electrode filled with 0·5 M-$CaCl_2$. This method enables controlled injection of a small amount of $Ca^{2+}$; although the exact amount of injected $Ca^{2+}$ is uncertain, relative amounts are known. The method has the technical difficulty that a $CaCl_2$ electrode tends to be blocked in the cytoplasm. The $CaCl_2$ electrode was bevelled to have a tip diameter of *ca.* 0·3 $\mu$m and a resistance of *ca.* 10 MΩ when it was filled with 3 M-KCl. The electrode was inserted into the egg by over compensation of the negative capacitance of the pre-amplifier. During this procedure Ca ions were injected, resulting in a transient hyperpolarization of the membrane (see Fig. 5*A*). The application of outward current pulses after that of inward current pulses was usually useful to prevent blockage of the electrode (Fig. 5*A*). Considering this effect as well as the small tip of the electrode, no backing current was applied to prevent the leakage of $Ca^{2+}$ from the electrode. However, the electrode still tended to become blocked during the experiment.

The membrane resistance was increased by the preliminary injection of $Ca^{2+}$ (Fig. 5*A*), probably because of the stabilization of the $CaCl_2$ electrode and the decrease in the leakage conductance. Test injections of $Ca^{2+}$ were performed with constant outward currents from the electrode, using a single 2 sec pulse or 0·5 sec pulses at 1 Hz. The upper limit of injection currents through the $CaCl_2$ electrode was about 2 nA. Changes in the membrane conductance were monitored by passing inward current pulses of 0·5 nA through the $CaCl_2$ electrode.

*Solutions.* A modified Krebs–Ringer solution (BWW medium) (Biggers, Whitten & Whittingham, 1971) was used primarily. The composition is (mM): NaCl, 94·6; KCl, 4·8; $CaCl_2$, 1·7; $MgSO_4$, 1·2; $KH_2PO_4$, 1·2; Na lactate, 22; Na pyruvate, 0·5; glucose, 5·6; $NaHCO_3$, 25·1. The medium was equilibrated with 5% $CO_2$ at pH 7·4. Covering the drop of medium with $CO_2$-equilibrated paraffin oil prevented the escape of $CO_2$ during the experiment. When the concentration of $Ca^{2+}$ or $K^+$ was changed, that of NaCl was changed isosmotically. When the concentration of divalent cations was raised higher than 3 mM without precipitation, NaCl was reduced in the following standard saline (mM): NaCl, 137; KCl, 5·5; $CaCl_2$, 1·7; $MgCl_2$, 1·2; glucose, 5·6; HEPES-NaOH buffered at pH 7·4, 7·5. For Cl-free solution, methanesulphonate was substituted for $Cl^-$ of the standard saline. In some experiments, NaCl and Na lactate of the BWW medium were replaced with LiCl, keeping $NaHCO_3$ unaltered. This solution will be denoted as 1/6 Na, 5/6 Li saline. Bovine serum albumin was added to all solutions before use (4 mg/ml.). The solution was changed by continuous perfusion while the electrode was kept inside the egg. The solution change was nearly complete within 60 sec, since the drop bathing the egg was 0·4 ml. and the egg was exposed directly to the flow of the introduced solution.

## RESULTS

### *I. Effects of external divalent cations on h.r.s*

#### *Effect of $[Ca^{2+}]_o$ on the frequency of h.r.s*

Fig. 1*A* shows a series of h.r.s during fertilization in BWW medium ($[Ca^{2+}]_o$ = 1·7 mM). The h.r. first appeared in coincidence with the cessation of flagellar motion of the first sperm which had attached to the egg surface (Fig. 1*A*, 1 st.) (Miyazaki & Igusa, 1981*a*). The succeeding two to four h.r.s appeared at intervals of 20–30 sec and h.r.s thereafter occurred at fairly constant intervals of

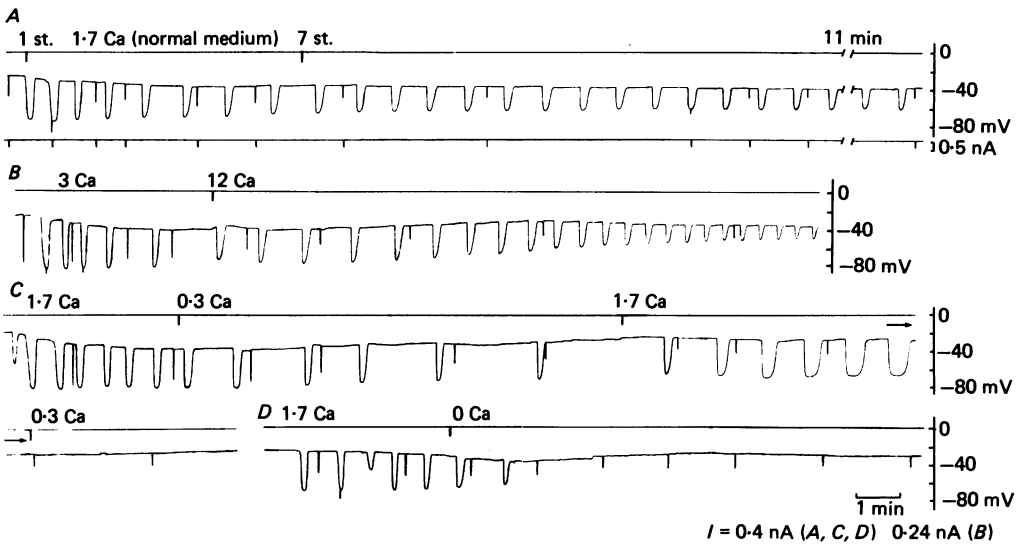


Fig. 1. Potential change during fertilization associated with five to ten sperm entries into the egg. Sperm suspension was added 1–2 min prior to the record, which is presented from the occurrence of the first h.r. 1 and 7 'st.' in *A* indicate the stopping of flagellar motion of the first and seventh sperm, respectively. External solution is indicated by the Ca concentration expressed in mM. The vertical bar on the top trace indicates the start of solution change. Current intensity of hyperpolarizing pulses is given at bottom right. These indications are common to subsequent Figures.

about 45 sec. The resting potential shifted gradually in the hyperpolarizing direction to −40 mV in about 10 min (h.s.). This was associated with a conductance increase as indicated by hyperpolarizing potential steps in response to current pulses. The series of periodic h.r.s was established if more than four to five sperm fused to the egg, and h.r.s were observed for longer than 1 hr, even when no further sperm attached to the egg.

In Fig. 1*B–D*, eggs were inseminated in 3 mM-Ca saline or BWW medium in such a way that several sperm fused to them in several minutes as in the case of Fig. 1*A* (see 7 st.), and then the medium was changed to media of different $[Ca^{2+}]$ after five to seven h.r.s had appeared. The primary effect of changing the $[Ca^{2+}]_o$ was a change in the frequency of h.r.s in a time- and concentration-dependent manner. The interval was shortened in 12 mM-Ca, especially at the later stage of the h.r. series (Fig. 1*B*),

whereas it was prolonged in 0·3 mM-Ca (Fig. 1*C*). The h.r. disappeared eventually in 0·3 mM-Ca during sustained perfusion or within 2 min after the introduction of Ca-free medium (Fig. 1*D*). These changes are summarized in Fig. 2*A* where the averaged number of h.r.s occurring in every 5 min during perfusion is plotted as a function of $[Ca^{2+}]_o$ in total forty-five eggs. The relationship between h.r. frequency and logarithm of $[Ca^{2+}]_o$ was roughly linear in the first 5 min of perfusion (filled circles), and the

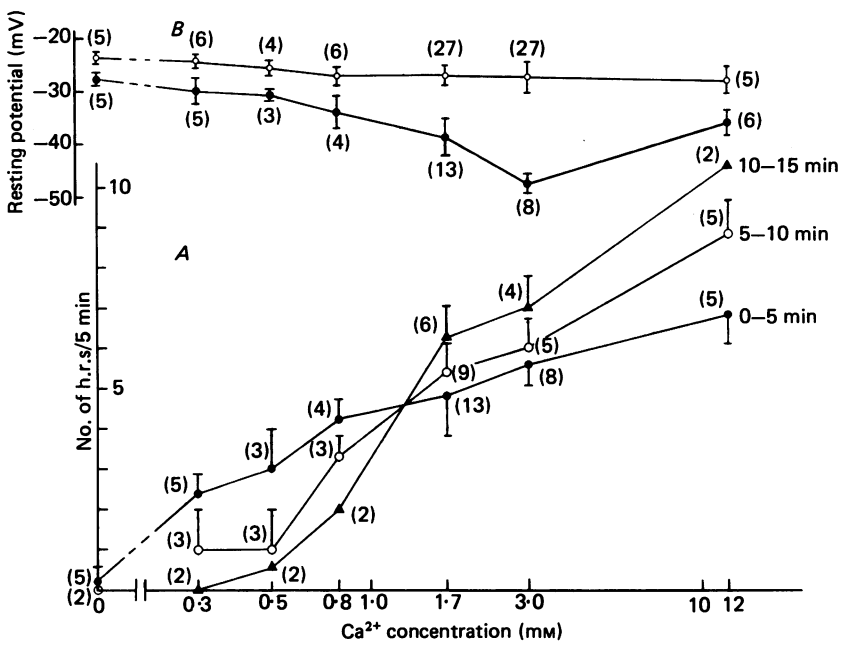


Fig. 2. The relationship between the h.r. frequency (*A*) or the resting potential (*B*) and logarithm of $[Ca^{2+}]_o$. Eggs were inseminated in BWW medium (1·7 mM-Ca) or 3 mM-Ca saline, and perfusion of a given $[Ca^{2+}]$ solution was started after five to seven h.r.s had appeared. Number of h.r.s occurring in every 5 min was counted between 0–5, 5–10 and 10–15 min after the start of perfusion, and plotted as mean ± S.D. against $[Ca^{2+}]$ of the perfusion solution. Parentheses indicate number of eggs for each point; results from only two eggs are shown by mean alone. Values for 1·7 and 3 mM-Ca were obtained by perfusion of the same solution used for insemination. In *B*, a filled circle is the resting potential of inseminated eggs at 5 min of perfusion, plotted in the same manner. An open circle is the resting potential of unfertilized eggs before insemination.

h.r. frequency in 12 mM-Ca was about three times the value in 0·3 mM-Ca. The dependence of the h.r. frequency on $[Ca^{2+}]_o$ became more pronounced with longer perfusion: the frequency in 12 mM-Ca during the third 5 min was more than five times that in 0·8 mM-Ca (triangles). At least 0·8 mM-$Ca^{2+}$ was required to maintain the h.r. series for longer than 15 min. Similar dependence on $[Ca^{2+}]_o$ was obtained for the initial stage of the h.r. series in eggs inseminated in low Ca media (not shown).

The frequency of h.r.s recovered on returning from lower Ca media to 1·7 mM-Ca (Fig. 1*C*). A successful recovery of the h.r. series was rarely obtained after perfusion with Ca-free medium. Since it has been shown that the hamster sperm is unable to fuse to the egg in the absence of $Ca^{2+}$ (Yanagimachi, 1978), the sperm–egg interactions

may be affected by the Ca-free medium, even though several sperm have already fused in BWW medium (Fig. 1*D*). The requirement of external $Ca^{2+}$ for the generation of the h.r. was confirmed by replacing $Ca^{2+}$ with other divalent cations (see later section).

*Effects of* $[Ca^{2+}]_o$ *on each h.r.*

In contrast to the change in the h.r. frequency, the amplitude of each h.r. was found to be little affected by changing the $[Ca^{2+}]_o$, considering that it decreased gradually even in the normal medium (Fig. 1*A*). Since the amplitude of the h.r. was affected by the resting potential and the membrane leakage conductance, the conductance increase during a h.r. was estimated by the subtraction of the membrane conductance just before or after the h.r. from that at its peak, assuming the *I–V* relation linear

TABLE 1. Conductance increase in the h.r. (mean ± S.D.)

| Solution | Conductance increase (nS) | No. of eggs |
|---|---|---|
| 0·3 mM-Ca | 37 ± 4·4 | 4 |
| 0·8 mM-Ca | 40 ± 3·6 | 4 |
| 1·7 mM-Ca | 42 ± 7·4 | 7 |
| 12 mM-Ca | *41 ± 4·8 | 5 |
| 3 mM-Ca | *37 ± 3·5 | 6 |
| 3 mM-Ca, 9 mM-Mn | 35 ± 4·0 | 3 |
| 3 mM-Ca, 9 mM-Co | 37 ± 5·2 | 5 |

Conductance increase in the h.r. which occurred between 4 and 6 min after the introduction of a given solution. The value was obtained by subtraction of the membrane conductance before or after the h.r. from that at its peak. * The h.r. became smaller progressively with time. This change tended to be a little more pronounced in salines made of Cl salts and buffered with HEPES than in media based on BWW medium.

(Miyazaki & Igusa, 1982*a*). Table 1 represents the conductance increase during the h.r. occurring at about 5 min after the introduction of a given medium. Mean values ranged between 35 and 42 nS at $[Ca^{2+}]_o$ between 0·3 and 12 mM: the conductance increase in the h.r. was little affected by $[Ca^{2+}]_o$. The reversal potentials of the h.r.s were obtained from the intersection of two steady-state *I–V* curves at the peak of the h.r. and immediately after the h.r., in the same manner as in Fig. 5*D* (see below). They ranged only between −86 and −83 mV, despite the fifteen-fold difference in $[Ca^{2+}]_o$ from 0·8 to 12 mM. The poor dependence of each h.r. on $[Ca^{2+}]_o$ strongly suggests that the h.r.s are not an immediate consequence of periodic enhancement of Ca influx, although they seem to be dependent on Ca influx.

In Fig. 1*C* the duration of each h.r. became slightly shorter in lower Ca media. On returning from lower Ca media to 1·7 mM-Ca, the duration of the h.r. was remarkably prolonged. The reason is unknown. The prolonged h.r.s recovered normal durations during sustained perfusion of 1·7 mM-Ca medium (not shown).

*Effect of membrane potential on h.r.s*

In Fig. 3 the membrane potential was held between +6 and −100 mV by passing d.c. current through the second electrode, after a relatively rapid series of h.r.s had been established in 12 mM-Ca. Depolarization from −35 mV to +6 mV prolonged the interval of the h.r.s from 40 to 80 sec (Fig. 3*a*), while hyperpolarization from

−33 mV to −60 mV (*c*) or −70 mV (*d*, dots) shortened the interval from 45 to 30 sec or from 35 to 25 sec, respectively. The h.r.s with reversed polarity appeared more frequently at −100 mV (Fig. 3*b*, dots), although some of them were not clear because of the fluctuation of the membrane potential. These findings suggest that the greater electromotive force of $Ca^{2+}$ increases Ca influx and thereby facilitates the occurrence of h.r.s.

*Effect of $[Ca^{2+}]_o$ on h.s.*

The resting potential shifted gradually from −28 mV to −40 mV in 1·7 mM-Ca associated with a conductance increase (Fig. 1*A*). The h.s. was also observed, when the egg was inseminated after introduction of Cl-free saline (not shown). The h.s. was

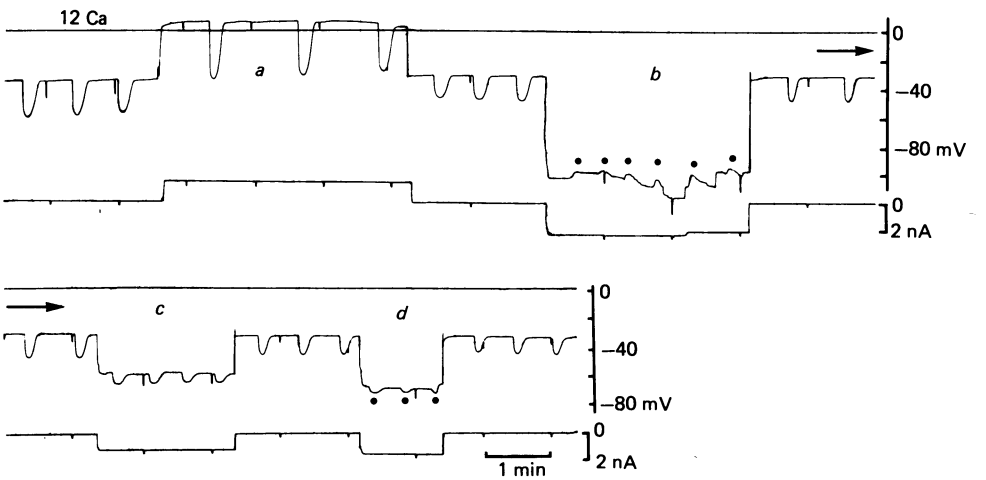


Fig. 3. Changes in h.r. frequency at various membrane potential levels held by passing d.c. current through a second electrode.

interrupted by iontophoretic injection of EGTA and the membrane was depolarized to nearly the original potential level before insemination (see Fig. 3 of Miyazaki & Igusa, 1982*a*). These findings suggest that the h.s. is at least partly due to a Ca-activated K conductance ($G_K$).

The dependence of the h.s. on $[Ca^{2+}]_o$ is summarized in Fig. 2*B* where a filled circle represents the mean resting potential of inseminated eggs at 5 min after the introduction of a given medium. The resting potential of unfertilized eggs (open circles) became more negative at higher $[Ca^{2+}]_o$, probably due to the reduction of leakage, the stabilizing effect of $Ca^{2+}$. Exceeding this effect, the resting potential of inseminated eggs became even more negative, depending on $[Ca^{2+}]_o$. Thus, the h.s. is likely to involve a counterbalance between the permeability to $Ca^{2+}$ ($P_{Ca}$) and the consequent Ca-activated $P_K$.

On raising the $[Ca^{2+}]_o$ to 12 mM-Ca, the resting potential shifted in the depolarizing direction, associated with frequent h.r.s (Fig. 1*B*). Fig. 2*B* shows the shift from −48 to −37 mV on changing the $[Ca^{2+}]_o$ from 3 to 12 mM. It should be noted that the depolarization is accompanied by an increase in the membrane conductance (Fig. 1*B*). Since the increase in leakage current is unlikely in 12 mM-Ca, the depolarization is considered to be based on $P_{Ca}$. In excess $[Ca^{2+}]_o$, $P_{Ca}$ may increase, and predominate the increased $G_K$.

*Effects of other divalent cations*

The requirement of external $Ca^{2+}$ for the generation of the h.r. was confirmed by substituting $Sr^{2+}$ or $Mn^{2+}$ for $Ca^{2+}$. These ions can replace $Ca^{2+}$ in the incorporation of sperm into the cytoplasm of the hamster egg (Yanagimachi, 1978). However, when 0 Ca, 3 mM-Sr saline was perfused, the interval of the h.r.s was remarkably prolonged progressively with time; no h.r. followed a small one seen after 10 min perfusion (Fig. 4*A*). With Ca-free but 5 mM-Mn saline (Fig. 4*B*), the h.r. disappeared soon after

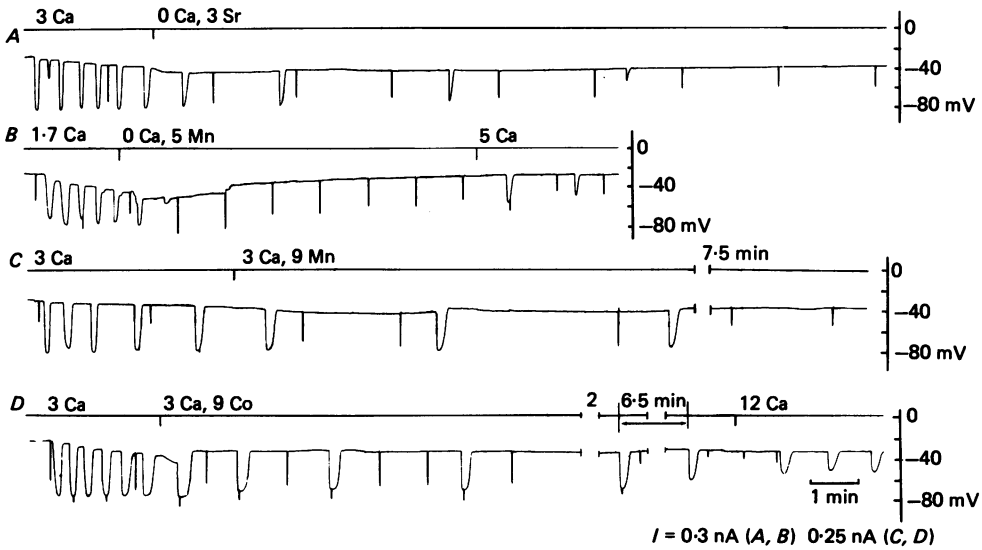


Fig. 4. The h.r. series in eggs inseminated in 3 mM-Ca saline or BWW medium and then perfused with Ca-substituted salines (*A* and *B*) or with 3 mM-Ca, 9 mM-Mn or Co saline (*C* and *D*).

perfusion. The recovery of the h.r. was obtained only when more than 5 mM-Ca was reintroduced within 10 min (Fig. 4*B*).

Fig. 4*C* and *D* shows records obtained in the presence of 3 mM-Ca and 9 mM-Mn or Co, respectively. The h.r. disappeared eventually in the presence of $Mn^{2+}$ but only after a period of 10 min. In the presence of $Co^{2+}$ the interval of the h.r. was prolonged up to 6·5 min, but the h.r. was not abolished. The reversibility of the h.r. frequency was shown by washing with 12 mM-Ca (Fig. 4*D*). It should be noted that the size of each h.r. was almost unaltered in the presence of $Mn^{2+}$ or $Co^{2+}$, although the interval was prolonged. Table 1 presents the conductance increase during the h.r. at about 5 min after perfusion of $Mn^{2+}$- or $Co^{2+}$-containing saline. The values are not significantly different from those in the absence of these ions. It should be also noted that the decrease of the h.r. frequency in all four records in Fig. 4 always coincided with the suppression of the h.s., compared with −48 mV in 3 mM-Ca saline (Fig. 2*B*).

### *II. Ca injection into unfertilized eggs*

*Regenerative h.r. induced by Ca injection*

An injection current pulse of 2 sec produced a depolarization, during which the membrane conductance was increased, as indicated by the decay of the depolarization (Figs. 5*B* and 6*A*). A hyperpolarization was seen at the cessation of the pulse. Fig. 5*C* is a control experiment with Mg injection and indicates that a depolarization

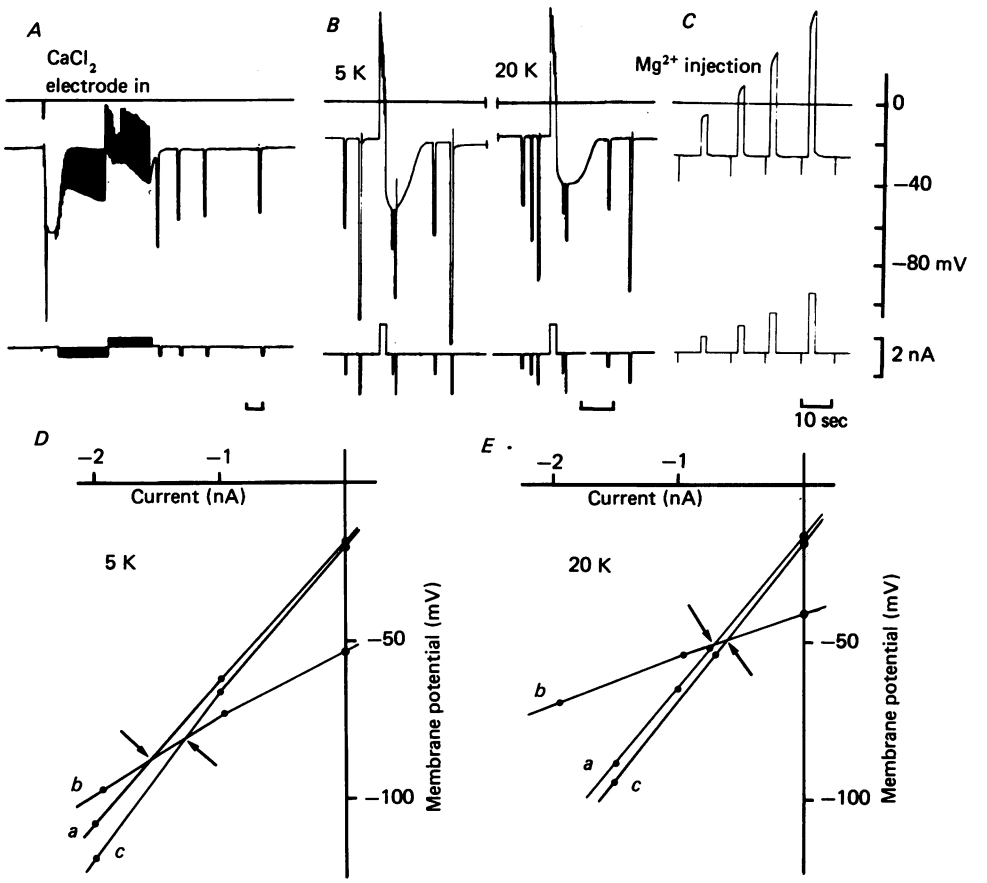


Fig. 5. *A*, procedure of the insertion and stabilization of the $CaCl_2$ electrode. *C*, control experiment with $Mg^{2+}$ injection. *B*, *D* and *E*, steady-state current–voltage relations at the peak of the h.r. and just before or after the h.r. The reversal potentials (arrows) of h.r.s in 5 mM and 20 mM-$K^+$ medium were obtained in the same egg. There was 4 min interruption on the record in *B*. $Ca^{2+}$ was injected with a single 2 sec pulse.

*per se* causes no hyperpolarization. Fig. 5*D* and *E* illustrate steady-state *I–V* curves before injection (curve *a*), at the peak of the hyperpolarizing response (h.r.) (curve *b*), and after recovery (*c*) in 5 and 20 mM-K saline in the same egg (see actual records in Fig. 5*B*). The membrane conductance was increased during the h.r. but slightly reduced immediately after the h.r. The reversal potential of the h.r. was estimated from the intersection of curve *b* with curve *a* or *c*, because the leakage conductance

at the peak of the h.r. was unknown. Curve *b* and *a* crossed at −87 mV, and curve *b* and *c* at −82 mV in 5 mM-K saline (Fig. 5*D*, arrows). Those values were −51 and −49 mV in 20 mM-K saline, respectively (Fig. 5*E*). The shift of the reversal potential was estimated to be between 36 and 33 mV. It is almost consistent with the value

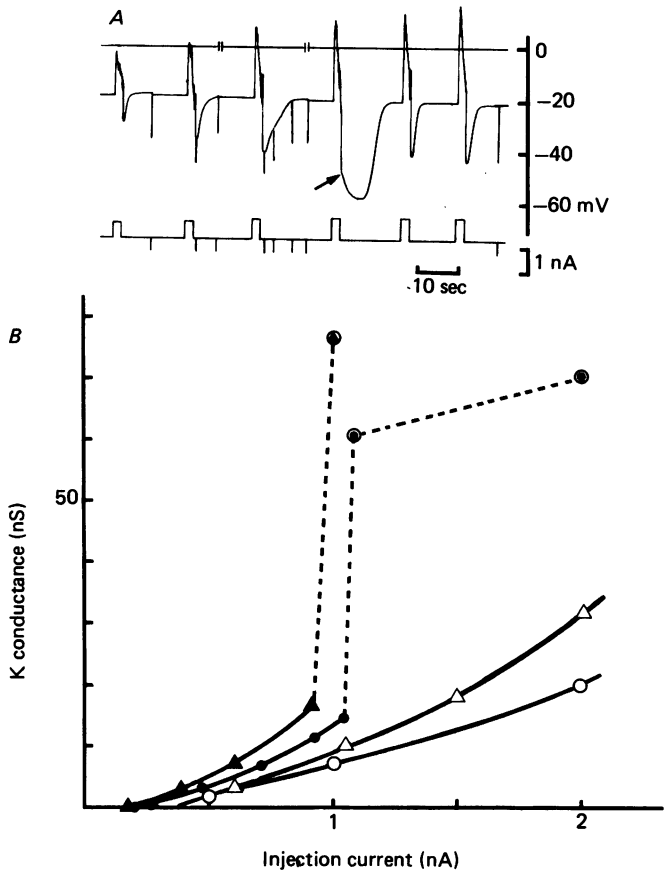


Fig. 6. *A*, a regenerative h.r. after the cessation of the injection pulse (arrow). A pair of vertical bars on the zero potential line indicates a 40 sec interruption of the record. *B*, relationship between K conductance ($G_K$) and injection current in two eggs (circles and triangles). Actual records are not shown here. $G_K$ was calculated by subtraction of the membrane conductance before or after each h.r. from that at the peak of the h.r. Filled symbols were obtained from subthreshold h.r.s before a full response was generated, and filled symbols with a circle were from full responses. Open symbols were from h.r.s in the refractory period 5–10 sec after the end of the full response induced by the critical current. To obtain three to four open symbols in each egg, $Ca^{2+}$ was injected once or twice after a full response. Therefore, two full responses which were almost identical were generated in each egg.

of 36·3 mV calculated from the Nernst equation at 31 °C. Thus, the h.r. induced by Ca injection is due to a Ca-activated K current, as are sperm-mediated h.r.s (Miyazaki & Igusa, 1982*a*).

When the injection current of $Ca^{2+}$ was increased little by little, the h.r. became larger, and eventually a much larger hyperpolarization was evoked at a critical

injection current (Fig. 6*A*). It should be noted that this hyperpolarization was augmented and prolonged *after* the cessation of the injection pulse (see arrow in Fig. 6*A*). The enhanced response, hereafter referred to as 'full response', had the peak potential of $-62 \pm 7$ mV (mean $\pm$ S.D.) and the duration of $11{\cdot}2 \pm 1{\cdot}8$ sec (sixty-one eggs). The decaying phase was 3–5 sec. The critical injection current for generating the full response was $1{\cdot}10 \pm 0{\cdot}22$ nA ($n = 55$).

Fig. 6*B* illustrates approximate relationships between K conductance ($G_K$) and injection current of $Ca^{2+}$ in two cases. The $G_K$ was calculated by subtraction of the membrane conductance just before or after injection from that at the peak of the h.r., considering the $I-V$ curves linear. Injection currents less than 0·2–0·3 nA were incapable of producing any conductance increase. With greater currents, the slope of the relationship became slightly larger with the increase in subthreshold currents, and the $G_K$ was abruptly increased by about 4·5 times at the critical injection current (dashed lines), corresponding to the full response. The $G_K$ was further increased with greater currents but the slope became much smaller again (double circles).

*Refractory period of full response*

Once a full response occurred, the same threshold pulse failed to generate a second full response for 1–2 min but produced a smaller h.r. which decayed as soon as the injection was ceased (Fig. 6*A*). This finding indicates a refractory period of the full response. The smaller h.r. elicited by threshold or suprathreshold current in the refractory period will be tentatively called the 'partial response' below. Fig. 7*A* shows changes in the h.r.s with constant injection currents, while the leakage conductance was almost constant (see potential steps in response to hyperpolarizing pulses). The recovery of the full response was not obtained until 60 sec after the end of the previous full response (response 1, 2 and 3). The recovery took about 50 sec even if the previous response was a partial response: response 5 was a partial response 20 sec after the end of the partial response 4 whereas response 8 was a full response 50 sec after the partial response 7. Since the response changed in its duration as well as in the amplitude, it was quantified by the area in the record. In Fig. 7*B* the area of a full response ($R_1$) and that of the succeeding response ($R_2$) were measured and the ratio $R_2/R_1$ was plotted against the interval between the end of the full response and the start of the next pulse. The ratio was 25–30 % at intervals shorter than 20 sec and recovered to 100 % in about 1 min (open symbols). The time course of the refractory period was affected by the size of the previous full response. When the full responses had a significantly larger area than those in Fig. 7*A*, $R_2/R_1$ remained at 10–20 % for about 1 min and the recovery took 2 min (filled symbols in Fig. 7*B*). Such full responses, however, were elicited only twice or three times in a single egg: they were reduced to a full response of usual size during successive injections, associated with a shortening of the refractory period (see filled and open triangles and squares).

The relationship between $G_K$ and injection current of $Ca^{2+}$ at the early phase of the refractory period is shown in Fig. 6*B* (open symbols): the $G_K$ at the peak of a h.r. is plotted as a function of a given current, at 5–10 sec after a full response elicited by about 1 nA. The relationship is approximately linear, or shows only a slight upward curvature, for currents up to 2 nA. The $G_K$ of the h.r. by 2 nA was 30–40 % of the value in the full response. Since the amount of injected $Ca^{2+}$ is thought to be roughly

proportional to the injection current, the steep non-linearity in Fig. 6*B* at the critical current for the full response strongly suggests a non-linear increase in $[Ca^{2+}]_i$ beyond the increase by injection.

In the curves of Fig. 6*B*, the slope was smaller for injections at the early phase of the refractory period (open symbols) than for subthreshold injections before induction of a full response (filled symbols); the ratio of the slopes was 0·45–0·5 at currents less than 1 nA. The reduction in the sensitivity of h.r.s to Ca injection suggests either inactivation of $G_K$ or enhancement of the Ca buffering power or both, immediately after the full response.

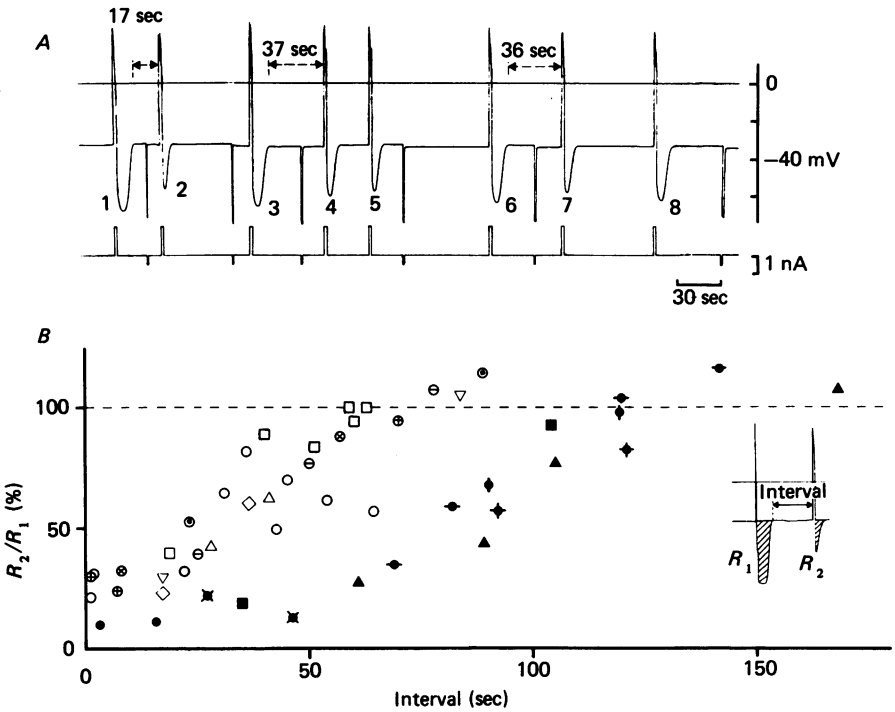


Fig. 7. Refractory period of the full response. *A*, the usual full and subsequent partial responses. *B*, ratio of the area of the responses ($R_2/R_1$) as a function of the interval of injections. Measurements are illustrated in the inset. Open or filled symbols indicate the cases of the usual sized full response or of the large full response, respectively. A simple open or filled circle was obtained from one egg. For other symbols, the same symbols were obtained from the same egg. Two eggs (triangle and square) showed large full responses but showed usual ones after successive injections.

*Some properties of the full response*

*Ca injections at two points.* Two $CaCl_2$ electrodes were inserted into the egg at two points about 50 $\mu$m apart, as illustrated in Fig. 8*A*. Since the interior of the egg is equipotential, a membrane potential change should be recorded with the third electrode without any spatial decay or time delay, wherever the membrane conductance is increased locally. The injection current from the first electrode, which induced the full response (1), produced partial responses (4, 5) immediately after the full response (3) elicited by the second electrode; the full response (6) was obtained again 70 sec after the previous injection. A similar result was obtained in the reversed

situation (compare responses 2, 7 and 8 with 3). The fact that Ca injections at two points 50 $\mu$m apart interfere each other in the egg of 70 $\mu$m diameter suggests that the increase in $[Ca^{2+}]_i$ for generating the full response covers nearly whole inner surface of the membrane.

*Removal of external $Ca^{2+}$.* A regenerative h.r. followed by a refractory period could occur in the absence of external $Ca^{2+}$ (Fig. 8*B*). However, a complete recovery of the full response was not obtained in the Ca-free medium. Fig. 8*B* illustrates the case which showed the most substantial recovery in twelve eggs examined, but the recovery was still partial. It seems that Ca transport from outside is necessary to prepare the next full response.

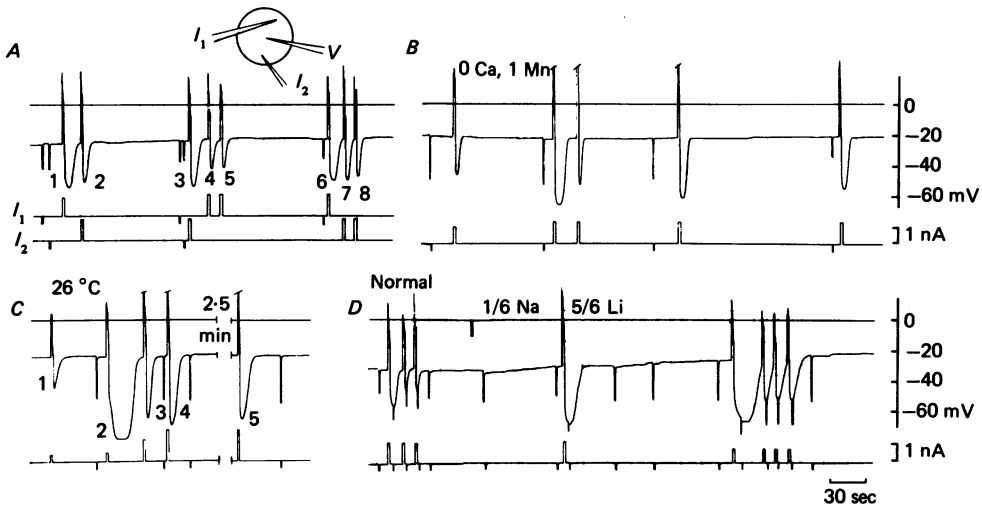


Fig. 8. *A*, Ca injections at two points about 50 $\mu$m apart. The inset shows the arrangement of three electrodes. *B*, full response in Ca-free medium. 1 mM-$Mn^{2+}$ was added, because the egg impaled with two electrodes became leaky in the Ca-free condition. *C*, a large and prolonged full response at 26 °C. *D*, prolongation of the recovery phase of the h.r. in Na-deficient medium in which 5/6 Na was replaced by Li. Further explanations are in the text.

*Effect of temperature.* When the temperature was lowered from usual condition (31 °C) to 26 °C within 1·5 min, a large and prolonged full response was generated (Fig. 8*C*). The critical injection current was $0{\cdot}57 \pm 0{\cdot}14$ nA($n = 5$), which was about half that at 31 °C. The full response is probably elicited more easily because of the reduced Ca buffering power at lowered temperature. In skeletal muscles a 'rapid-cooling contracture' is observed in the presence of caffeine, due to the release of $Ca^{2+}$ from the sarcoplasmic reticulum (Sakai, 1965; Sakai, Kurihara & Yoshioka, 1974). Kuba (1980) has demonstrated a Ca-dependent hyperpolarization on the rapid cooling of bull-frog sympathetic neurones bathed in caffeine-containing solution, consistent with intracellular Ca release. A similar mechanism may operate in the hamster egg. In Fig. 8*C* the refractory period after the end of the full response is clearly demonstrated by injection with currents three or four times greater than the critical current (responses 3, 4). Once a full response occurred at 26 °C, a comparable full response

could not be obtained even with 2 nA current (the upper limit of currents through the $CaCl_2$ electrode), and even after a 3 min interval (see response 5).

*Removal of external $Na^+$.* When 5/6 Na of the BWW medium was replaced by Li, the duration of the full response became 3–4 times longer: both the peak phase and the recovery phase were prolonged (Fig. 8*D*). Similar changes were obtained in Na-free solution when NaCl was replaced with Tris OH–HCl. These findings suggest that the increased $[Ca^{2+}]_i$ is actively buffered and that one of the buffering systems involves

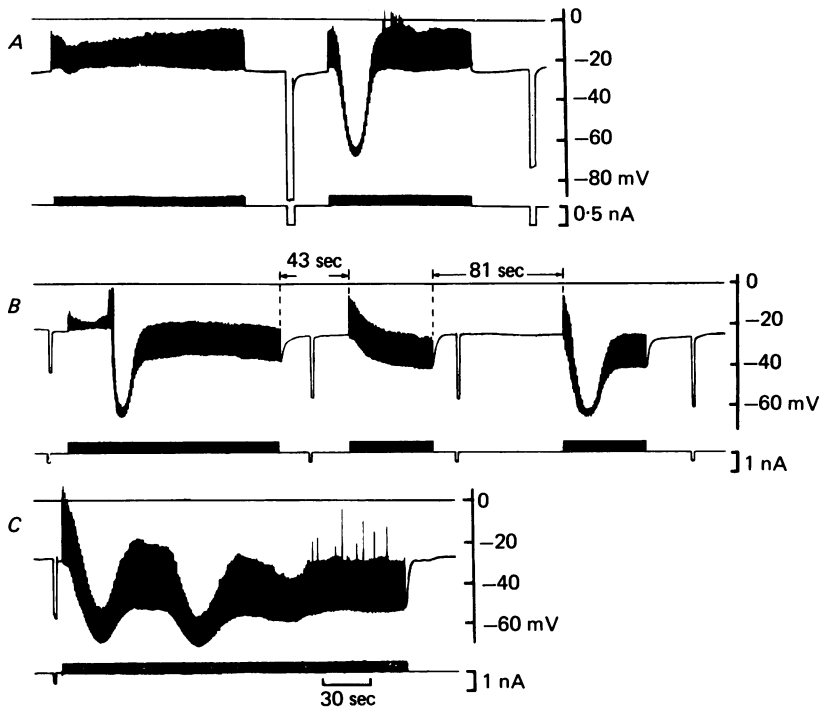


Fig. 9. Hyperpolarization by repetitive Ca injections (0·5 sec pulse, 1 Hz). In the record of *B* (left) the early depolarizing steps by repetitive pulses were small and suddenly became larger; it is likely that partial block of the $CaCl_2$ electrode was removed.

$Na^+$–$Ca^{2+}$ exchange, which is known to be suppressed by the replacement of external $Na^+$ with $Li^+$ (see Baker, 1972 for review). Further analysis of the contribution of $Na^+$–$Ca^{2+}$ exchange is required.

*Hyperpolarization by repetitive injections of $Ca^{2+}$*

Ca ions were injected with 0·5 sec pulses at 1 Hz to examine whether periodic h.r.s can be generated in the unfertilized egg. The lower envelope of potential steps indicates the membrane potential. With constant pulses, a phasic hyperpolarizing response corresponding to the full response by a single pulse was generated in an all-or-none fashion (Fig. 9*A*). The peak of the phasic responses by pulses of various current intensities averaged $-65{\cdot}5 \pm 5{\cdot}5$ mV ($n = 49$) and ranged between $-75$ and $-55$ mV. The critical injection current was $0{\cdot}41 \pm 0{\cdot}09$ nA ($n = 11$). Repetitive pulses less than 0·3 nA produced no phasic response, even if they were continued for 4 min

(Fig. 9*A*, left). With suprathreshold pulses, the phasic response was followed by a sustained hyperpolarization (Fig. 9*B*, left), the level of which was dependent on the injection current. To obtain a second phasic response, a pause of 60–80 sec was necessary after the end of the previous injections (Fig. 9*B*, right). When the pause was insufficient, the membrane potential only reached the same steady level as that for the previous injections (Fig. 9*B*, middle). In some cases two phasic responses were produced at the interval of about 1 min between the peaks, but the membrane potential eventually reached a steady level (Fig. 9*C*). Thus, Fig. 9 indicates that periodic h.r.s are not generated in the unfertilized egg.

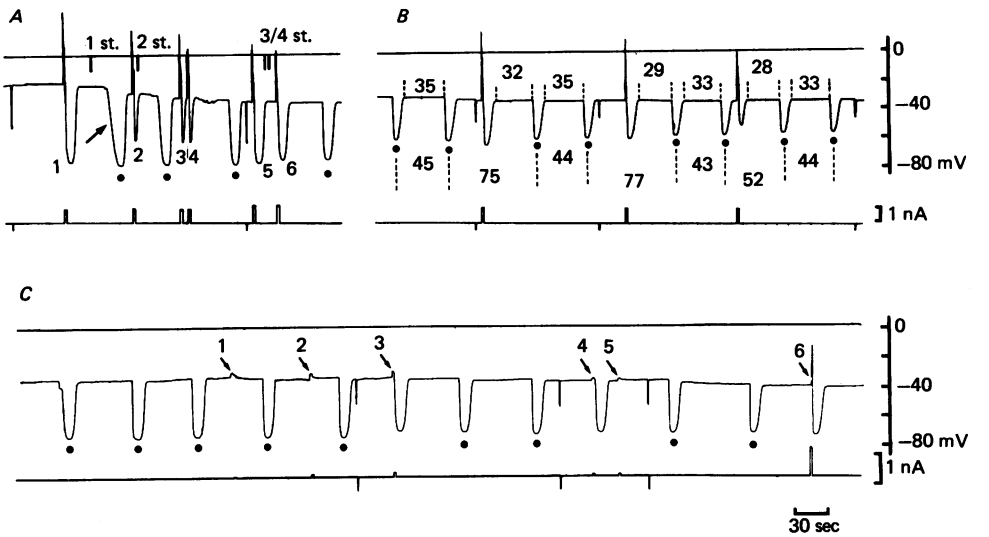


Fig. 10. Ca injection in inseminated eggs. S.–h.r.s are indicated by dots. *A*, early stage of the h.r. series. 1–4 'st.'s indicate the stopping of flagellar motion of the first to fourth sperm which had attached to the egg surface. *B* and *C*, later stages of the h.r. series. Records are presented from about 4 min after the occurrence of the first h.r. Figures in *B* indicate the intervals between vertical dotted lines, in seconds. Arrows in *C* indicate Ca injection. Each record was obtained from different egg. Further explanations in the text.

### *III. Ca injection into inseminated eggs*

#### *Interference between Ca-induced h.r. and sperm-mediated h.r. (s.–h.r.)*

Inseminated eggs show periodic h.r.s (Fig. 1). Hereafter, these h.r.s will be tentatively described as 'sperm-mediated h.r.s' (s.–h.r.s), although each h.r. may not be mediated by an action of sperm. In Fig. 10 the h.r. by Ca injection was interposed between s.–h.r.s. In Fig. 10*A*, the first s.–h.r. (dot) started 15 sec after the cessation of flagellar motion of the first sperm (1 st.). Despite a full response induced by Ca injection (response 1) 30 sec earlier, the first s.–h.r. was as large as the full response, although the rate of potential fall was smaller than usual (arrow). The critical injection current for the full response 1 failed to produce a full response immediately after s.–h.r.s (see responses 2–4), but larger pulses could generate full responses (5 and 6) after an interval of 15 sec. The initial stage of fertilization seems to provide a favourable condition for the occurrence of the full response. Later s.–h.r.s appeared

at a fairly constant interval of 45 sec (Fig. 10*B*). The periodicity was interrupted by an interposed, Ca-induced h.r., but it was restored about 30 sec after the Ca-induced h.r. whenever $Ca^{2+}$ was injected after the previous s.–h.r. Therefore, the rhythm of s.–h.r.s is considered to be determined by the intracellular processes regulating cytoplasmic $Ca^{2+}$ of the egg.

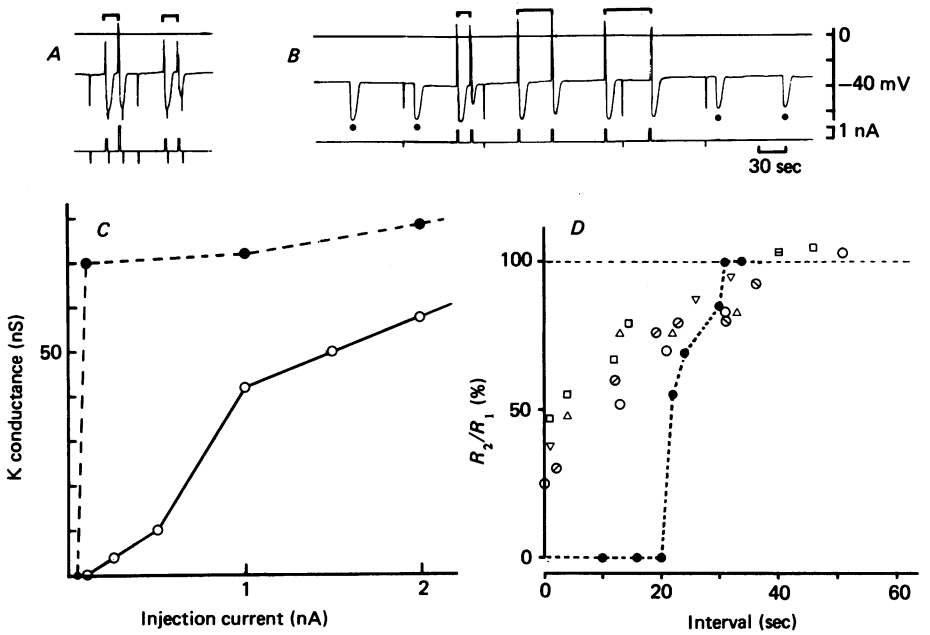


Fig. 11. *A*, h.r. by a given current 5 sec after the end of the full response by an 1 nA pulse. *B*, three pairs of the full response and partial response with 1 nA pulses at different intervals. *C*, relation between $G_K$ and injection current, obtained in the same manner as in Fig. 6*B*. Filled circle, $G_K$ for subthreshold h.r.; double circles, full responses; open circles, partial response 5 sec after the end of each full response by an 1 nA pulse as shown in *A*. *D*, refractory period obtained in the same manner as in Fig. 7*B*. Filled circles and dashed line, h.r.s produced by 0·1 nA pulses after the end of each full response triggered by a 0·1 nA pulse; open symbols, h.r.s produced by 1 nA pulses after the end of each full response elicited by an 1 nA pulse as shown in *B*. The same symbols were obtained from the same eggs. All records were obtained at the pauses of s.–h.r.s in inseminated eggs.

*Regenerative h.r. and refractory period*

In Figs. 10*C* and 11 regenerative h.r.s by Ca injection were analysed at the pause of s.–h.r.s. Since the interval between the end of a s.–h.r. and the start of the next s.–h.r. was 45–55 sec, injections were limited within at most 40–50 sec after the end of a s.–h.r. Fig. 10*C* shows that a large h.r. is induced in an all-or-none fashion with the critical current of 0·15 nA at 30 sec after the end of a s.–h.r. (arrow 3), while smaller currents produce no hyperpolarization at all (arrows 1 and 2). The longer the interval between the end of the s.–h.r. and the time of injection, the smaller the critical current. When the interval was 40 sec, a 0·1 nA pulse evoked the regenerative h.r. (arrow 4). Although these Ca-induced h.r.s were slightly smaller than s.–h.r.s, the regenerative h.r. at about 40 sec after the s.–h.r. was taken as the full response. An injection current of 0·1 nA evoked the full response in ten eggs, and 0·03 nA was

enough in one case. Thus, the critical injection current in inseminated eggs was less than one-tenth of that in unfertilized eggs. The size of the full response of $G_K$ was little increased by further increase in injection current (see arrow 6 in Fig. 10*C* and double circles in Fig. 11*C*).

Fig. 11*D* shows the refractory period in the same manner as Fig. 7*B*. The 0·1 nA pulse produced no hyperpolarization until 20 sec after the end of the full response by the identical current (filled circles and dashed line, see arrows 4 and 5 in Fig. 10*C*). When the interval was prolonged by only 2 sec, the same pulse generated a h.r. as large as 55 % of the full response. A 100 % recovery was obtained with the interval of 30 sec. Thus, an all-or-none like recovery was demonstrated with 0·1 nA pulses. Fig. 11*B* shows three pairs of h.r.s induced by 1 nA pulses at different intervals. The occurrence of s.–h.r. was disturbed as long as $Ca^{2+}$ was injected successively, if the interval between injections was shorter than that of s.–h.r.s. With comparable injection currents (*ca.* 1 nA), the refractory period in inseminated eggs was 30–40 sec (Fig. 11*D*, open symbols), in contrast to 60 sec in unfertilized eggs (Fig. 7*B*).

In Fig. 11*D* the partial response immediately after the full response was 30–50 % of the full response, different from 10–30 % in unfertilized eggs (see Fig. 7*B*). The $G_K$ in the partial response was examined at 5 sec after the end of the full response elicited by an 1 nA pulse (Fig. 11*A*), under conditions similar to that for Fig. 7*B*. The relation between $G_K$ and injection current is approximately linear except between 0·5 and 1 nA, where the slope is steeper (Fig. 11*C*, open circles). The $G_K$ in partial responses produced by currents greater than 1 nA was more than half that in the full response. In inseminated eggs the non-linear increase in $[Ca^{2+}]_i$ seems to occur to some extent immediately after the full response, if the amount of $Ca^{2+}$ injected is increased.

*Periodic h.r.s by repetitive injections of $Ca^{2+}$*

Fig. 12*A* illustrates that repetitive injections can produce periodic h.r.s superimposed on a gradual, hyperpolarizing shift of the membrane potential. The amplitude of the h.r. decreased progressively. The interval of the h.r.s is dependent on the injection current; 23 sec for 0·35 nA (*a*) and 33 sec for 0·2 nA (*b*). The hyperpolarizing shift of the membrane potential at the pauses of h.r.s was greater in *a* than in *b*. These findings are quite similar to the effect of elevated $[Ca^{2+}]_o$ on the s.–h.r. frequency (Fig. 2*A*). Repetitive injections induced periodic h.r.s in Ca-free, 3 mM-Mn saline (Fig. 12*B*). In this condition, s.–h.r.s had been abolished, as shown in Fig. 4*B*. The interval of the Ca-induced h.r.s tended to be longer than that in normal medium, even when injection currents were identical (compare Fig.12*B* with *A*, *b*).

## DISCUSSION

*Regenerative h.r.*

The present study reveals that the occurrence of the h.r. at fertilization requires the presence of external $Ca^{2+}$, and that the frequency of the h.r.s is dependent on $[Ca^{2+}]_o$. However, the amplitude, conductance increase and reversal potential of each h.r. are little affected by the change in $[Ca^{2+}]_o$. No depolarizing phase precedes each h.r. These findings strongly suggest that the increase in $[Ca^{2+}]_i$ reflected in the h.r.

is not caused directly by a transient enhancement of Ca influx but is mediated by intracellular processes such as Ca release from stores. A regenerative h.r. with an apparent threshold was produced in unfertilized eggs by incremental Ca injections. The steep jump in the relationship between $G_K$ and injected $Ca^{2+}$ is considered to be due to a non-linear increase in $[Ca^{2+}]_i$ rather than a non-linear dependence of $G_K$ on linear increase in $[Ca^{2+}]_i$, since the relationship is almost linear during the refractory period of the regenerative h.r. In the analysis of Ca-activated $G_K$ in snail

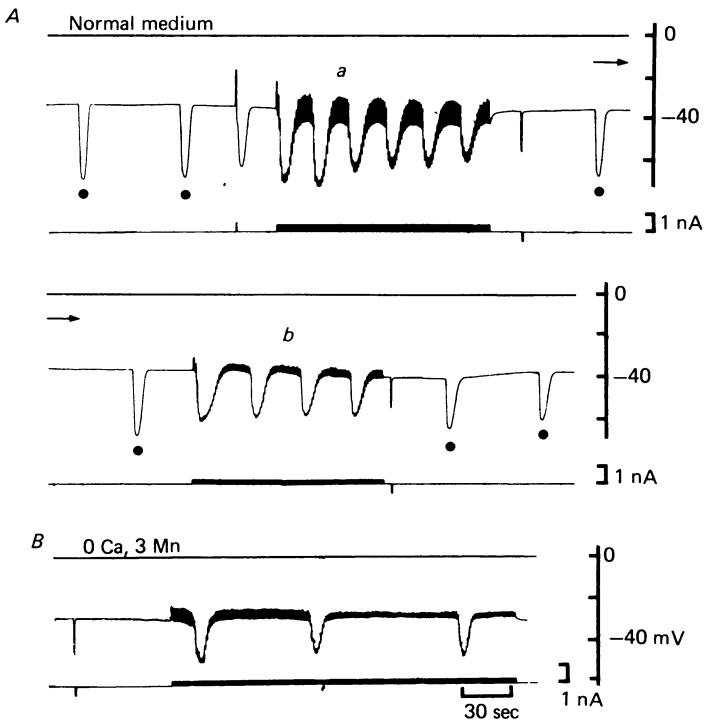


Fig. 12. *A*, periodic h.r.s by repetitive injections of $Ca^{2+}$ in inseminated eggs. Upper record is continuous with the one below (see arrows). *B*, periodic h.r.s in 0 Ca, 3 mM-Mn saline in another egg.

neurones by Meech & Thomas (1980), such a steep jump was not seen when known quantities of $Ca^{2+}$ were injected. The regenerative increase in $G_K$ seems to occur over the whole membrane and is induced by smaller Ca pulses upon lowering the temperature. Furthermore, in inseminated eggs the full response is elicited by Ca injection with such small pulses that they produce no hyperpolarization by themselves during the refractory period. Thus, the enhancement of the increase in $[Ca^{2+}]_i$ does not simply result from injected $Ca^{2+}$ *per se* transiently exceeding the buffering capacity; rather it involves the release of $Ca^{2+}$ from intracellular stores. The mechanism seems to be similar to the 'Ca-induced Ca release' which has been found in the sarcoplasmic reticulum (Endo, Tanaka & Ogawa, 1970; Ford & Podolsky, 1970; see Endo, 1977). The interference between s.–h.r.s and the Ca-induced h.r. indicates that both h.r.s are based on process(es) at common sites. Therefore, the periodic s.–h.r.s are considered to be a consequence of periodic Ca release from intracellular stores.

*Refractory period*

The recovery phase of the h.r. is considered to reflect the removal of the increased $[Ca^{2+}]_i$ by Ca buffering systems, one of which may be Ca extrusion mediated by Na–Ca exchange. The full response to Ca injection was followed by a refractory period, which was about 1 min in unfertilized eggs and was prolonged to 2 min after the greater full response. One of the factors determining the refractory period is presumably the re-uptake of Ca stores. This is supported by the all-or-none like recovery of the full response in inseminated eggs, when very small, threshold injection currents were used. Further support comes from the prolongation of the refractory period upon lowering the temperature. In Ca-free medium, complete recovery of the full response required more than 3 min in unfertilized eggs, suggesting that $Ca^{2+}$ supplied from outside is an important factor in loading the Ca stores. Another factor determining the refractory period may be a rise in the threshold or inactivation of Ca-induced Ca release. This speculation is supported by the finding in unfertilized eggs that periodic h.r.s are not produced by repetitive injections with constant pulses: apparently the refractory period persists as long as $Ca^{2+}$ is injected.

*Difference between inseminated eggs and unfertilized eggs*

The regenerative h.r. was observed more clearly in inseminated than in unfertilized eggs. The critical injection current of $Ca^{2+}$ for the full response is less than one-tenth, indicating that the threshold for Ca-induced Ca release becomes much lower in inseminated eggs, although the reason is unknown. The refractory period is shortened to 30–40 sec, which is comparable to the period of s.–h.r.s. It seems probable that the re-uptake of Ca stores is augmented in inseminated eggs (see below). An enhanced Ca re-uptake and the lowered threshold of Ca-induced Ca release could explain the occurrence of periodic h.r.s by repetitive injections of $Ca^{2+}$.

*Dependence of s.–h.r.s on external $Ca^{2+}$*

The fact that no s.–h.r.s occur in Ca-free solution indicates the requirement of either Ca influx or the binding of $Ca^{2+}$ with sites on the outer membrane surface. The present results support the role of Ca influx. A regenerative h.r. was induced by Ca injection in Ca-free solution. With repetitive Ca injections into inseminated eggs, similar periodic h.r.s were induced by an extrinsic, continuous supply of $Ca^{2+}$, associated with the dependence of the frequency on the amount of $Ca^{2+}$ injected or on the presence of external $Ca^{2+}$. These findings indicate that increased Ca influx, when present, can produce periodic h.r.s. The h.s. appears to be an indicator of the continuous Ca influx because of its dependence on $[Ca^{2+}]_o$ and its suppression by injection of EGTA. The h.s. was decreased by the addition of $Mn^{2+}$ or $Co^{2+}$, associated with a remarkable decrease in the h.r. frequency but not the amplitude of each h.r. These ions are likely to suppress the Ca influx.

At fertilization the sperm induces an increase in membrane permeabilities of eggs to various ions ($Na^+$, $K^+$, $Ca^{2+}$ or $Cl^-$), generating the fertilization potential (Hagiwara & Jaffe, 1979). The dominant ionic species are different from animal to animal. It is plausible that $P_{Ca}$ is increased in the hamster egg. The Ca transport system is probably not the voltage-gated Ca channel, which has been shown to be

present in the hamster egg membrane (Miyazaki & Igusa, 1981*b*), because the frequency of the h.r. is increased on hyperpolarization and the h.r.s can occur from the holding potential of −160 mV (Miyazaki & Igusa, 1982*a*). Further studies are necessary to identify the putative Ca transport system.

*Possible mechanism of periodic h.r.s*

The present findings may be interpreted to be the result of a linkage of continuous Ca influx to intracellular Ca release: transported Ca ions are immediately sequestered in intracellular stores adjacent to the plasma membrane, and the accumulated $Ca^{2+}$ is released by an appropriate rise in $[Ca^{2+}]_i$. These processes could be repeated periodically. The finding that the periodicity of s.–h.r.s is reset by the Ca-induced h.r. indicates that the interval of s.–h.r.s corresponds to the refractory period revealed by Ca injections. Therefore, it seems probable that the interval of the periodic increase in $[Ca^{2+}]_i$ is determined by the balance between the loading of Ca stores and the basal $[Ca^{2+}]_i$ as an inducer of Ca release, and that the Ca influx is capable of determining the rhythm of h.r.s by changing the rate of Ca accumulation in the stores. This model is supported by the observation that the frequency of s.–h.r.s is increased on raising the $[Ca^{2+}]_o$ and decreased by lowering the $[Ca^{2+}]_o$ or by adding $Mn^{2+}$ or $Co^{2+}$, with little change in the amplitude of each s.–h.r.

It has been shown in both invertebrate (Steinhardt *et al.* 1977) and vertebrate (Ridgway, Gilkey & Jaffe, 1977; Gilkey *et al.* 1978) eggs that sperm–egg fusion triggers a transient Ca release. In the hamster egg the first h.r., which coincides with the cessation of flagellar motion of the first sperm (Miyazaki & Igusa, 1981*a*), seems to be induced by similar mechanism. The inseminated egg then undergoes remarkable changes such as the increase in $P_{Ca}$ and the fall of the threshold of Ca-induced Ca release. The succeeding periodic h.r.s may occur without a specific action of sperm to trigger each Ca release.

It has been found that hyperpolarizing responses occur in fibroblastic L cells either spontaneously or by electrical or mechanical stimuli. Okada, Tsuchiya & Inoue (1979) and Okada, Tsuchiya, Yada, Yano & Yawo (1981) have concluded that the primary source of $Ca^{2+}$ is external. On the other hand, Henkart & Nelson (1979) have suggested Ca release from the endoplasmic reticulum apposed to the surface membrane. They proposed that external $Ca^{2+}$ is necessary to bind somewhere in the membrane and, thereby, to play a 'permissive' role for surface stimuli to cause the h.r. The present work in the hamster egg supports a linkage of Ca transport to Ca release, although morphological bases for Ca stores have not yet been studied. A similar model has been proposed for sympathetic ganglion cells (Kuba & Takeshita, 1981). These mechanisms for regulating the $[Ca^{2+}]_i$ may play important roles in mediating cellular functions of non-muscle cells.

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