# Depolarizing Response of Rat Parathyroid Cells to Divalent Cations

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ABSTRACT Membrane potentials were recorded from rat parathyroid glands continuously perfused in vitro. At 1.5 mM external $Ca^{++}$, the resting potential averages $-73 \pm 5$ mV (mean ± SD, $n = 66$). On exposure to 2.5 mM $Ca^{++}$, the cells depolarize reversibly to a potential of $-34 \pm 8$ mV (mean ± SD). Depolarization to this value is complete in ~2–4 min, and repolarization on return to 1.5 mM $Ca^{++}$ takes about the same time. The depolarizing action of high $Ca^{++}$ is mimicked by all divalent cations tested, with the following order of effectiveness: $Ca^{++} > Sr^{++} > Mg^{++} > Ba^{++}$ for alkali-earth metals, and $Ca^{++} > Cd^{++} > Mn^{++} > Co^{++} > Zn^{++}$ for transition metals. Input resistance in 1.5 mM $Ca^{++}$ was 24.35 ± 14 MΩ (mean ± SD) and increased by an average factor of 2.43 ± 0.8 after switching to 2.5 mM $Ca^{++}$. The low value of input resistance suggests that cells are coupled by low-resistance junctions. The resting potential in low $Ca^{++}$ is quite insensitive to removal of external $Na^+$ or $Cl^-$, but very sensitive to changes in external $K^+$. Cells depolarize by 61 mV for a 10-fold increase in external $K^+$. In high $Ca^{++}$, membrane potential is less sensitive to an increase in external $K^+$ and is unchanged by increasing $K^+$ from 5 to 25 mM. Depolarization evoked by high $Ca^{++}$ may be slowed, but is unchanged in amplitude by removal of external $Na^+$ or $Cl^-$. Organic (D600) and inorganic ($Co^{++}$, $Cd^{++}$, and $Mn^{++}$) blockers of the $Ca^{++}$ channels do not interfere with the electrical response to $Ca^{++}$ changes. Our results show remarkable parallels to previous observations on the control of parathormone (PTH) release by $Ca^{++}$. They suggest an association between membrane voltage and secretion that is very unusual: parathyroid cells secrete when fully polarized, and secrete less when depolarized. The extraordinary sensitivity of parathyroid cells to divalent cations leads us to hypothesize the existence in their membranes of a divalent cation receptor that controls membrane permeability (possibly to $K^+$) and PTH secretion.

## INTRODUCTION

It is well known that the rate of parathyroid hormone secretion (PTH) is inversely related to serum $Ca^{++}$ levels. In vivo (Mayer and Hurst, 1978;

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Patt and Luckhardt, 1942) and in vitro (Brown et al., 1976; Morrissey and Cohn, 1978; Sherwood et al., 1970) experiments have demonstrated that a slight decrease (on the order of tenths of millimoles) in external $Ca^{++}$ concentration evokes a strong secretory response from parathyroid cells. Conversely, an increase in extracellular $Ca^{++}$ reduces the rate of PTH secretion. The curve relating secretion to $Ca^{++}$ concentration is very steep, with the full secretion rate at 0–0.5 mM and maximal suppression at 2–2.5 mM (Brown et al., 1976; Habener and Potts, 1976; Morrissey and Cohn, 1978). The steepness of the curve suggests the existence in parathyroid cells of an unusual and very sensitive Ca-sensing mechanism, the nature of which has not been discovered.

In many secretory cells, external $Ca^{++}$ is essential for secretion. Physiological stimulation results in $Ca^{++}$ entry into the cell interior, which is responsible for triggering exocytosis (Douglas, 1968; Petersen, 1980; Rubin, 1970). This general scheme may not apply to parathyroid cells. An increase in external $Ca^{++}$, as well as $Mg^{++}$, decreases PTH secretion (Brown et al., 1976; Habener and Potts, 1976; Sherwood et al., 1970) and full secretory activity can be observed in the absence of external $Ca^{++}$ (Fischer et al., 1982; Pintado and Wallace, personal communication). In addition, application of the calcium ionophore A23187, which presumably increases cytosolic $Ca^{++}$ (Reed and Lardy, 1972), inhibits PTH secretion (Brown et al., 1980; Fischer et al., 1982; Habener et al., 1977).

Membrane electrical activity plays an important role in stimulus-secretion coupling in many gland cells (Petersen, 1980). Electrophysiological studies on the parathyroid have been performed only recently and the two reports available show contradictory findings. In mouse parathyroid gland, in vitro intracellular recordings by Bruce and Anderson (1979) show that cells hyperpolarize when the external $Ca^{++}$ concentration decreases. In contrast, it has been reported that goat parathyroid cells maintained in culture depolarize in low $Ca^{++}$ and that voltage-dependent $Ca^{++}$ entry may trigger PTH exocytosis (Sand et al., 1981).

We have performed experiments in rat parathyroid glands in vitro and have found a very clear and reversible depolarization in response to increased external $Ca^{++}$ in the physiological range for rats (Roth and Raisz, 1964). Our results thus agree qualitatively with those found in the mouse (Bruce and Anderson, 1979). Depolarization is also observed after addition of all divalent cations tested, both alkali-earth and transition metals, including the $Ca^{++}$ channel blockers $Cd^{++}$, $Mn^{++}$, and $Co^{++}$. The depolarization evoked by increased $Ca^{++}$ is accompanied by an increase in input resistance and may be due to a suppression of $K^+$ permeability. The possibility that a divalent cation receptor located on the external surface of the membrane controls membrane permeability and, in turn, PTH secretion, is considered.

## METHODS

### *Preparation*

Experiments were performed on parathyroid glands obtained from adult, male

Wistar rats 100–200 g in weight. Animals were killed with ether and the gland was excised under a dissecting microscope. Parathyroid glands in the rat are located bilaterally in the lateral border of the thyroid gland and appear as pale ovoids ~1–1.5 mm in diameter partially embedded in the thyroid tissue (Greene, 1935). The parathyroid gland and part of the thyroid tissue adjacent to it were transferred to the recording chamber and secured to the bottom by small stainless-steel pins. The thin connective capsule covering the gland was removed with the aid of a stereomicroscope.

*Experimental Setup*

The recording chamber and part of the experimental setup are schematically represented in Fig. 1. The chamber was made of lucite and divided into three

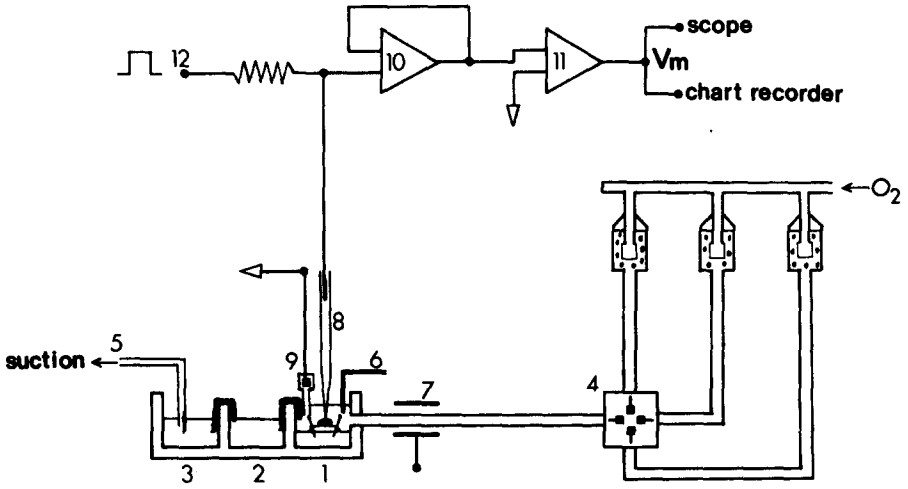


FIGURE 1. Schematic drawing of the apparatus used for intracellular recording in the continuously perfused parathyroid gland. 1: recording chamber. 2: intermediate reservoir. 3: reservoir where the suction (5) is applied. Fluid flows between 1, 2, and 3 by way of cotton wicks. 4: stopcock to select between five different solutions. 6: microthermistor permanently attached to the recording chamber. 7: heating filament that warms up the inflow solution. 8: glass microelectrode. 9: 3 M KCl agar bridge. 10: voltage follower. 11: stage for electronic compensation of electrode capacitance and resistance. 12: square current pulses (obtained from a constant current pump) that can be injected through the microelectrode.

compartments. The gland was placed in the first compartment (1), sitting on a layer of Sylgard (Dow Corning Corp., Midland, MI). This compartment contained the inlet for the external fluid. A thin wall of plastic attached to the bottom of the chamber (not illustrated in the figure) was placed as a baffle between the gland and the inlet aperture to direct the solution stream away from the microelectrode. The recording compartment had a volume of ~0.5 $cm^3$ and the bathing solution flow rate was 1 ml/min. Solution flowed from compartment 1 to a buffer compartment (2) and from this to a third compartment (3), where suction was applied. Flow between compartments was by way of cotton wicks. A silk thread glued at the outlet needle (5) was used to break the meniscus, making the suction regular and smooth.

The external perfusion system was mounted separately from the recording table. Any one of five solutions could be applied while recording in a given parathyroid cell. Switching was accomplished without interrupting the solution flow, and the level of solution in the recording compartment was thus kept constant. The dead space of the perfusion system was <0.1 ml and its washout time was 1 or 2 s. The temperature in the recording compartment was monitored with a microthermistor (6) and controlled by a circuit of our own design. This circuit passed the appropriate current through a coil (7) placed around the solution inlet, thus maintaining the recording compartment at 37°C.

### *Solutions*

Solution compositions are shown in Table I. Modified Krebs solutions with variable $Ca^{++}$ concentrations (between 0.5 and 2.5 mM) were made by mixing solutions A and B. In experiments where the effects of divalent cations other than $Ca^{++}$ or $Mg^{++}$ were tested, $SrCl_2$, $BaCl_2$, $MnCl_2$, $CdCl_2$, $CoCl_2$, or $ZnCl_2$ were substituted equimolarly for NaCl. Cl-free solutions (98% Cl-free) with variable $K^+$ concentrations were made by mixing solutions E, F, G, and H. In

TABLE I

*Composition of Solutions (in mM)*

| | A Krebs 0.5 Ca | B Krebs 2.5 Ca | C Na-free 1.5 Ca | D Na-free 2.5 Ca | E Cl-free, 0 K, 2 Ca | F Cl-free, 0 K, 4 Ca | G Cl-free, 100 K, 2 Ca | H Cl-free, 100 K, 4 Ca |
|---|---|---|---|---|---|---|---|---|
| NaCl | 120 | 117 | — | — | — | — | — | — |
| KCl | 5 | 5 | 5 | 5 | — | — | — | — |
| K-glutamate | — | — | — | — | — | — | 100 | 100 |
| Na-fumarate | 2.7 | 2.7 | — | — | 2.7 | 2.7 | 2.7 | 2.7 |
| Na-pyruvate | 4.9 | 4.9 | — | — | 4.9 | 4.9 | 4.9 | 4.9 |
| Na-glutamate | 4.9 | 4.9 | — | — | 130 | 127 | 30 | 27 |
| $MgSO_4$ | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| $CaCl_2$ | 0.5 | 2.5 | 1.5 | 2.5 | 2.0 | 4.0 | 2.0 | 4.0 |
| Glucose | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 |
| HEPES | 10 | 10 | — | — | 10 | 10 | 10 | 10 |
| Trizma (pH 7.5) | — | — | 142.5 | 141.5 | — | — | — | — |

some experiments variable amounts of D600 from a 1-mM stock solution were added to the modified Krebs solution until reaching final concentrations between 30 and 150 $\mu$M. The pH of all solutions was adjusted to 7.4 except for the Na-free solutions (C and D), where Trizma, pH 7.5, was used as buffer and as Na substitute. Solutions were continuously bubbled with 100% $O_2$ and had an osmolality of 270–290 mosmol/kg. The junction potentials of all solutions were measured with the electrode placed in the recording chamber. In solutions with differences only in divalent cation concentration, the junction potential differed by <1 mV. When switching from a modified Krebs solution to a Na-free solution, a junction potential of +4 mV was observed.

### *Recording*

Parathyroid cells were impaled with glass microelectrodes filled with 3 M KCl and having 100–200 MΩ DC resistance. The microelectrodes were pulled with a vertical puller from thick-walled borosilicate glass (OD = 2 mm, ID = 0.7 mm) with an inner filament for ease of filling. The microelectrodes were electrically connected to an FET operational amplifier (AD515; Analog Devices, Inc.,

Norwood, MA) wired as a voltage follower and placed close to the preparation. External voltage was sensed by a 3 M KCl agar bridge placed in the recording compartment of the chamber. The membrane potential was simultaneously monitored by an oscilloscope and a digital meter, and recorded on a chart recorder (Brush Instruments, Gould Inc., Cleveland, OH) with a frequency response of ~100 Hz. Square pulses of 200 ms duration and variable amplitude (between 0.1 and 0.25 nA) obtained from a constant-current pump were injected through the microelectrode to measure input resistance. Stray capacitance and voltage drop across the microelectrode were electronically compensated. The recording chamber and micromanipulator were placed on a heavy granite table sitting on inner tubes for shock absorption. The recording table was electrically shielded to prevent noise.

### *Experimental Procedure*

Cells were successfully impaled as deep as 250–300 μm from the surface of the gland. Most impalements only lasted for a few seconds, but occasionally the electrode stayed in the cell and a stable value of the membrane potential was reached in 2 or 3 min. Good impalements were characterized by a sudden change in the membrane potential. In all cases, the recorded voltage was balanced to zero before entering the gland. After an impalement, a check for voltage drift was made by withdrawing the electrode from the gland. In every cell from which a stable recording could be made, the response to changes in external $Ca^{++}$ concentration was tested. This was used as an indication that the recordings were made in a qualitatively homogeneous population of parathyroid cells. After this test, the rest of the experimental protocol was performed. We have recorded more than 60 parathyroid cells for periods from 10 min to >3 h. In long penetrations it was possible to test several solutions with different ionic compositions. In many other cells, stable recordings lasting several minutes were obtained, but the electrode came out of the cell before a solution change could be accomplished.

## RESULTS

### *Resting Potential in Parathyroid Cells*

Most cells were impaled while the gland was bathed with a solution containing 1.5 mM $Ca^{++}$. In many of these cells, the resting potential stabilized in the first minute after the penetration, reaching an average value of $-73 \pm 5$ mV (mean ± SD, $n = 66$).

### *Membrane Potential Depends on External $Ca^{++}$*

In all parathyroid cells exposed to more than one solution, the magnitude of the membrane potential was strongly dependent on the concentration of external $Ca^{++}$. This is illustrated in Fig. 2 by a continuous recording of membrane voltage from a single cell. The cell was initially impaled in Krebs 2.5 mM $Ca^{++}$, yielding a resting potential of −27 mV. Solution changes are indicated by the arrows. The records show very clearly that membrane potential depends strongly and reversibly on external $Ca^{++}$. The cell depolarizes in response to an increase in external $Ca^{++}$ after a

delay required for washout and equilibration. Once the membrane potential started to change, the time required to attain the new steady state level was between 1.5 and 3 min, depending on each particular cell.

Membrane voltage is sensitive to external $Ca^{++}$ in a narrow concentration range, between 1 and 2.5 mM $Ca^{++}$. $Ca^{++}$ concentrations of <1 mM were only tested in a few cells because, for unknown reasons, perhaps chance, it was difficult to obtain stable intracellular recordings. In the lower part of Fig. 2, switching from 1.5 to 0.5 mM $Ca^{++}$ solution does

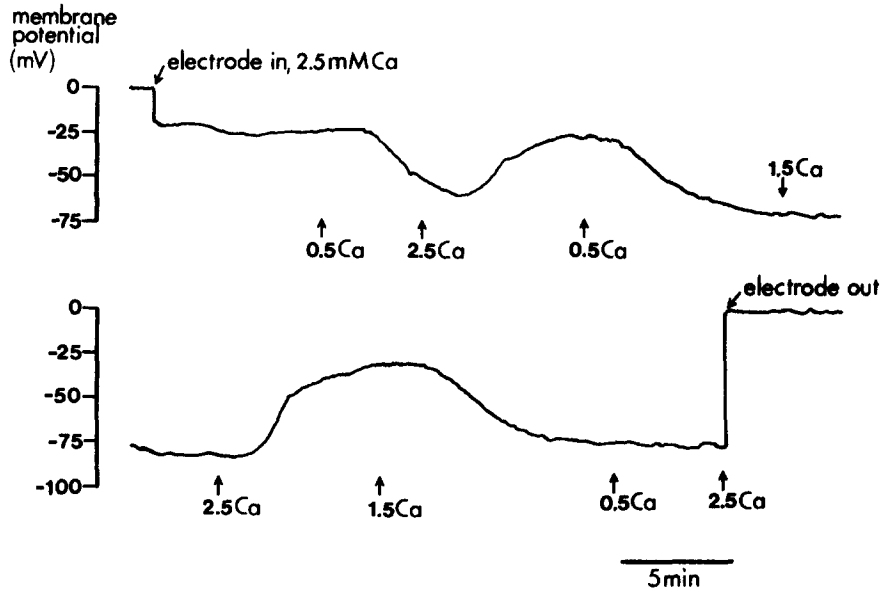


FIGURE 2. Modifications of membrane potential by changes of external $Ca^{++}$ concentration. The traces are a continuous intracellular recording from a parathyroid cell exposed to different external $Ca^{++}$ concentrations at the time indicated. The cell was impaled in a solution containing 2.5 mM $Ca^{++}$. The steepest voltage response occurs between 1.5 and 2.5 mM $Ca^{++}$. In 0.5 mM $Ca^{++}$, the membrane potential is about the same as in 1.5 mM $Ca^{++}$, or slightly more positive. Different $Ca^{++}$ concentrations were obtained by mixing solutions A and B of Table I. Osmolality of all solutions was between 270 and 290 mosmol/kg, pH 7.4, temperature 37°C.

not have an appreciable effect on the membrane potential. Some cells even showed a slight depolarization when the bathing solution was changed from 1.5 to 0.5 mM $Ca^{++}$.

The mean resting potential of several cells as a function of external $Ca^{++}$ concentration is shown in Fig. 3. The values in *A* are from cells continuously recorded for 10–120 min and exposed to at least two solutions with different $Ca^{++}$ concentrations. Extreme values of potential were $-34 \pm 8$ mV (mean ± SD) in 2.5 mM $Ca^{++}$ and $-80.5 \pm 2$ mV (mean ± SD) in 1.0 mM $Ca^{++}$.

Fig. 3*B* shows the same curve compiled from penetrations maintained for only 1–10 min, too short a time for a solution change to be accomplished. The $Ca^{++}$ concentration in the bathing solution for each penetration is given on the axis. The measurements by the two methods are in excellent agreement and clearly demonstrate that in parathyroid cells a 1.5 mM change in external $Ca^{++}$ in a definite range (between 1 and 2.5 mM) evokes a mean potential change of 46 mV. The relationship between membrane potential and external $Ca^{++}$ in that range is very steep.

One of the most striking characteristics of this electrical response is its nearly perfect reversibility. Some cells were exposed several times to

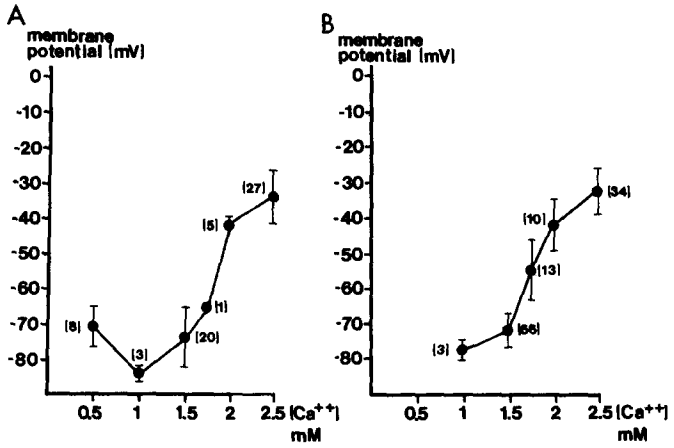


FIGURE 3. Membrane potential in parathyroid cells as a function of external $Ca^{++}$. (*A*) Data from single penetrations that were held long enough (10 min to 3 h) to record resting potential in at least three solutions with different $Ca^{++}$ concentrations. Each point represents the mean ± SD of 3–27 measurements as given by the numbers in parentheses. (*B*) Data from penetrations too brief to allow a change of the bathing solution. The penetrations were performed in the $Ca^{++}$ concentration specified on the axis, and the resting potential remained stable for 1–10 min. Each point represents the mean ± SD of 1–66 measurements, and the numbers of measurements are in parentheses. Composition of solutions is the same as in Fig. 2.

solutions with 1.5 and 2.5 mM $Ca^{++}$ and the membrane potential was measured at a given time after the solution change. In a typical cell the mean membrane potential values of eight measurements done 5–6 min after switching to the new solution were −79.7 ± 2.4 mV (mean ± SD) in 1.5 mM $Ca^{++}$ and −32.2 ± 2.5 mV (mean ± SD) in 2.5 mM $Ca^{++}$. The extremely small dispersion around the mean indicates a nearly perfect reversibility of the response.

On three occasions stable intracellular recordings from the tissue surrounding the parathyroid gland (presumably from thyroid cells) were obtained. The resting potential of these cells was about −65 mV and did

not show any appreciable modification in response to changes in external $Ca^{++}$ concentration in the range of 0.5–2.5 mM $Ca^{++}$.

*Absence of Spike Activity*

The electrical response evoked by increasing or decreasing external $Ca^{++}$ always showed the qualitative features referred to above and no spiking activity was ever seen. In some cells that showed a very low resting potential (−40 mV in 1.5 mM $Ca^{++}$), irregular jumps and spikes were observed in the first seconds after the impalement that were probably due to an imperfect penetration of the cell, since either the microelectrode came out of the cell shortly after the impalement or the membrane sealed around the electrode and the "spikes" disappeared as membrane potential increased.

*Calcium Effects Are Mimicked by Other Divalent Cations*

Other alkali-earth cations have the same qualitative effect on parathyroid cell membrane potential as $Ca^{++}$. This is illustrated in Fig. 4*A*, which shows a continuous intracellular recording from a single cell. The initial impalement was done in a solution containing 1.5 mM $Ca^{++}$, yielding a resting potential of −76 mV. At the time indicated by the arrows, the bathing solution was changed to one containing, in addition to 1.5 mM $Ca^{++}$, 1 mM of another divalent cation. All the cations caused a reversible depolarization, but with different time courses and magnitudes. The effectiveness for the response was $Ca^{++} > Sr^{++} > Mg^{++} > Ba^{++}$. None of these divalent cations was as effective as $Ca^{++}$ in depolarizing parathyroid cells. $Sr^{++}$ and $Ba^{++}$ responses were, respectively, ~60 and ~30% of the depolarization observed with $Ca^{++}$. $Mg^{++}$ yielded an intermediate value. The difference in the time course is also very apparent. Whereas the depolarization induced by 1 mM additional $Ca^{++}$ reached the steady state in 2.5 min, the $Ba^{++}$-induced depolarization was three times smaller and required ~6 min to attain the steady state. The same experimental protocol shown in Fig. 4*A* has been repeated in three different cells with similar results.

We have also studied the effect of other divalent cations, and an example is shown in Fig. 4*B*. The record belongs to another parathyroid cell impaled in 1.5 mM $Ca^{++}$, with an initial membrane potential of −75 mV. The experimental protocol was the same as before: in addition to 1.5 mM $Ca^{++}$, 1 mM of a test divalent cation was added to the bathing solution. In all cases the cell depolarized when the test cation was added. In this case the effectiveness of the response is $Ca^{++} > Cd^{++} > Mn^{++} > Co^{++}$. $Cd^{++}$ and $Mn^{++}$ seem to be slightly more effective than $Sr^{++}$, and the effectiveness of $Co^{++}$ is about the same as with $Ba^{++}$. The effects of $Cd^{++}$, $Mn^{++}$, and $Co^{++}$ have been tested in two different cells with identical qualitative results. $Zn^{++}$ was tested in a cell (not illustrated) and evoked a depolarization of about the same magnitude as $Co^{++}$. Fig. 4*A* and *B* shows unequivocally that membrane potential in parathyroid cells is affected not only by external $Ca^{++}$ but by all the divalent cations tested.

*Input Resistance and Cell-to-Cell Coupling*

Input resistance has been measured in several cells by passing hyperpolarizing square current pulses through the recording microelectrode. For these measurements the voltage drop across the microelectrode resistance

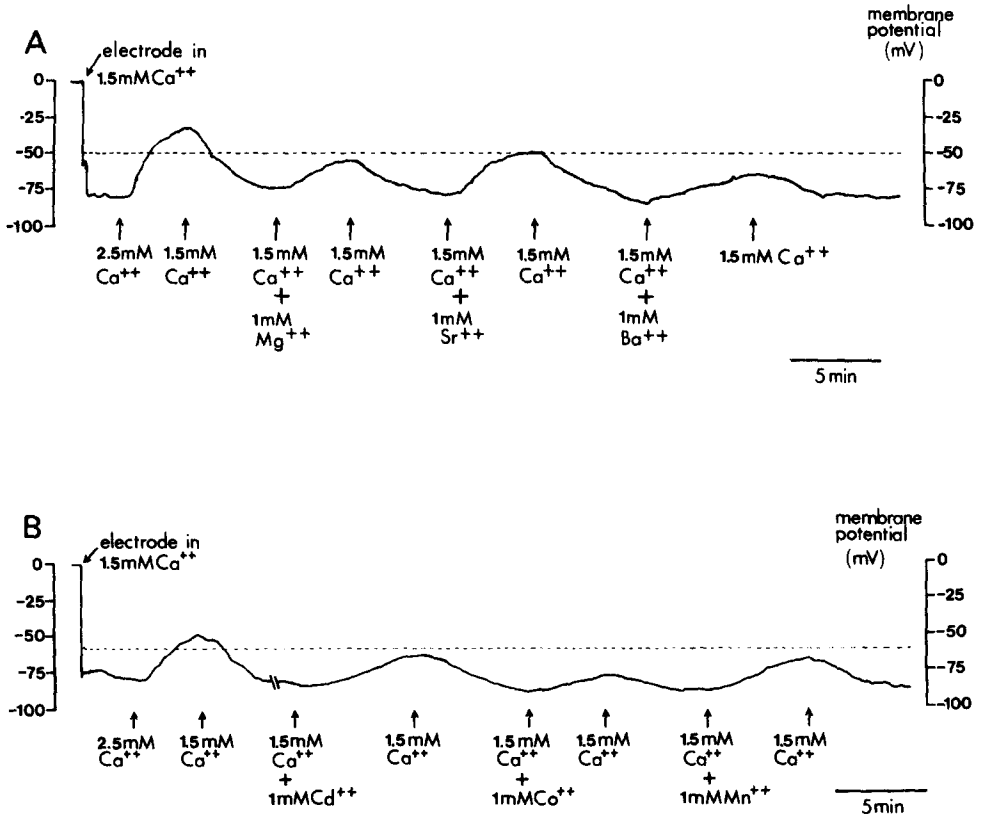


FIGURE 4. Effect of various divalent cations on membrane potential of parathyroid cells. *A* shows a continuous intracellular recording from a cell bathed with solutions containing the indicated concentration of divalent cations. The cell was impaled in 1.5 mM $Ca^{++}$, and switches to other solutions are indicated by the arrows. Solution composition is the same as in Fig. 1, except that $MgCl_2$, $SrCl_2$, $BaCl_2$, or additional $CaCl_2$ were substituted equimolarly for NaCl. The dashed horizontal line at −50 mV membrane potential is drawn for convenience of comparison. *B* shows a continuous recording of another cell exposed to the indicated transition elements. The time between the parallel bars in the record was 2.5 min, and the dashed line at −57 mV membrane potential is drawn for convenience.

was subtracted out electronically. The subtraction circuit was adjusted before penetrating the cell; the adequacy of the subtraction was tested after the electrode was withdrawn. The measurement was discarded if electrode resistance changed significantly during the penetration.

The mean value for input resistance was 24.3 ± 14 MΩ (mean ± SD, 10 cells) at 1.5 mM $Ca^{++}$. Taking into account the small size of parathyroid cells (~8 μm diam; Roth and Raisz, 1964), the expected input resistance for a single cell is ~500 MΩ, assuming a membrane resistance of 1,000 Ω $cm^2$. For a single cell of this diameter, a 24-MΩ input resistance would imply an unreasonably low value for membrane resistance, 48 Ω $cm^2$. The input resistance thus strongly suggests that in the whole gland, cells are coupled by low-resistance junctions. Input resistance would then reflect both membrane and junctional resistance of the coupled cells (see Discussion).

*Input Resistance Changes During the Depolarization Induced by High $Ca^{++}$*

The electrical response to $Ca^{++}$ in parathyroid cells is accompanied by a change in input resistance, but, as illustrated in Fig. 5, the correlation

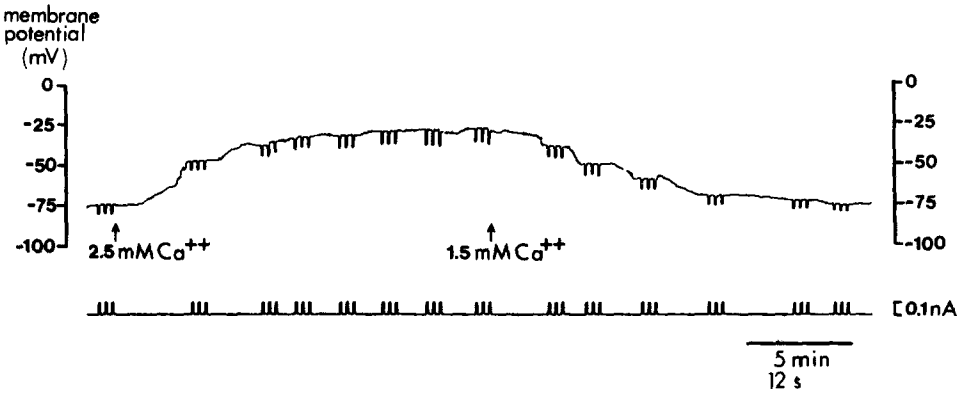


FIGURE 5. Input resistance increases on exposure to 2.5 mM $Ca^{++}$. The cell was impaled in a solution containing 1.5 mM $Ca^{++}$, and solution changes occurred at the arrows. The composition of solutions was the same as in Fig. 2. Input resistance was measured by injecting hyperpolarizing square current pulses of 0.1 nA amplitude and 200 ms duration, and the voltage drop across the microelectrode was electronically subtracted. The input resistance is thus proportional to the voltage deflection during the current pulse. The 12-s time scale is applicable during the pulse trains, and the 5-min scale at other times.

between the two variables is not very precise. Input resistance was determined from the voltage deflection (upper trace) recorded during the injection of hyperpolarizing current pulses of 0.1 nA amplitude (lower trace). It can be seen that input resistance increases by a factor of 2 or 3 during the depolarization induced on changing from 1.5 to 2.5 mM $Ca^{++}$. After switching back to the 1.5 mM $Ca^{++}$ solution, input resistance decreases as the cell progressively hyperpolarizes and, in this case, falls somewhat below the initial value.

In cells where input resistances in 1.5 and 2.5 mM $Ca^{++}$ were measured, it was higher in 2.5 mM $Ca^{++}$ in every case, and the average factor was

2.4 ± 0.8 (mean ± SD, 10 cells). In most cases the time course of the input resistance change did not exactly parallel the voltage change. It is of course possible that the resistance change is a result of the $V_m$ change caused by $Ca^{++}$, rather than being a direct effect of $Ca^{++}$.

*Complete Replacement of External $Na^+$ Does Not Prevent the Depolarization Induced by High $Ca^{++}$*

To investigate which ions participate in the electrical response induced by $Ca^{++}$, intracellular potentials have been recorded in cells perfused with solutions in which one permeant external ion was replaced by another that was presumed to be less permeant. The effect of complete replacement of external $Na^+$ by $Tris^+$ was tested in three cells, and in all cases similar qualitative results were obtained. An example is shown in Fig. 6.

In this cell, membrane potential was continuously recorded for ~35 min and it was possible to switch eight different times to solutions with different ionic compositions. The impalement was done in a Na-free solution containing 1.5 mM $Ca^{++}$ and the initial resting potential was −68 mV, which is close to the usual value found at this $Ca^{++}$ concentration in cells perfused with the modified Krebs solution. Switching to a full-$Na^+$ solution with the same $Ca^{++}$ concentration (1.5 mM $Ca^{++}$) caused only small fluctuations in the membrane potential. The introduction of 2.5 mM $Ca^{++}$, Na-free solution evoked a depolarization of ~35 mV, which was reversed on returning to 1.5 mM $Ca^{++}$. At this time the introduction of a full-$Na^+$ solution with 2.5 mM $Ca^{++}$ induced a depolarization to the same voltage as before, but with a faster time course than in the absence of $Na^+$. The second run with the same solutions caused the same effects: relatively slow depolarization by Na-free 2.5 mM $Ca^{++}$, slow repolarization by 1.5 mM $Ca^{++}$, and fast depolarization by 2.5 mM $Ca^{++}$.

Although full-$Na^+$ and Na-free solutions have been compared in only three cells, we think it is safe to say that at 1.5 mM $Ca^{++}$, external $Na^+$ does not make an important contribution to the resting potential in parathyroid cells. The magnitude of the depolarization induced by 2.5 mM $Ca^{++}$ is about the same in the presence or absence of $Na^+$ in the external solution. However, the depolarization induced by Na-free, 2.5 mM $Ca^{++}$ probably has a slower time course than the one observed with full-Na, 2.5 mM $Ca^{++}$. This difference in time course, although very clear for the cell shown in Fig. 6, was not so apparent in the other two cells, where $Na^+$-free and full-$Na^+$ solutions were compared, and therefore no definitive conclusion can be drawn at present. In cells perfused with $Na^+$-free solutions, the repolarization after switching to 1.5 mM $Ca^{++}$ was slower than in cells never exposed to $Na^+$-free solutions. This slower repolarization may be due to temporary inactivation of the Na-K pump in cells partially depleted of internal $Na^+$ after several minutes of being perfused with $Na^+$-free solution.

*Depolarization Induced by $Ca^{++}$ Is Not Abolished by $Ca^{++}$ Channel Blockers*

Since the depolarization induced by $Ca^{++}$ was present even in the absence

of external $Na^+$, the hypothesis that a $Ca^{++}$ conductance could be partially responsible for this response was tested in several cells. It has been shown previously (see Fig. 4) that several divalent cations reputed to be $Ca^{++}$ channel blockers ($Cd^{++}$ and $Co^{++}$, and, often, $Mn^{++}$) simulate the effects of $Ca^{++}$ in parathyroid cells. In this section we show that neither inorganic nor organic $Ca^{++}$ channel blockers modify the depolarization induced by 2.5 mM $Ca^{++}$.

The effects of two inorganic $Ca^{++}$ channel blockers are shown in Fig. 7. Part *A* compares the depolarization induced by 2.5 mM $Ca^{++}$ with and without 2 mM $Co^{++}$. The cell was impaled in 1.5 mM $Ca^{++}$ (not illustrated

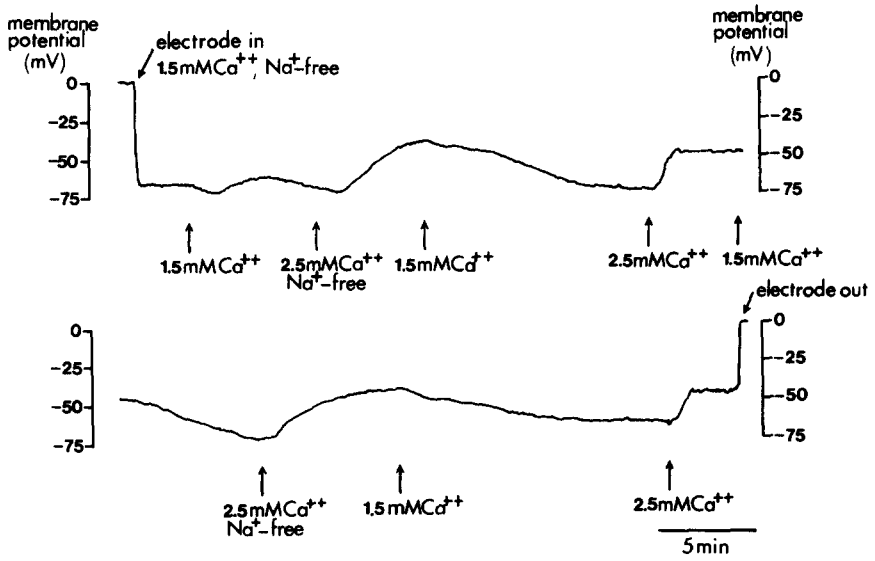


FIGURE 6. Effect of complete replacement of external $Na^+$ on the depolarization induced by high $Ca^{++}$. The cell was initially impaled in a 1.5 mM $Ca^{++}$, $Na^+$-free solution, and changes to other solutions are indicated by the arrows. Both panels are continuous in time and belong to the same cell. The composition of solutions is given in Table I. The time courses of the depolarization and repolarization evoked by high and low $Ca^{++}$, respectively, in the absence of $Na^+$ are discussed in the text.

in the figure), and several minutes later a solution with 2.5 mM $Ca^{++}$ evoked a reversible depolarization of 33 mV. A second run in 2.5 mM $Ca^{++}$, but with the addition of 2 mM $Co^{++}$, yielded a depolarization of 40 mV. The effect of $Ca^{++}$ and $Co^{++}$ was additive and the depolarization was larger. In part *B*, from another cell, 2.5 mM $Ca^{++}$ depolarizes by ~40 mV even in the presence of 0.5 mM $Cd^{++}$.

The effects of several concentrations of D600 have been studied in three cells and two of them are illustrated in Fig. 8. In *A*, the depolarization evoked by 2.5 mM $Ca^{++}$ can be compared in the absence and in the presence of 30, 80, and 100 $\mu$M D600. The record in *B* is from

another cell, where a very large concentration (150 μM) of D600 was used. The response was only slightly smaller than in the control records taken before and after the addition of D600. The effect of 40 μM D600 in 1.5 mM $Ca^{++}$ was tested in two cells, and no appreciable change of membrane potential was observed. The records in Fig. 8 clearly show that large concentrations of D600 have very little, if any, effect on membrane potential depolarization induced by high $Ca^{++}$. These results suggest that in parathyroid cells, a $Ca^{++}$ current through the usual voltage-

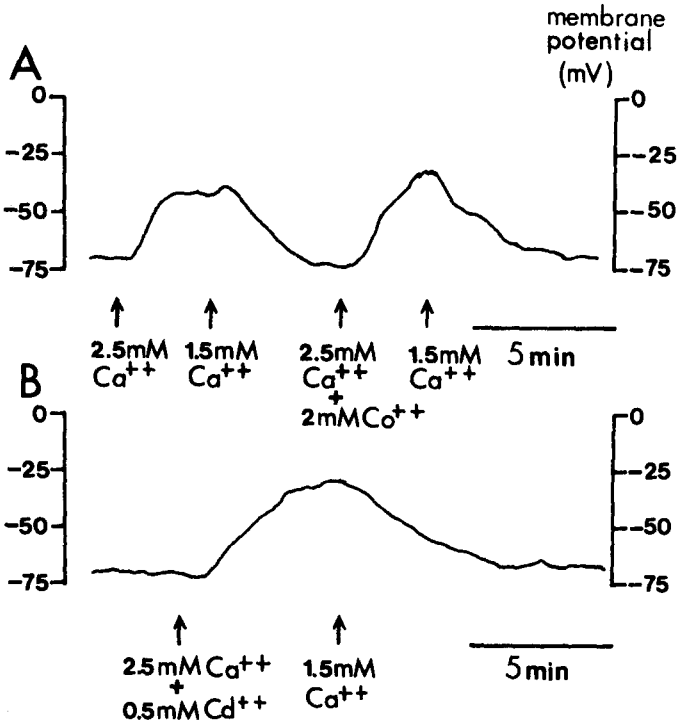


FIGURE 7. Effects of inorganic $Ca^{++}$ channel blockers on the depolarization evoked by high $Ca^{++}$. *A* shows the membrane potential of a cell initially impaled in 1.5 mM $Ca^{++}$, with solution changes at the arrows. *B* is from a different cell, initially penetrated in 1.5 mM $Ca^{++}$. The composition of solutions is the same as in Fig. 2, except that $CoCl_2$ and $CdCl_2$ were substituted equimolarly for NaCl.

dependent $Ca^{++}$ channels does not participate in the depolarization seen in high $Ca^{++}$. This is further supported by the observation that $Ba^{++}$ is less effective than $Ca^{++}$, even though $Ba^{++}$ is more effective than $Ca^{++}$ in carrying current through Ca channels (Hagiwara et al., 1974).

*Effects of Chloride Substitution*

In several experiments, 98% of the external $Cl^-$ was replaced by glutamate and the effects of variable concentrations of $Ca^{++}$ and $K^+$ on parathyroid

cell membrane potential were studied. Complete replacement of $Cl^-$ was done to test whether its presence was necessary for the voltage changes induced by $Ca^{++}$ and to avoid possible hysteresis in the response when studying the effects of alterations in external $K^+$ (Hodgkin and Horowicz, 1959). The $Ca^{++}$ concentrations used in these solutions were higher than those used in the modified Krebs solution because high concentrations of glutamate decrease $Ca^{++}$ activity. $Ca^{++}$ concentrations used for the low- and high-$Ca^{++}$ solutions were 2 and 4 mM, respectively. These concentra-

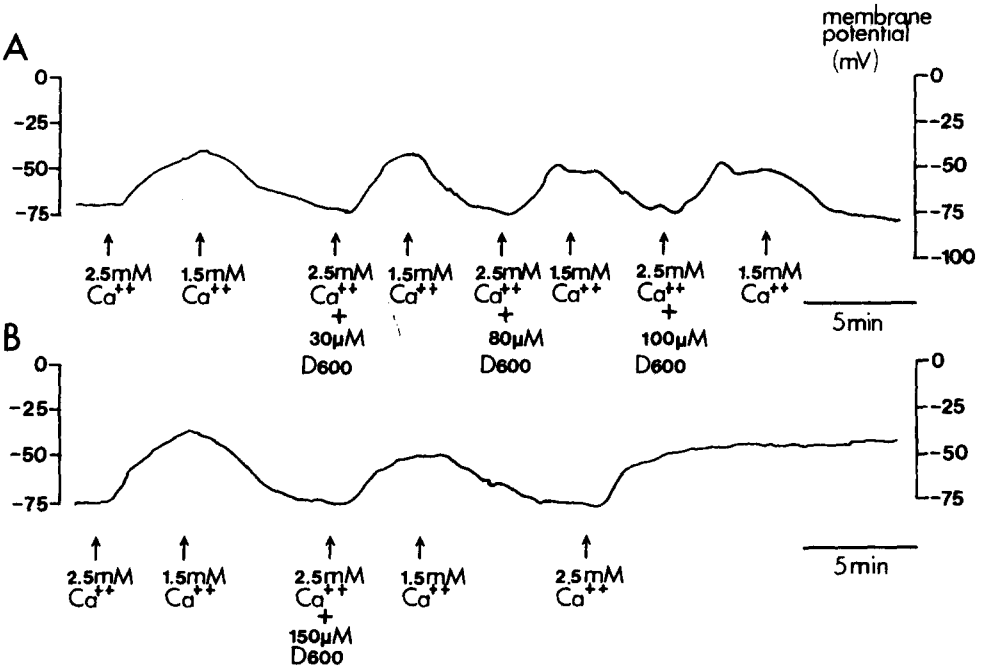


FIGURE 8. The organic $Ca^{++}$ channel blocker D600 does not affect the response to $Ca^{++}$ changes. *A* is a continuous recording from a cell initially impaled in 1.5 mM $Ca^{++}$ with changes to new solutions indicated by the arrows. *B* is from another cell, where a larger concentration of D600 was tested. The composition of solutions is the same as in Fig. 2. Variable quantities of D600 from a 1-mM stock solution were added to the 2.5 mM $Ca^{++}$ solution to obtain the desired final concentration of D600.

tions were chosen empirically because they yielded membrane potential values about the same as 1.5 and 2.5 mM $Ca^{++}$ in the Krebs solution. An estimate with a calcium electrode gave similar calcium activities for both sets of solutions.

Removal of $Cl^-$ does not seem to affect appreciably the resting potential measured in either low or high $Ca^{++}$ at a constant (5 mM) $K^+$ concentration. Mean values of potential were $-78.6 \pm 6.6$ mV (mean ± SD, $n = 14$) in 2 mM $Ca^{++}$ and $-39.0 \pm 7.3$ mV (mean ± SD, $n = 13$) in 4 mM $Ca^{++}$. Thus, the mean depolarization induced by high $Ca^{++}$ was ~39 mV,

which is not very far from the value measured with the Krebs solutions (40 mV) at the same concentration of potassium.

*Effects of Alteration in External Potassium*

The effects of varying external $K^+$ at low and high $Ca^{++}$ are illustrated by the records of Fig. 9. Part *A* shows a continuous intracellular recording

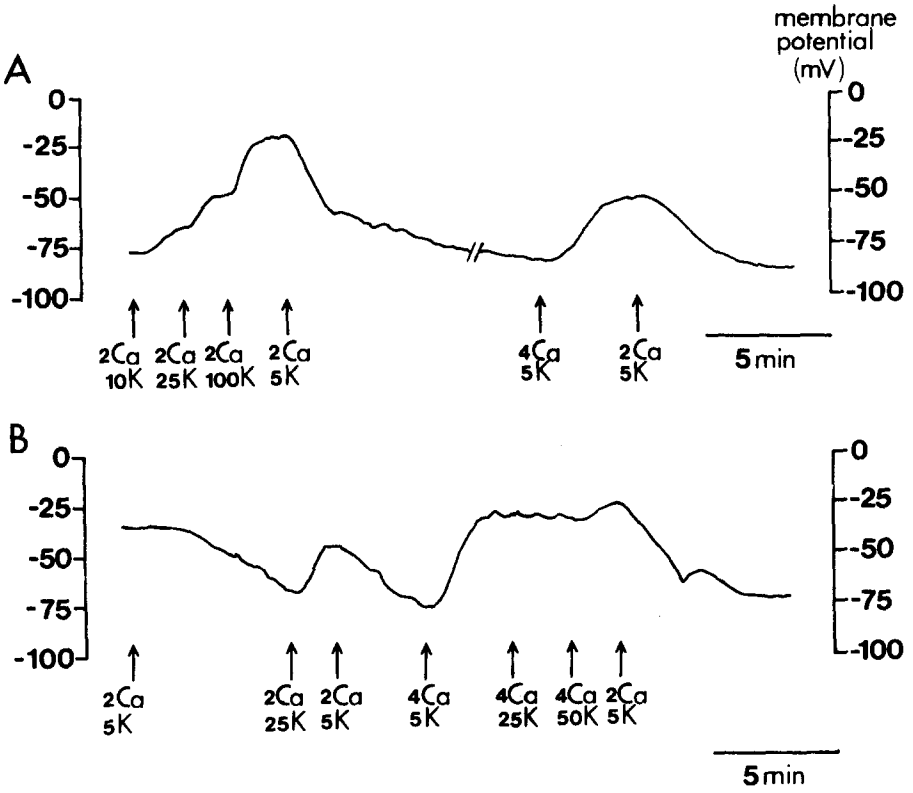


FIGURE 9. Effects of external $Cl^-$ replacement and variable external $K^+$ concentrations on the electrical activity in parathyroid cells. *A* is a recording from a cell initially impaled in a 2 mM $Ca^{++}$, 5 mM $K^+$, $Cl^-$-free solution with changes to new solutions at the time indicated by the arrows. The time between the parallel bars in the record was 2 min. *B* is from another cell first impaled in a 4 mM $Ca^{++}$, 5 $K^+$, $Cl^-$-free solution with changes to new solutions at the time pointed out by the arrows. The composition of solution is given in Table I. In all cases, 98% of external $Cl^-$ was substituted by glutamate.

of a cell initially impaled in 2 mM $Ca^{++}$, 5 mM $K^+$ solution, with a resting potential of −76 mV. Successive changes to solutions with 10, 25, and 100 mM $K^+$ at the same $Ca^{++}$ concentration are indicated by the arrows. The cell depolarizes upon an increase in external potassium to a level determined by the concentration. After switching back to the 2 mM $Ca^{++}$,

5 mM $K^+$ solution, the cell repolarizes to approximately the original level. In all cells depolarized by elevated external $K^+$, the repolarization showed a slower time course than the depolarization. Fig. 9*A* also shows that the same cell was depolarized reversibly by 4 mM $Ca^{++}$ at a constant (5 mM) $K^+$ concentration by ~36 mV.

Fig. 9*B* illustrates a continuous intracellular recording from another cell, which was initially impaled in 5 mM K, 4 mM Ca and had a resting potential of −33 mV. $V_m$ went negative by 34 mV on changing to 5 mM K, 2 mM Ca. The addition of 25 mM K, 2 mM Ca reversibly depolarized the cell by 28 mV. After recovery in 5 mM K, 2 mM Ca, the cell was subsequently depolarized by 43 mV in 5 mM K, 4 mM Ca, reaching a membrane potential of about −30 mV. At this point the addition of 25 and 50 mM $K^+$, keeping constant the external $Ca^{++}$, had little, if any, effect on the membrane potential. The reintroduction of 5 mM K, 2 mM Ca solution repolarized the cell to the previous resting potential level (−74 mV). Fig. 9*B* also shows that the time course of membrane depolarization and hyperpolarization induced by $Ca^{++}$ is very similar to the voltage changes observed by the introduction or the withdrawal of high $K^+$ solution.

The records in Fig. 9 are typical examples of the response of parathyroid cells to changes in external $Ca^{++}$ and $K^+$ in Cl-free solutions, and they suggest that external $Cl^-$ does not make an important contribution to either the resting potential or the $Ca^{++}$-induced electrical response. In low $Ca^{++}$, the membrane potential is highly sensitive to external $K^+$, but once the cells are depolarized by high $Ca^{++}$, external $K^+$ seems to have little effect on the membrane potential.

The effects of external $Ca^{++}$ and $K^+$ in Cl-free solutions have been studied in many cells and a summary is given in Fig. 10. In the figure, membrane potential in 2 mM (filled symbols) and 4 mM (open symbols) $Ca^{++}$ has been plotted vs. the logarithm of external $K^+$. The vertical line represents the standard deviation of the mean and the numbers of observations are in parentheses. In low $Ca^{++}$, the membrane potential is very sensitive to external $K^+$. Fitting by eye, the straight part of the curve (above 25 mM $K^+$) yields an ~61 mV change in membrane potential for a 10-fold change in external $K^+$, which is similar to the value predicted by the Nernst equation for a potassium electrode at 37°C. Extrapolating the straight line to 0 mV membrane potential gives an estimate of the intracellular potassium concentration of 160 mM. At high $Ca^{++}$, the number of measurements is smaller, but it is very clear that membrane potential is less sensitive to external $K^+$. Thus, it seems that at low $Ca^{++}$, parathyroid cells behave as a potassium electrode and $P_K$ is the dominant permeability. At high $Ca^{++}$, the ratio of $P_K$ to other permeabilities probably decreases and thus membrane potential is less sensitive to external potassium. The possible participation of different ionic permeabilities in the voltage change seen in parathyroid cells will be analyzed in the Discussion.

*Effects of Isoproterenol*

It is known that the beta agonist isoproterenol, at concentrations between 1 and 10 $\mu$m, increases PTH secretion rate in dispersed bovine parathyroid cells (Brown et al., 1977). We have studied the effects of variable concentrations of isoproterenol (between 10 and 40 $\mu$M) added to the modified Krebs solution in four cells whose electrical response to external $Ca^{++}$ had previously been observed. These concentrations of isoproterenol did not have an important effect on the membrane potential either at low or at high $Ca^{++}$. In one cell there was no voltage change at all. In another cell membrane potential hyperpolarized by 6–7 mV, and in the

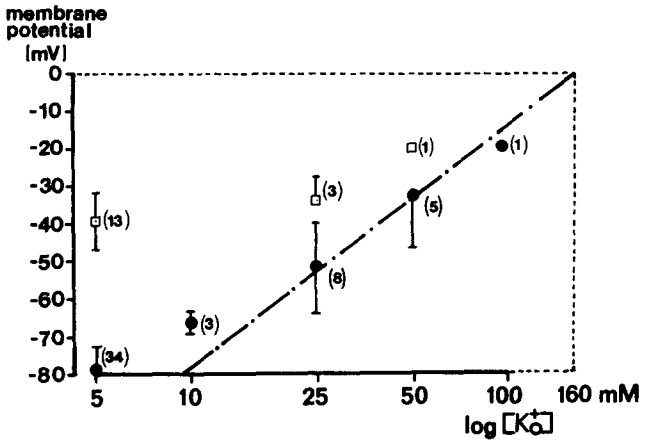


FIGURE 10. Dependence of membrane potential (ordinate) on the external concentration of $K^+$ represented in a semilogarithmic scale (abscissa). Filled circles are measurements in low $Ca^{++}$ (2 mM) and open squares are measurements in high $Ca^{++}$ (4 mM). Every point represents the mean and the vertical bars represent the standard deviation of several measurements obtained from records like those shown in Fig. 10. The number of measurements is in parentheses. The straight line has been drawn by eye and has a slope of ~61 mV per 10-fold increase in external $K^+$ concentration. The intercept of this line with the 0-mV axis gives an estimate of the internal concentration of $K^+$.

other two cases a 4–5-mV depolarization was observed. Although the number of observations is small and no definitive conclusion can be drawn, our data suggest that the mechanism for isoproterenol stimulation of parathyroid cells is different from that evoked by lowering divalent cations (see Discussion).

## DISCUSSION

The effects of external calcium concentration on parathyroid cell membrane potential have been reported only twice, with contradictory conclusions. Mouse parathyroid cells hyperpolarize on lowering external $Ca^{++}$

(Bruce and Anderson, 1979), but cultured goat parathyroid cells are said to do the opposite, having a resting potential of −29.2 mV in 0.8 mM $Ca^{++}$ and −44.7 mV in 2.4 mM $Ca^{++}$ (Sand et al., 1981). Our measurements on rat parathyroid agree qualitatively with observations in the mouse, but the magnitude of our resting potentials is ~1.5 times larger than reported for mouse, and twice as large as the reported values for cultured goat cells.

There have been no reports of spontaneous spike activity, regardless of $Ca^{++}$ concentration, and we have found none, even though our cells had resting potentials of very healthy magnitude. In cultured cells, regenerative responses are said to be triggered by injection of depolarizing current through the microelectrode (Sand et al., 1981). The high electrode resistance and low input resistance of the gland prevented us from stimulating effectively through the microelectrode.

Parathyroid cells in the rat are ~8 μm in diameter (Roth and Raisz, 1964). Using 1,000 Ω $cm^2$ as a guess of membrane resistance (Cole, 1968), the input resistance would be 498 MΩ were the cells not coupled to each other by junctions of lower resistance. For goat parathyroid cells in culture, the reported figure is 503 ± 218 MΩ in 2.4 mM $Ca^{++}$ and 290 ± 198 MΩ at 0.8 mM $Ca^{++}$. We report a much lower value for input resistance for cells in the gland, 24.3 ± 14 MΩ. This strongly suggests that in the parathyroid, as in other glands (Petersen, 1980), cells are interconnected by low-resistance junctions. Freeze-fracture replicas of rat parathyroid cells have confirmed the existence of numerous gap junctions (Ravazzola and Orci, 1977; López-Barneo and Franzini-Armstrong, 1982).

*Possible Origins of the Depolarization Induced by Divalent Cations*

It is first necessary to consider possible but uninteresting explanations of the sensitivity of membrane potential to $Ca^{++}$ concentration. The large magnitude of the resting potential in low $Ca^{++}$ makes it unlikely that we have problems with the seal between membrane and microelectrode. Further, the input resistance is higher in 2.5 mM $Ca^{++}$, when the cell is depolarized. The depolarization is thus unlikely to result from a failure of the membrane-electrode seal. A second possibility is that raising $Ca^{++}$ causes sufficient $Ca^{++}$ entry to reduce coupling between cells (De Mello, 1975; Iwatsuki and Petersen, 1977; Rose and Loewenstein, 1975). This could lead to depolarization of the impaled cell by the shunt resistance around the electrode, when the impaled cell is no longer supported by its neighbors. This possibility can be discarded on the grounds that $Mg^{++}$, which is normally present in the cell at high concentration and does not cause uncoupling (De Mello, 1975; Iwatsuki and Petersen, 1977), has an effect on membrane potential similar to $Ca^{++}$. Transition elements also depolarize parathyroid cells, although they are very impermeant and do not directly affect cell coupling. On occasion, they even compete with $Ca^{++}$ and restore cell coupling (Petersen, 1980). Thyroid follicular cells

from the rat are coupled and have an input resistance value of 11 MΩ (Green and Petersen, 1981). Both in the mouse (Bruce and Anderson, 1979) and in the rat (present experiments), the resting potential of thyroid cells was not affected by changes in external $Ca^{++}$ in the range of interest. Thus, both of these uninteresting explanations are unlikely.

The potential changes observed are much too large to be accounted for as a direct result of a change in the equilibrium potential of $Ca^{++}$. Even if the membrane were exclusively $Ca^{++}$ permeable, changing $Ca^{++}$ from 1.5 to 2.5 mM would change the membrane potential by only 6.7 mV, much less than the observed change. Nor does it seem likely that $Ca^{++}$ channels of the type reported in other tissues play a part in the response. The selectivity of the $Ca^{++}$ channel for alkali-earth divalent cations (Hagiwara et al., 1974; Hagiwara, 1975) contrasts with our findings in parathyroid cells, where no qualitative distinction between the four cations ($Ca^{++}$, $Sr^{++}$, $Mg^{++}$, and $Ba^{++}$) was seen. $Ba^{++}$ is not very effective in depolarizing parathyroid cells although it permeates well through the Ca channel (Hagiwara et al., 1974; Reuter, 1973). In addition, $Co^{++}$, $Cd^{++}$, and $Mn^{++}$, which are $Ca^{++}$ channel blockers (Baker et al., 1973; Kohlhardt et al., 1973; Hagiwara, 1975), mimic the effects of $Ca^{++}$ rather than blocking the response. Finally, the $Ca^{++}$ channel blocker D600 (Kohlhardt et al., 1972) is without effect on the response to raising $Ca^{++}$.

Thus, it seems that parathyroid cells possess an unusual calcium-sensing mechanism of extraordinary sensitivity, not observed in other biological preparations. A similar but much smaller response is seen in the resting secretory membrane of freshwater lamellibranch mantles. A 10-fold decrease in external calcium (from 6 to 0.6 mM) hyperpolarizes by 6 mV (Sorenson et al., 1980).

The order of effectiveness of divalent cations in depolarizing parathyroid cells is very similar to the affinity of Ca-binding proteins and calmodulin-like receptors for several divalent cations. It is known that $Mn^{++}$, $Sr^{++}$, $Mg^{++}$, and $Ba^{++}$ can bind to a brain $Ca^{++}$ regulatory protein (Wolff et al., 1977). In molluscan neurons, activation of potassium permeability by internal $Ca^{++}$ (an effect probably mediated by a $Ca^{++}$ receptor located in the inner side of the membrane) is simulated by other divalent cations (Meech, 1974; Meech and Standen, 1975). $Ca^{++}$ is the most effective ion, followed by $Cd^{++}$, $Sr^{++}$, and $Mn^{++}$. The cations $Ba^{++}$, $Co^{++}$, $Mg^{++}$, and $Zn^{++}$ have been found to be very ineffective (Gorman and Hermann, 1979). About the same order has been found in the exchange of various divalent cations for troponin-bound $^{45}Ca^{++}$ (Fuchs, 1971).

These findings suggest that the interaction of divalent cations with parathyroid cells is not based on their different permeability through the membrane and point toward the possible existence of a divalent cation receptor in the external surface of these cells. This receptor could be a Ca-binding protein with a different affinity for each of the divalent cations that affect membrane voltage. Modifications in membrane permeability

by conformational changes of the receptor after it binds to one or several divalent cations would result in the electrical events associated with alterations in the external concentration of divalent cations. Recent morphological studies on rat parathyroid glands using the freeze-fracture technique (López-Barneo and Franzini-Armstrong, 1982; C. Franzini-Armstrong, unpublished observations) have revealed the existence in the cell membrane of particles that are densely packed, regularly distributed throughout the plasmalemma, and unusually uniform in size. One might speculate that these unusual particles are receptors, whose dense packing confers on these cells their extraordinary $Ca^{++}$ sensitivity.

*Input Resistance and Membrane Resistance*

The input resistance ($R_i$) in a tissue that is electrically interconnected by gap junctions depends on both junctional ($R_j$) and membrane resistance ($R_m$). The change that we observe on raising external $Ca^{++}$ could arise from altering either factor. We know no mechanism by which extracellular $Mg^{++}$ or transition elements could raise junctional resistance, and therefore we suspect that divalent cations alter membrane resistance.

The magnitude of the $R_m$ change signaled by a 2.4-fold increase in $R_i$ depends on the ratio $R_j/R_m$ (cf. Holman and Neild, 1979, for a somewhat different conclusion). To approach this problem we used a simple model of the tissue, which was adequate for our purposes. The model (Fig. 11*A*) assumes that the penetrated cell (I) is a sphere of radius 4 μm, surrounded by spherical shells of 8 μm thickness. The shells contain successive generations of neighbors. Fig. 11*B* illustrates the electrical circuit that corresponds to this three-dimensional array assuming that (*a*) $R_m$ (resistance of the impaled cell) and $R_j$ (resistance of its junctions to all of its nearest neighbors) are the same for all cells, and (*b*) the resistivity of extracellular and intracellular media is negligible (Bennett, 1966; Socolar and Loewenstein, 1979). To facilitate computations, only three shells of cells (in addition to the impaled one) have been considered. The values of $R_{m1,2,\text{and}3}$ and $R_{j1\text{and}2}$ (see Fig. 11*B*) used are given in the figure legend.

Solving this equivalent circuit yields the plot shown in Fig. 11*C*. The ordinate represents the ratio $R_i/R_m$ at different values of $R_j/R_m$, as given on the abscissa in a semilog scale. The curve shows that when $R_j/R_m$ is large, $R_i/R_m$ approaches unity, since the impaled cell is well isolated from its neighbors and input resistance reflects only the membrane resistance of the impaled cell. When $R_j/R_m$ is very small, $R_i/R_m$ again approaches unity because the recording is being made from what is in essence a large single cell. For intermediate values of $R_j/R_m$, it can be seen that $R_i/R_m$ is much smaller than unity and therefore a given increase in $R_m$ is signaled by a smaller change in $R_i$. The same qualitative relationship between $R_m$, $R_j$, and $R_i$ is obtained with a two-dimensional model, but the minimal $R_i/R_m$ occurs at a different ratio of junctional to membrane resistance.

*Permeability Changes Involved in the Response to High Calcium*

In the absence of other information, the voltage change induced by high

$Ca^{++}$ could be due to either an increase of a depolarizing conductance or a decrease in a polarizing conductance, e.g., $P_K$. Neglecting the contribution of anions, it can be calculated by the Goldman (1943) equation

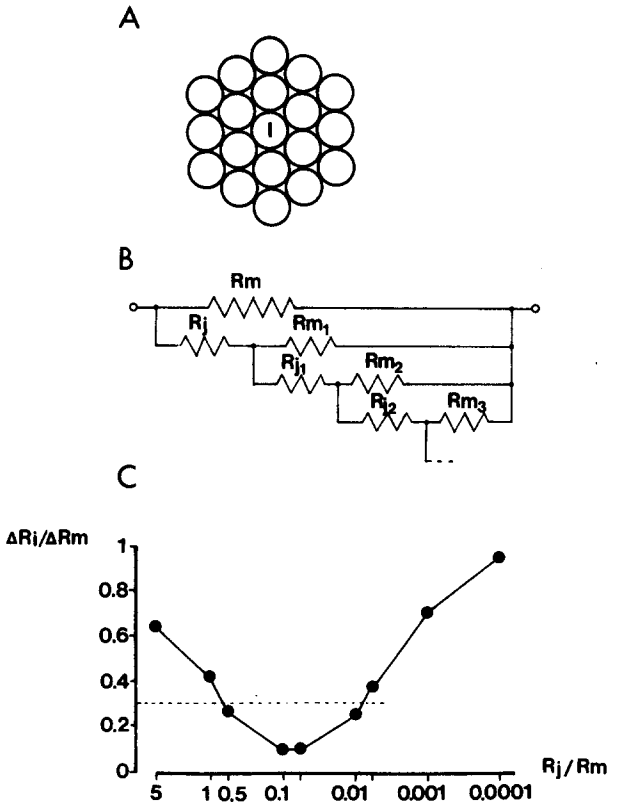


FIGURE 11. Cell-to-cell coupling in the parathyroid gland. *A* shows a model of cell topology in the parathyroid gland. The impaled cell (I) is surrounded by spherical shells of closely packed cells. The first, second, and third shells contain 25, 97, and 216 cells. *B* shows the equivalent electrical circuit of this model. $R_m$ is the membrane resistance of a single cell, and $R_j$ is the parallel resistance of the junctions to all of its neighbors. $R_{m_1}$, $R_{m_2}$, and $R_{m_3}$ are the parallel membrane resistance of the first, second, and third shell surrounding the penetrated cell and are, respectively, $R_m/25$, $R_m/97$, and $R_m/216$. $R_{j_1}$ and $R_{j_2}$ are the resultant junctional resistance between the first and the second shells and between the second and the third shells. These values are $25R_j/97$ and $25R_j/216$, respectively. *C* illustrates that the ratio of the change in input resistance to the change in membrane resistance ($R_i/R_m$) depends on the ratio $R_j/R_m$ (abscissa). The interrupted line is drawn at a ratio $R_i/R_m$ of 0.3 (a given change in $R_m$ results in a change in $R_i$ that is smaller by a factor of 0.33).

that if a depolarization of 39 mV (−73 mV at 1.5 $Ca^{++}$ and −34 mV at 2.5 mM $Ca^{++}$) were due to a decrease in $P_K$, a 7.9-fold increase in membrane permeability would be observed that would be reflected in an

$R_m$ change of about the same size. This calculation is done assuming 10 mM as the intracellular concentration of Na and 160 mM (estimated from the plot in Fig. 10) as the intracellular concentration of $K^+$.

In a coupled tissue, an 8-fold increase in $R_m$ can result in a 2.4-fold $R_i$ change, only 30% as large as the $R_m$ change, as discussed above. The interrupted line in Fig. 11*C* shows that with the three-dimensional model, two different $R_j/R_m$ ratios could give an $R_i/R_m$ ratio of this value. $R_j/R_m$ near 0.01 seems a reasonable ratio for a well-coupled tissue. Therefore, it is not impossible that the depolarization seen in high $Ca^{++}$ is due to a suppression of $K^+$ permeability. Clarification of this point must await more explicit data on $R_m$, and this will necessarily involve electrically isolated cells.

*Relationship Between Membrane Electrical Events and PTH Secretion*

In the mouse (Bruce and Anderson, 1979) and in the rat (present experiments), the relationship between membrane potential and external $Ca^{++}$ is very similar to the relationship between PTH release and external $Ca^{++}$ observed in the cow, both in vivo (Mayer and Hurst, 1978) and in vitro (Brown et al., 1976; Habener and Potts, 1976). PTH release and membrane potential as a function of external $Ca^{++}$ are illustrated in Fig. 12. Part *A* has been redrawn from Habener and Potts (1976) and part *B* is based on our experimental data. Both curves are very steep and comparable, although there is a shift of 0.2–0.3 mM along the $Ca^{++}$ concentration axis when they are superimposed. This shift may be related to differences between animal species or to differences in calcium activity between solutions used in biochemical and electrophysiological experiments.

We are not aware of the existence of information relating PTH release and external $Ca^{++}$ in the rat. However, the $Ca^{++}$ range in which a steep change in membrane voltage is seen (1–2.5 mM) is very similar to the range over which reversible morphological changes are seen in cultured rat parathyroid glands (1.3–2.7 mM; Roth and Raisz, 1964).

Other divalent cations also have parallel effects on membrane voltage and PTH secretion. $Mg^{++}$ is 2.5 times less efficient than $Ca^{++}$ in decreasing PTH secretion (Habener and Potts, 1976; Sherwood et al., 1970), and we find that it is ~2.4 times less potent than $Ca^{++}$ in depolarizing parathyroid cells. It has recently been observed that $Mn^{++}$ and $Sr^{++}$ are very effective in inhibiting PTH secretion and that $Ba^{++}$ is a poor $Ca^{++}$ substitute in decreasing PTH release (Wallace and Scarpa, 1982). This corresponds quite well with their depolarizing potency in our experiments.

Other points of parallelism between membrane voltage and secretion are that ouabain inhibits PTH secretion, possibly by depolarizing the cells (Brown et al., 1981; Fischer et al., 1982), and that complete removal of external $Na^+$ (which we have shown does not substantially affect the membrane potential) does not alter the modulation by external $Ca^{++}$ of

PTH secretion in dispersed cells from the cow (Fischer et al., 1982; Wallace and Scarpa, 1982). Taken together, these data strongly suggest that in parathyroid cells, hyperpolarization is associated with increased secretion, and depolarization with decreased secretion.

A separate mechanism, mediated by cAMP, may also exist. Isoproterenol is a potent secretagogue for PTH release (Brown et al., 1977; Fischer et al., 1982), although it does not seem to modify membrane potential. Isoproterenol activation of PTH release, in contrast to low-$Ca^{++}$-activated secretion (Fischer et al., 1982), is accompanied by a large increase in

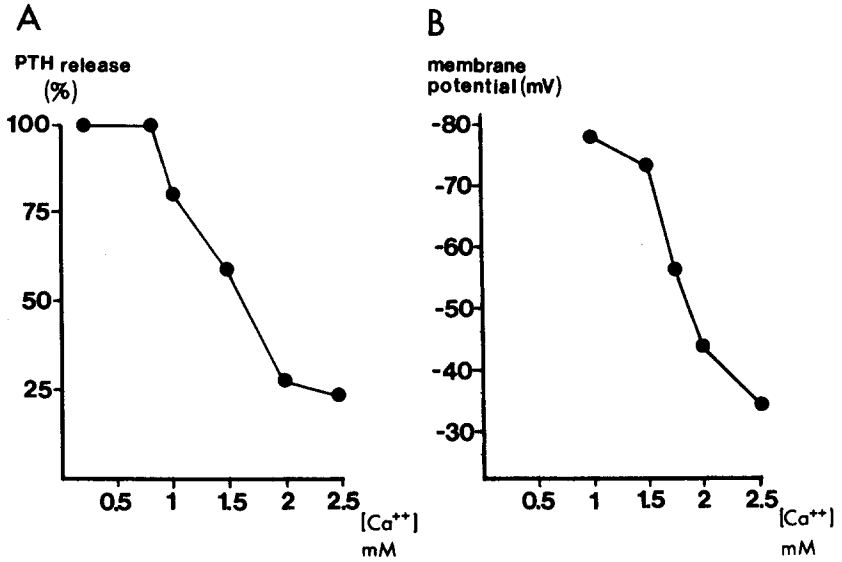


FIGURE 12. $Ca^{++}$ alterations affect PTH secretion and membrane potential in parallel. *A* has been redrawn from the data of Habener and Potts (1976) on slices of calf parathyroid glands maintained in vitro. The percent of immunoreactive PTH is given in the ordinate and $Ca^{++}$ concentration in the abscissa. The $Mg^{++}$ concentration was held constant at 0.8 mM. Points represent the means of four to six measurements. *B* is from our results on the rat. The $Mg^{++}$ concentration was held constant at 1.1 mM. Points represent the means of 3–66 measurements.

intracellular cAMP (Brown et al., 1977; Fischer et al., 1982).

This correspondence between membrane voltage and secretion contrasts with the usual sequence of events found in other glands, where the physiological stimulants for secretion evoke a membrane depolarization that opens voltage-dependent $Ca^{++}$ channels. $Ca^{++}$ entry through these channels seems to be responsible for triggering exocytosis, and removal of divalent cations from the external solution turns off secretion (Petersen, 1980; Rubin, 1970). There seem to be some exceptions to this usual pattern. For example, juxtaglomerular cells hyperpolarize by 8–10 mV

after addition of adrenaline, which is a stimulant for renin secretion (Fishman, 1976). In these cells, complete removal of external $Ca^{++}$ and increase of $Mg^{++}$ stimulate renin release (Fray, 1977).

In addition to the electrophysiological findings and the effect of divalent cations on PTH secretion, other evidence supports the view that the mechanism for stimulus secretion-coupling in the parathyroid gland is somehow unusual. Fully reversible secretory activity can be observed in dispersed cells in the absence of $Ca^{++}$. Further, large doses (up to $10^{-4}$ M) of the $Ca^{++}$ channel blocker D600 have no effect on PTH secretion activated by low $Ca^{++}$ (Wallace et al., 1983). Finally, it is known that the divalent cation ionophore A23187, which presumably increases cytosolic $Ca^{++}$ (Reed and Lardy, 1972), inhibits PTH secretion (Brown et al., 1980; Fischer et al., 1982; Habener et al., 1977).

In summary, membrane potential of parathyroid cells is steeply dependent on external $Ca^{++}$ in the physiological range. It seems highly likely that the membrane potential change is related to a change in secretory activity (Fig. 12). The relation between membrane voltage, calcium, and secretion is very unusual and suggests the existence of a stimulus-secretion coupling mechanism that has no precedent in the literature. The mechanism of this coupling and the permeabilities involved in the $Ca^{++}$-induced depolarization remain to be solved.

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