Different Roles of Membrane Potentials in Electrotaxis and Chemotaxis of Dictyostelium Cells

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Running title: Membrane Potentials and Electrotaxis

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Many types of cells migrate directionally in direct current (dc) electric fields (EFs); a phenomenon termed galvanotaxis or electrotaxis. The directional sensing mechanisms responsible for this response to EFs however, remain unknown. Exposing cells to an EF causes changes in plasma membrane potentials (Vm). Exploiting the ability of Dictyostelium cells to tolerate drastic Vm changes, we investigated the role of Vm in electrotaxis and in parallel, in chemotaxis. We used three independent methods to control Vm - extracellular pH, extracellular [K+], and electroporation. Changes in Vm were monitored with microelectrode recording techniques. Depolarized Vm was observed under acidic (pH 5.0) and alkaline (pH 9.0) conditions, as well as higher extracellular [K+] conditions. Electroporation permeabilized the cell membrane and significantly reduced the Vm, which gradually recovered over 40 minutes. We then recorded the electrotactic behaviors of Dictyostelium cells with defined Vm using these three techniques. The directionality (Directedness of electrotaxis) was quantified and compared to that of chemotaxis (Chemotactic Index). We found that a reduced Vm significantly impaired electrotaxis, without significantly affecting random motility or chemotaxis. We conclude that extracellular pH, [K+] and electroporation all significantly affected electrotaxis, which appeared to be mediated by the changes in Vm. The initial directional sensing mechanisms for electrotaxis therefore differ from those of chemotaxis and may be mediated by changes in resting Vm.

KEY WORDS: membrane potentials, electrotaxis/galvanotaxis, chemotaxis

Abbreviations: membrane potentials: Vm; electric field(s): EF(s); direct current: dc; K+ concentration: [K+]
INTRODUCTION

Cells migrate directionally in response to many extracellular cues including chemical gradients (chemotaxis), topography, mechanical forces (mechanotaxis/durataxis), and electrical fields (EFs) (electrotaxis/galvanotaxis) (1, 3, 8, 15, 27). Electric fields have long been suggested to be a candidate directional signal for cell migration in development, wound healing, and regeneration. The mechanisms used by cells to sense the weak dc EFs however, have remained very poorly understood.

One of the immediate effects felt by a cell upon exposure to an EF is a change in the cell membrane potentials (Vm). In an EF, the plasma membrane facing the cathode depolarizes, while the membrane facing the anode hyperpolarizes (17, 18). It has been proposed that the changes in Vm may underlie electrotaxis. In a cell with negligible voltage-gated conductance, the hyperpolarized membrane facing the anode attracts Ca$^{2+}$ by passive electrochemical diffusion. This side of the cell then contracts, thereby propelling the cell towards the cathode. In a cell with voltage-gated Ca$^{2+}$ channels, channels near the cathodal (depolarized) side open, thereby allowing Ca$^{2+}$ influx. Intracellular Ca$^{2+}$ levels will rise both on the anodal side and on the cathodal side in such a cell. The direction of cell movement in this situation will depend on the balance between the opposing contractile forces (17). The role of Vm in electrotaxis has not yet been directly tested.

In this report, we used Dictyostelium cells to test this directly. Dictyostelium cells show a robust electrotaxis and tolerate significant changes in Vm, while maintaining good motility under conditions of differing extracellular pH, ion concentration, and even following electroporation (20, 25, 29). These features make Dictyostelium cells a unique testing model. We quantified
electrotaxis and chemotaxis of cells with well controlled Vm using three independent methods. We found that the Vm indeed regulated electrotaxis, while having no effect on chemotaxis. We thus identified a contrasting role of Vm between electrotaxis and chemotaxis, which may underlie the mechanisms used by cells to sense weak dc EFs.
MATERIALS AND METHODS

Cell culture and development

*D. discoideum* AX3 were grown axenically in HL5 medium. Vegetative cells were washed and starved in development buffer (DB), and then were pulsed with 50 nM cAMP every 6 minutes for an additional 4 hours (29). All procedures were carried out at room temperature (~22°C).

Micropipette chemotaxis assay

Chemotaxis experiments were performed as reported (4, 10). Briefly, 20 µl of cells (1-4 × 10⁵ cells/ml) in DB were seeded onto a coverslip chamber. Bathing solutions with different pH or different K⁺ concentrations were then introduced. A Femtotip microinjection needle filled with 10 µM cAMP was placed into the field and a positive pressure of 25 psi was applied via a connected microinjector. Chemotaxis was recorded by time-lapse video using an inverted microscope (CKX41; Olympus) with a 10x objective lens. Images were taken every 30 seconds for 30 minutes.

Electrotaxis assay

Electrotaxis experiments were carried out as described (21, 28, 29). Developed cells were seeded into an electrotactic chamber. After 10 minutes of incubation, unattached cells were removed by gently washing with DB. Cells were then bathed in defined buffers as indicated with different pH or different [K⁺], in parallel with the chemotaxis assay. For cells treated with electroporation, normal DB (pH 6.5, 5 mM K⁺) was used and the EF was switched on 10 minutes after seeding. The applied EF was maintained at 12 V/cm for 30 minutes. Time-lapse images of cell
migration were acquired using an inverted microscope (Axiovert 40, Carl Zeiss) equipped with a 
CCD camera (C4742-95, Hamamatsu Corporation) and a motorized X, Y, Z stage (BioPoint 2, 
Ludl Electronic Products Ltd.), and controlled by SimplePCI 5.3 imaging software.

**Quantitative analysis of electrotaxis and chemotaxis**

Chemotaxis and electrotaxis were analyzed as previously described (4, 29).

Chemotactic index and electrotactic index were used to quantify how directionally cells 
migrated towards cAMP or in response to an EF, respectively. To calculate the chemotactic 
index or electrotactic index, the cosine of the angle between the direction of movement and the 
direction of the chemoattractant gradient or electric vector was determined (29). For migration 
speed we used trajectory and displacement speeds (29). Persistency was further calculated as 
the shortest linear distance between the start and end points of the migration path divided by 
the total distance traveled by a cell. All motile isolated cells were analyzed. At least 30 cells 
from three independent experiments were analyzed.

**Membrane potential (Vm) measurements**

Cells were seeded on a sterile glass coverslip and observed with a 60x objective. Vm 
measurements were conducted using fine-tipped glass-pipette microelectrodes. Two types of 
recording – transient and continuous recording were used to verify each other. The pipettes were 
pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) using a PP-830 
pipette puller (Narishige International, Inc., New York, NY), and the resistance was ~20 MΩ 
when filled with 3M KCl solution, as measured in the DB. Recordings were performed using a 
GeneClamp 500 amplifier (Axon Instrument/Molecular Devices, Union City, CA). The signals 
were digitally filtered at 1 KHz and digitized at 2 KHz using a Digidata 1322A digitizer and
pClamp 9.0 software (Axon Instrument/ Molecular Devices).

DB solution was used as the standard recording solution. The solutions with higher or lower pH values were obtained by adding HCl or NaOH. The solutions with different [K^+] were made by using 3 M KCl and normal DB. All experiments were conducted at room temperature and the recording was repeated in 16 or more cells.

Our measurements (both transient impalement, and continuous recording) showed similar Vm values, which were consistent with other published recordings (24).

Modulation of Membrane potential (Vm)

The first method used to control Vm was to maintain cells in four bathing solution with pH values of 5.0, 6.5, 7.5, and 9.0. All solutions were autoclaved and stored at room temperature until use. Before measurement of Vm, or chemotaxis and electrotaxis experiments, cells were bathed in a defined solution for ~10 minutes. The second method modulated Vm by adjusting recording buffer [K^+] at three concentrations (5 mM, 25 mM, and 50 mM). The K^+ concentrations were verified with an ion-selective probe. All solutions were autoclaved. The third method was electroporation, which was carried out as previously described (7, 11). Electroporation was performed in a Gene Pulser Xcell Electroporation System (Bio-Rad, USA) with two pulses of 0.85 kV/25 μF with an RC-time of 1 millisecond, separated by a 5 second interval. For Vm measurements, 20 μl of cell suspension was immediately taken out of the electroporation chamber and placed in a Petri dish in DB, and measured 10 minutes later.

Statistics

Pearson's correlation coefficient and the chi-square test were performed when pertinent. All data points were presented as mean ± SEM averaged from at least 3 measurements.
RESULTS

Extracellular pH and $K^+$ concentration regulated $V_m$ in *Dictyostelium* cells.

A typical negative peak potential was transiently observed upon impalement of a *Dictyostelium* cell with a microelectrode. $V_m$ transiently reached a peak value within several milliseconds of impalement, the potential quickly decreased due to leakage (Fig. 1A). The initial peak value was used to reflect the $V_m$ as in previous studies (24, 25). The values were verified with the continuous measurement. We achieved continuous recording using microelectrodes with an extremely fine tip (resistance up to 30 MΩ) penetrates a cell so the $V_m$ can be reliably monitored continuously for a much longer time; usually several minutes compared to milliseconds in the first method (Fig. 1B, C).

We first quantified the effect of extracellular pH and $[K^+]$ on $V_m$. Being bathed in solution of differing pH values and $[K^+]$ resulted in significant and consistent changes in $V_m$ (Fig. 1). An example of continuous recording of the dynamic change in $V_m$, in which extracellular pH dropped from 7.5 to 5.0, then went back up to 7.5, showed that the $V_m$ remained stable and was responsive to pH changes over several minutes (Fig. 1B). The membrane significantly depolarized when pH either dropped to 5.5 or increased to 9.0 (Fig. 1D). The maximal $V_m$s were recorded at pH 7.5 and 5mM $K^+$.

Chemotaxis and electrotaxis with different bathing solution pH

To test the effects of depolarizing $V_m$ on chemotaxis, we used a needle chemotaxis assay and quantitatively analyzed the directional cell migration. Without cAMP gradient, cells bathed in different solutions showed a similar pattern of migration in random directions. In buffer solutions of pH5.0, pH6.5, and pH9.0, no significant differences in cell morphology, behavior, or trajectory speed were observed among cells in response to cAMP gradient (Fig. 2A&D, Table 1).
This is consistent with the previous report (25).

The electrotaxis of Dictyostelium cells however is significantly altered by pH of the bathing solution. The chemotactic index for cells bathed in DB of pH 5.0, pH 6.5 and pH 9.0 were 0.56±0.01, 0.58±0.01 and 0.52±0.01, respectively. However, the electrotaxis was significantly affected by bathing solution pH (Fig. 2 C&D). Cells in solutions of pH 6.5 had more negative Vms, and showed the best electrotaxis. Cells in pH 5.0 or pH 9.0 showed significantly reduced electrotaxis, with directedness values of 0.51±0.01 and 0.30±0.01, respectively (Fig. 2D).

Chemotaxis and electrotaxis of K⁺-induced depolarized Dictyostelium cells.

We then examined the effect of extracellular [K⁺] on chemotaxis and electrotaxis. Cells bathed in buffer of 5 mM K⁺, moved towards cAMP with a typically polarized morphology. In buffer of 50 mM K⁺, cells near the micropipette tip moved towards the cAMP source. Cells further away from the tip appeared to move less directionally towards the pipette. Nonetheless, directional migration was evident (Fig. 3A). Cells in 50 mM K⁺ migrated with a reduced chemotactic index of 0.30±0.01, compared to that of 0.58±0.01 for cells in 5 mM K⁺ (Fig.3D).

Extracellular [K⁺] significantly affected electrotaxis. Cells bathed in buffer with 5 mM K⁺ showed robust electrotaxis with a directedness value of 0.93±0.01 (Fig.3D). The directedness gradually decreased to 0.64 in 25 mM K⁺, and 0.24 in 50 mM K⁺ (Fig.3D). Cells bathed in buffer with 50 mM K⁺ had a significantly lower directedness value, representing a decrease of 73%.

Although increasing extracellular [K⁺] appeared to inhibit both chemotaxis and electrotaxis, it seemed to have a more significant effect on electrotaxis (Table 2).

Electroporation depolarized Vm and abolished electrotaxis.

To further verify the role of Vm in electrotaxis, we used electroporation to depolarize
Dictyostelium cells. Electroporation with high-voltage pulses permeabilizes the cell plasma membrane, thus significantly depolarizing Vm by causing a large increase in non-ion-selective membrane permeability. Measurements in electroporated cells showed that the membrane is significantly depolarized following electroporation. In the data shown in Fig. 4 the starting time (0 minute) was set as the time of electroporation. All cells measured at 10 minutes following electroporation showed a significantly reduced Vm, which recovered gradually. The recovery took roughly 30-40 minutes following electroporation (Fig. 4A).

Electrotaxis in electroporation-induced depolarized cells was lost. Batch-matched control cells had good directional migration with a directedness value of 0.96±0.01 within 10 minutes in an EF, which was maintained through 20-30 minutes (Fig. 4C). Following electroporation, electrotaxis was completely inhibited (Fig. 4B). In the first 20 minutes following electroporation, the directedness of cells in an EF was almost zero. At 30-40 minutes, some directedness re-appeared, as Vm recovered to a certain degree (Fig. 4D).

We analyzed the relationship between these parameters and Vm. We found that Vm correlates significantly well with electrotaxis with a correlation coefficient of -0.77 (Fig. 5A), whereas chemotaxis did not appear to correlate with the changes in Vm (Fig. 5B).
We tested the effects of extracellular pH and [K⁺] on electrotaxis, using Dictyostelium cells, which have the unique property of tolerating changes in extracellular pH, [K⁺], and even electroporation, while maintaining good motility. We found that 1) changes in extracellular pH, [K⁺], and electroporation significantly affected Vm; 2) reduced Vm following these three independent methods significantly inhibited electrotaxis. The inhibitory effect on electrotaxis correlated well with the reduced Vm, but chemotactic effects did not.

In developed Dictyostelium cells, cAMP binds G protein-coupled receptors, activates Gαβγ, small GTPase, PI3K (class 1 phosphatidylinositol-3 kinases), and thereby phosphorylates PI(3,4)P2 into PI(3,4,5)P3, and finally induces F-actin polymerization, resulting in pseudopod development. Several other pathways may also contribute to chemotaxis (9, 22).

We demonstrated that Dictyostelium cells also show robust electrotaxis and are a good model for dissecting the molecular/genetic basis of electrotaxis (19, 29).

Extracellular pH, [K⁺], and electroporation significantly affected Vm, and correspondingly reduced or abolished electrotaxis. When Vm recovered, electrotaxis was restored. Vm in Dictyostelium cells are mainly generated by electrogenic proton pumps (24, 25). By varying extracellular pH, we controlled the Vm with good reproducibility. The Vm values were smaller than those reported before (24, 25). We used two different recording methods to confirm the measurements. This difference may be due to: 1) different strains of cells: AX3 was used here, while NC4 was used before; 2) different bathing solution: we used DB buffer while Van Duijn and co-workers used a Na⁺-saline (40 mM NaCl, 5mM-KCl, 1 mM-CaCl₂ and 5 mM-Hepes-NaOH, pH7.0); 3) different development protocols (24, 25). Concentration of
extracellular [K⁺] affects Vm (26). Differing extracellular [K⁺] regulated Vm; the more [K⁺], the lower the Vm (Fig. 1).

At 50 mM K⁺, electrotaxis was significantly inhibited (Fig. 3). Depolarization of cells following electroporation abolished the electrotactic response, while recovery of Vm restored the electrotactic response (Fig. 4). Chemotaxis of the cells with an altered Vm, modulated by changes in extracellular pH or [K⁺], was largely unaffected. This is consistent with the previous report (25). Collectively these results support the theory that the inhibition of electrotaxis by changes in extracellular pH, [K⁺], and electroporation, appears to be a specific effect caused by changes to Vm. The genome of Dictyostelium cells shows at least two possible TRP channel genes, a Ca²⁺ channel gene, and several K⁺ channel genes (14). Several signal transduction pathways related to electrotaxis could depend on Vm caused by the interactions between ion channels and other signaling proteins such as integrins (2, 5, 6, 12, 13, 16, 23). It may involve different membrane proteins such as ion channels, transporters, receptors and the actin cytoskeleton, and may also involve Ca²⁺ signaling (20). The reduced Vm might inhibit Ca²⁺ signaling and thereby affect the electrotaxis. Another possibility is that Vm may control the sensors that detect the EFs. We are currently using a high throughput strategy to screen for such sensing molecules in electrotaxis.

In conclusion, changes in extracellular pH, [K⁺], and electroporation all had significant effects on electrotaxis. When the Vm was depolarized, electrotaxis was significantly inhibited. Extracellular pH, [K⁺], and electroporation all had significant effects on electrotaxis which appeared to be mediated by the changes in Vm. The initial directional sensing mechanisms for electrotaxis therefore differ from those in chemotaxis and may be mediated by changes in Vm.
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Author contribution: MZ, PD, AM. XZ and RG designed research; RG, XZ, YS, and YK performed research; MZ, RG, XZ, YS analyzed data; RG, XD and MZ wrote the paper with help from YS.

The authors declare no conflict of interest.
REFERENCES


Figure legends

Fig.1 Extracellular pH and K+ concentration regulate the membrane potential (Vm) of Dictyostelium cells. Developed Dictyostelium cells were bathed in developing buffer (DB) with different pH values and K+ concentrations. Vm was measured by microelectrode impalement. A. Typical peak-shaped potential transient recorded upon microelectrode penetration of a Dictyostelium discoideum cell bathed in DB with different pH values or K+ concentrations. B, C. Continuous recording of Vm with an extra-fine electrode showed stable stationary Vm of D. discoideum. The dotted vertical line indicates the time when the bathing solution was replaced with a buffer of different pH value or different K+ concentration. D. Averaged Vm from 21 cells (pH 5.0), 16 cells (pH 6.5), 16 cells (pH 7.5), and 16 cells (pH 9.0). #: P<0.001 when compared to that in pH 7.5; #: P<0.001 when compared to that in pH 6.5. E. Averaged Vm from 13 cells (5 mM K+), 16 cells (25 mM K+), and 24 cells (50 mM K+). #: P<0.001 when compared to that in buffer with K+ concentration of 0.5 mM K+. #: P<0.001 when compared to that in K+ concentration of 5 mM K+.

Fig.2 Extracellular pH plays different roles in chemotaxis and electrotaxis in Dictyostelium cells. A. cAMP gradients were formed from the tip of a micropipette filled with 10 µM cAMP. Trajectories of cell migration towards cAMP in DB buffer with different pH values were indicated. Dark spots represented the position of the micropipette. Scale bar represents 20 µm. B. Cell migration in random directions in control condition without an EF, although they were bathed in DB buffer with different pH values as indicated. C. Cell migration trajectories in which the start point of each cell is set as the origin. Cells migrated cathodally in a pH of 6.5 in
an EF. However, directed cell migration was significantly impaired under acidic (pH 5.0) or alkaline (pH 9.0) conditions. D. The effects of extracellular pH on electrotaxis in *Dictyostelium* cells correlate with the effects on Vm. *Dictyostelium* cells bathed in pH 6.5 showed a greater Vm than that of the cells in pH 5.0 or pH 9.0, and significantly better electrotaxis. The changes in pH and corresponding changes in Vm did not significantly affect the chemotaxis (Chemotactic Index). *:P<0.001 when compared to that in pH 6.5.

**Fig. 3** Extracellular K+ significantly affects electrotaxis but does not affect chemotaxis of *Dictyostelium* cells. A. Chemotaxis of *Dictyostelium* cells in buffer with different extracellular K+ concentrations. Cells in buffers with 5 mM or 50 mM K+ were able to undergo chemotaxis. Trajectories of cell migrate towards cAMP. Dark spots represented the positions of micropipette tips. Scale bar represents 20 µm. B. Cells migrate in random directions in butters with 5 mM or 50 mM K+ without an applied EF. C. Cells migrated cathodally in DB with 5 mM and 50 mM K+. When K+ concentration increased (Vm decreased, see Fig. 1), the electrotaxis was significantly reduced. D. The effects of extracellular K+ on electrotaxis in *Dictyostelium* cells correlated with the effects on Vm. Cells in buffer with 50 mM K+ had a significantly reduced Vm as well as significantly reduced electrotaxis. Slightly reduced chemotactic activity was seen in higher concentration of K+ solution.

**Fig. 4** Electroporation depolarizes membrane potential (Vm) and abolishes electrotaxis. A. Representative Vm measured at pH 6.5 after electroporation. The moment of electroporation was set as time 0. B. Electroporated cells lost the electrotactic response, which showed some
degree of recovery 30 min later. See supplementary video 6. **C.** Control cells showed robust 
electrotaxis. **D.** Loss of electrotaxis correlated well with Vm. When Vm recovered, electrotaxis 
recovered significantly. Ctl = Vm in control cells not electroporated. EF = 12 V/cm. *: P<0.001 
when compared to that of cells not being electroporated. #: P<0.001 when compared to that of 
cells after 40 min of recovery following electroporation.

**Fig. 5 Significant correlation is between Vm and electrotaxis but not chemotaxis. A.** 
Scatter plot shows electrotactic directedness and resting membrane potential (r = -0.77). The 
best-fit line is shown. **B.** Scatter plot shows chemotactic index and membrane potential (r = 
-0.51).
A Chemotaxis

B No EF

C Electrotaxis (12 V/cm)

D

Gao et al., Fig. 2
A  
Chemotaxis

B  
No EF

C  
Electrotaxis (12 V/cm)

D  
Membrane potential

Gao et al., Fig. 3
A  Vm decrease and recovery after electroporation

B  Electrotaxis lost after electroporation

C  Electrotaxis of non-electroporation control

D  Comparison of membrane potential and directedness

Gao et al., Fig. 4
Electrotaxis correlates well with membrane potentials

![Graph A](image)

Chemotaxis does not correlate well with membrane potentials

![Graph B](image)

Gao et al., Fig. 5
Table 1. The effects of extracellular pH and K+ on chemotaxis.

<table>
<thead>
<tr>
<th>Developing buffers</th>
<th>Trajectory speed [μm/min]</th>
<th>Displacement speed [μm/min]</th>
<th>Chemotax index</th>
<th>Persistency</th>
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<td>2.07±0.02 #</td>
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<td>50mM [K+]</td>
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<td>1.62±0.03 **</td>
<td>0.30±0.01 **</td>
<td>0.65±0.01</td>
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</table>

The data represent mean±SEM. *: P<0.01; **: P<0.001 when compared to that in buffer with K+ of 5 mM; #: P<0.01; ##: P<0.001 when compared to that in pH6.5.
Table 2. The effects of pH and K⁺ on electrotaxis.

<table>
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<tr>
<th>Buffers</th>
<th>Developing Trajectory speed [μm/min]</th>
<th>Displacement speed [μm/min]</th>
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<th>Persistency</th>
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<td>0.51±0.01 ***#</td>
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<td>pH9.0</td>
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<td>0.30±0.01 ***##</td>
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<td>5mM K⁺</td>
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<td>25mM K⁺</td>
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<tr>
<td>50mM K⁺</td>
<td>6.46±0.01 &amp;&amp;</td>
<td>2.95±0.01 &amp;&amp;</td>
<td>0.24±0.01 &amp;&amp;</td>
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The data represent mean±SEM. *:P<0.01; **:P<0.001 when compared to that in pH7.5; #: . P<0.01; ##:P<0.001 when compared to that in pH6.5; &:P<0.01; &&:P<0.001 when compared to that in buffer with K⁺ of 5mM.