Relations between Ameboid Movement and Membrane-Controlled Electrical Currents

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ABSTRACT We have studied the pattern of electrical currents through amebas (mainly *Chaos chaos*) with an ultrasensitive extracellular vibrating probe. Amebas drive both steady currents and current pulses through themselves.

Relatively steady current with an average surface density of 0.1–0.2 $\mu A/cm^2$ enters the rear quarter of an ameba and leaves its pseudopods. Streaming reversals are preceded by changes in this current pattern and the region with the largest new inward current becomes the new tail. Ion substitution studies suggest that some of the steady inward current is carried by calcium ions.

Characteristic stimulated pulses of current sometimes follow the close approach of the vibrating probe to the side of an advancing pseudopod. Such a pulse enters the cytoplasm through a small patch of membrane near the probe (and seems to leave through the adjacent membrane), is usually followed by hyaline cap and then by pseudopod initiation, is calcium dependent, lasts about 5–10 s, and has a peak density of about 0.4 $\mu A/cm^2$.

Spontaneous pulses of similar shape and duration may enter or leave any part of an animal. They are much less localized, tend to have higher peak densities, and occur in physiological salt solutions at about 0.2–4 times per minute. Retraction of a pseudopod is always accompanied or preceded by a spontaneous pulse which leaves its sides.

INTRODUCTION

As long ago as 1896 Verworn found that the polarity of *Amoeba proteus* could be controlled by an imposed electrical field. When a current is driven through an ameba by an external field as small as 0.9 V/cm (about 50 mV per average ameba length) the ameba moves towards the negative pole (Hahnert, 1932). It was clear that ameboid movement could be strongly influenced by electrical currents. Since that time there have been several studies on the electrophysiology of the ameba using intracellular microelectrodes, as well as some extracellular work by Tasaki and Kamiya (1964). However, these investigations did not uncover any relationship between the observed membrane-controlled electrical events and the morphological changes in the streaming ameba. We have developed a vibrating probe method for measuring local extracellular currents which allows constant observation and extracellular measurement without disturbing or constricting the ameba. This makes it possible to bridge this gap, revealing an

intimate relationship between the natural electrical currents and ameboid movement. An abstract of this work has already appeared (Nuccitelli et al., 1975).

MATERIALS AND METHODS

Preparation

A. proteus and *Chaos chaos* were obtained from Carolina Biological Supply Co. and cultured at 20°C in 200 ml of Carolina Biological spring water with four boiled wheat grains and an ample supply of *Paramecium*. An approximately 12-h light-dark cycle was maintained.

All experiments were conducted in Marshall's medium (Bruce and Marshall, 1965): 0.15 mM K_2HPO_4, 0.11 mM KH_2PO_4, 0.5 mM $CaCl_2$, 0.05 mM $MgSO_4$. The media were prepared with reagent grade salts and glass-distilled deionized water. Most of the studies were conducted in a medium adjusted to pH 5.5 in which *Chaos* usually had two to five pseudopodia. Monopodial forms could be obtained by lowering the pH to 4.8 (Käppner, 1961). Ca^{++}-free medium was buffered with 0.1 mM EGTA (ethylene-glycolbis-(β-aminoethyl ether) *N,N'*-tetraacetic acid) to guarantee less than 10^{-8} M free Ca^{++} even with 0.01 mM Ca^{++} contamination (Caldwell 1970), and $MgCl_2$ replaced $CaCl_2$. In Mg^{++}-free medium, $CaSO_4$ replaced $MgSO_4$ and in [Mg^{++},Cl^-]-free the only Ca^{++} salt was 0.5 mM $CaSO_4$. In 50× K^+ medium 10 mM K_2SO_4 was added to normal Marshall's and in 50× KCl medium 10 mM KCl was added to normal Marshall's.

During the experiments the amebas were placed in 3-ml dishes made with cover slip glass bottoms. Each dish was normally first rinsed with polylysine at 1 mg/ml to promote adhesion to the glass. However, some of the measurements were made without this initial rinse, and the current characteristics were identical. The amebas were transferred from the culture to this dish through at least three washes of the experimental medium to be used. They were then placed on a Zeiss UPL inverted microscope and observed at ×60 or ×100 while the current measurements were made. In order best to study the correlation between the electrical events and the observed morphological changes, a motion picture is needed. However, in this first study we used only frequent photographs while describing the movements of the amebas verbally on a tape recorder. Only the most easily observed events such as pseudopod formation and retraction could be correlated with electrical events in this way, and the time resolution of these correlations is about 1 s. The medium could be easily changed over a 30-s period by using a coupled, two-syringe system. 9 ml were exchanged each time to assure complete medium replacement in the 3-ml chambers.

Instrumentation

The sensor of our extracellular current measuring system is a vibrating probe with a spherical, 25–30-μm platinum-black electrode at its tip (Jaffe and Nuccitelli, 1974). The oscillating voltage between this tip and a coaxial reference electrode 3 mm behind the tip is measured while the probe is vibrated at 370 cps in a horizontal plane between two extracellular points 30 μm apart. Vibration between these points converts any steady voltage between them into a sinusoidal output whose amplitude is measured with the aid of a lock-in amplifier (Princeton Applied Research Corp., Princeton, N. J., model 122) tuned to the vibration frequency. This conversion of a direct voltage to an alternating one serves to eliminate any drift or low-frequency noise originating at the probe's surface. Furthermore, at the probe's vibration frequency, its impedance and hence its thermal or Johnson noise are greatly reduced compared to the static impedance.

The measured local voltage difference, ΔV, can then be directly converted to I, the local current density in the vibration direction, because the electrical field, E, is practically constant over the relatively small vibration distance, d. Thus Ohm's law gives:

$$I = E/\rho = V/d\rho, \quad (1)$$

where ρ is the resistivity of the medium. For example, a measurement of 0.9 μV while vibrating over 30 μm in 6,000 Ω-cm Marshall's medium indicates a current density of 0.9 μV/(0.003 cm $\times$ 6,000 Ω-cm) = 0.05 μA/cm^2 in the center and direction of vibration.

The pattern given by a group of such measurements around the cell was then extrapolated to the cell's surface to give the size and pattern of the transcellular current. Since the limiting resistance to this current is through the plasma membrane, this current will be primarily independent of the medium's resistivity while the measured voltage will vary with this resistivity. We will therefore express our voltage measurements in terms of the more independent and far more meaningful current densities, using Eq. (1).

Fig. 1 C serves to illustrate the output of the vibrating probe system in response to known current densities in Marshall's medium. In this case current was injected from an electrode bearing a 50-μm diam Ag-AgCl sphere at its tip, and the center of this artificial

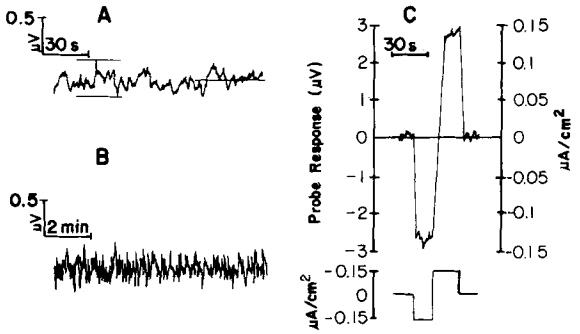


FIGURE 1. Vibrating probe performance. A, The probe's output when in a reference position far from any current source in Marshall's medium with a 1-s time constant. The lines on the left indicate the peak-to-peak noise over a 30-s interval. The line on the right indicates the average value of this signal. B, The probe's output in the same conditions as A but at a slower chart speed. C *above*, response of the vibrating probe (in Marshall's medium) to the known current density shown in C *below*.

source was placed 230 μm from the probe's vibration center. Similar measurements were routinely used to calibrate the system, and a detailed account of its responses to known currents can be found in the paper by Jaffe and Nuccitelli (1974), particularly in Fig. 6.

The probe was translated with a Line Tool model H2 XYZ-micropositioner calibrated at 2.5 μm per division. In this way it could generally be kept at a reasonably constant distance from the surface of an ameba, despite the animal's rapid movements. The amplifier's output was recorded on a Sargent model SRG chart recorder (Sargent-Welch Co., Skokie, Ill.). When a measurement of the current density near a cell was made, the average value of the recorder trace was determined by equalizing areas above and below a hairline as shown on the right of Fig. 1 A. We then compared this average value with the reference position values obtained both before and afterwards. Reference positions were 1–3 mm away from any ameba.

Noise, Drift, and Artifacts

Fig. 1 A actually illustrates the system's output when the vibrating probe is in a reference position. We routinely measured the peak-to-peak (ptp) noise in a reference position, in Marshall's medium, over a 1-min interval with the amplifier's time constant set at 1 s. This

ptp noise averaged about 0.03 $\mu A/cm^2$ over several months of experimentation. Note that this corresponds to a root mean square noise value of about 0.006 $\mu A/cm^2$.

Fig. 1 B shows a typical 10-min record of the system's output in a reference position on a slower time scale. This output normally drifts at the practically negligible rate of only a few tenths of a nanoA/cm² per minute. Just after a change of medium, the drift may be as much as 100 times higher, but even this can be compensated for by averaging the reference values measured before and after the measurement near a cell.

The piezoelectric vibrator or bender was somewhat modified from that described previously (Jaffe and Nuccitelli, 1974), so as further to reduce so-called "barrier artifacts." In particular, the driving voltage was now applied to the metal strip inside the bender (instead of its outer surfaces) while its entire outer surface was now grounded. This and other refinements (to be detailed elsewhere) serve to reduce current leaks to the probe either from the vibrator or from corrosion batteries elsewhere in the instrument. Such leakage currents can introduce a significant artifactual component into the measured signals when these currents are reflected from cells or other electrical barriers. To check for such barrier artifacts, we preceded each study of the fields around live amebas by measuring the apparent (and actually echo) currents around either a glutaraldehyde-fixed ameba or a glass bead of comparable size and shape. A typical result of such a control study is shown in the inset to Fig. 3 B. In this case, the echo current measured normal to and 50 μm away from a glass bead was an outward one with a barely detectible density of about 0.003 $\mu A/cm^2$. In general, these echo current values ranged from undetectable up to 0.01 $\mu A/cm^2$ and were always outward. No echo currents were ever detected when the probe was vibrated tangent to a barrier's surface.

Extrapolation to the Cell's Surface

Our probe measures the current density at the center of its vibration which was generally about 80 μm from the ameba's surface. In order to know what the current density is at the membrane, we must extrapolate the measured current densities to the surface on the basis of the field's fall-off with distance from the surface. The spatial pattern of the steady current is that of a current dipole since current enters one region and leaves another. Such a field falls off as the inverse cube of the distance from the center of the dipole (Nuccitelli and Jaffe, 1975). The problem is to determine the center of the ameba's dipole when the animal's shape is continually changing. We empirically determined the average distance between the tail surface and dipole center by plotting measured current densities at various distances from the surfaces of several amebas as shown in Fig. 2. The solid lines represent the theoretical inverse cube fall-off for four different positions of the dipole center. As expected, the data points do not fall on any one line because of the constantly shifting pattern. Nevertheless, they all fall between a value for the dipole's radius, a, of 200–500 μm. Therefore the extrapolation factor will vary from $((80 + 200)/200)^3 = 2.7$ to $((80 + 500)/500)^3 = 1.6$. We will use an average factor of 2. All of the steady current densities and spontaneous pulses (which appear to have a similar spatial pattern) reported in this paper have been multiplied by this factor to obtain the surface current density. When the probe vibration axis does not coincide with the dipole axis this extrapolation factor will be reduced by a $\cos\theta$ term. Since we have no way of accurately determining the dipole axis we could not make this correction. These approximations in the extrapolation procedure will introduce a 50% uncertainty in the actual values given here.

RESULTS

Extracellular current measurements around the amebas indicate a myriad of electrical events with both pulse and steady components. These fall into three basic categories: steady currents, spontaneous pulses, and stimulated pulses. It

was quickly found that polypodial amebas generate far more spontaneous pulses than do monopodial forms. Consequently, polypodial amebas were selected for the investigation of pulse amplitudes and frequencies, and monopodial forms were used to study the steady currents which could be obscured by pulsing. In the course of this work more than 100 specimens of *C. chaos* were studied along with several of *A. proteus*. All three types of electrical events were found in both species, but *Chaos* was used for most of this study because of its larger size.

Steady Current Polarity

The steady current is the most consistent component of the electrical measurements. In every ameba studied we found that a relatively steady current entered[1]

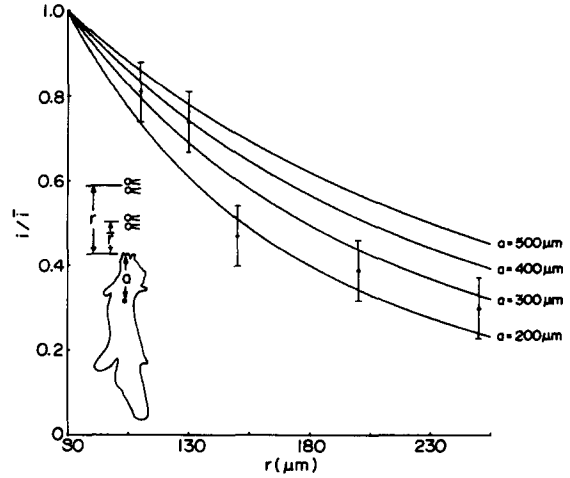
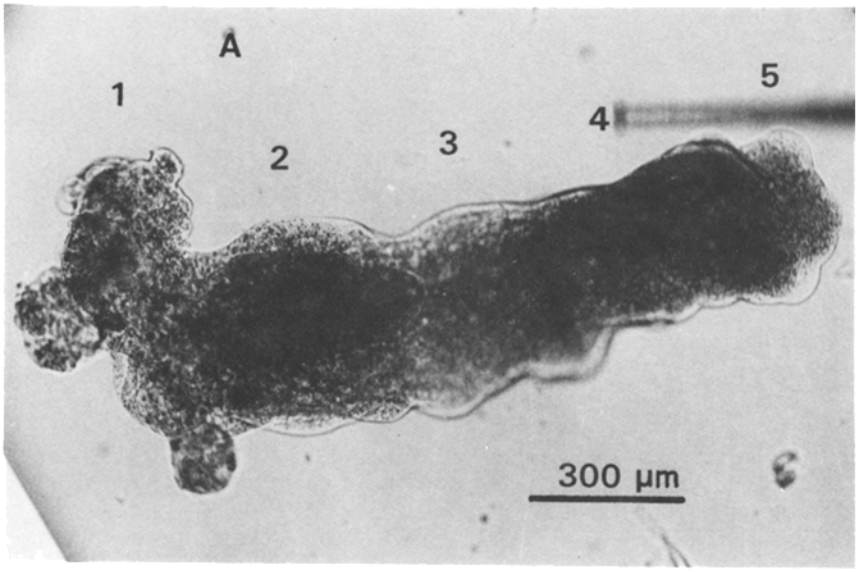


FIGURE 2. Relative current density vs. radial distance from the tail surface. Reference current density, ī, was measured at a distance r̄ = 80 μm from the surface. Bars indicate standard errors. Solid lines are plots of $i/\bar{i} = ((a + r)/(a + \bar{r}))^{-3}$ for a = 200, 300, 400, 500 μm.

the tail or uroid zone of the ameba while leaving the pseudopods. This inward tail current density varied over a large range of 0.03–0.4 μA/cm² with an average of 0.14 ± 0.03 (SE) μA/cm² for 10 measurements in normal Marshall's taken from Fig. 3 and Table II. Fig. 3 shows the probe vibrating normal to the surface of a typical monopodial ameba and illustrates the current measurements at several positions along the pseudopod. Current normally enters the rear quarter of the animal rather uniformly while leaving the front three-quarters. A sketch of the basic overall current pattern is shown in Fig. 3 C. It is given primarily as an aid to interpreting the record shown in Fig. 3 B.

The actual current pattern is constantly changing as the morphology changes and is not likely to be as symmetrical as that pictured. This, together with our inability to measure the current's vertical component, made it impossible exactly to confirm the necessary equality of net inward and outward current. Neverthe-

[1] We use the normal convention here of current as the flow of positive charges.


A
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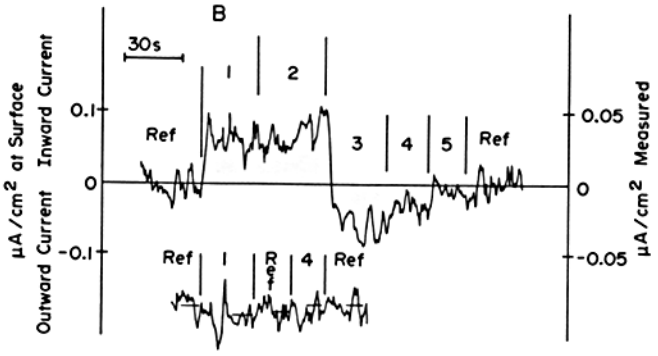
300 μm


B
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μA/cm² at Surface Outward Current Inward Current
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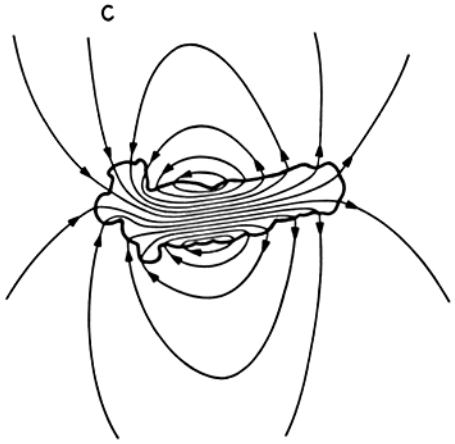
C

less, two observations are at least consistent with this expected equality: (*a*) the inward current density measured along 20–30% of the ameba is always larger than the outward current densities found along the rest of the animal; (*b*) polypodial amebas with increased pseudopod surface area always had a correspondingly smaller outward current density along the pseudopods than did monopodial forms.

The steady current pattern is closely related to the polarity of the ameba. This relationship is particularly striking during the frequent spontaneous polarity reversals found in monopodial forms of *C. chaos*. Such overall streaming reversals are usually preceded by a steady current reversal in the pseudopod. Specifically, we have observed seven cases of streaming reversal while studying both tail and pseudopod ends with the vibrating probe. In all seven, the region which developed the largest inward current density became the new tail. Fig. 4 illustrates one of these cases. At about the time of streaming reversal, the pseudopod current reverses so that current enters at both the tail and the pseudopod tip while presumably leaving in the middle region of the animal. As soon as this new inward pseudopod current exceeds the current entering the tail, the streaming stops and then reverses so that the region with the largest inward current becomes the new tail. Minutes later, the old tail current also reverses its direction. Therefore, the original tail current reverses only slowly, after the streaming reversal occurs.

In the case shown in Fig. 4 the probe was above the tail when the streaming reversed so it was not evident whether the current reversal at the pseudopod occurred before or after the streaming reversal. However, in three other cases we have observed that current reversal at the pseudopod coincides with the cessation of streaming and precedes the streaming reversal by about 20 s. One such case is shown in Fig. 5 where the probe was moved back and forth between the tail and pseudopod tip during a temporary streaming reversal. The pseudopod current reversed from outward to inward about 20 s before the first streaming reversal. The reversal itself occurs when the inward current at the pseudopod tip is slightly larger than at the tail. The inward pseudopod current does not persist, however, and about 2 min after the streaming reversal it is slightly smaller than the tail current. About 30 s later, the streaming again reverses—and again the region with the largest inward current becomes the tail. We have observed transient, local streaming reversals in a small part of a

FIGURE 3. The steady current measured around a monopodial ameba. A, Photomicrograph of the ameba studied. The vibrating probe is at position no. 4 and the other positions studied are indicated by the numbers. B, The actual recording of the extracellular current densities measured in the indicated positions. Abscissa: time; ordinate: current density. The scale at right indicates the measured values while the left scale gives the extrapolated current at the animal's surface. The probe was always vibrated normal to the surface and the reference position (Ref) was 1 mm away from the ameba. *Inset* shows a control record made with the probe in two of the same positions around a glass bead. C, The spatial current pattern inferred from the measurements in B along with the field fall-off study and tangential measurements. Relative line densities indicate relative current densities.

pseudopod which occurred without an overall current reversal. However, all seven complete endoplasmic streaming reversals were accompanied by the current reversal at the new tail region and in three of these we were in the proper measuring position to determine that the current reversal preceded the streaming reversal.

The steady current is not dependent on streaming. In quiescent amebas (found occasionally in all media) which show no streaming at all, measurements on three amebas give an average current of 0.12 ± 0.03 (SE) $\mu A/cm^2$ entering the

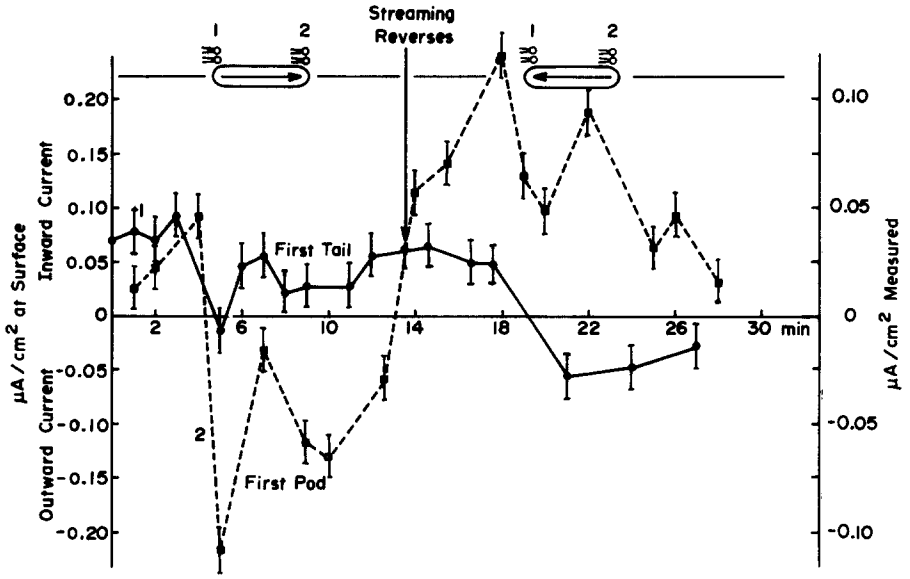

FIGURE 4. The current density normal to the two ends of a monopodial ameba during a reversal in the streaming pattern. Abscissa: time (min); ordinate: current density. The streaming direction is indicated by the arrow in the sketch on top. The probe was moved back and forth between the original tail end (solid line) and the original pseudopod tip (dotted line) in order to follow the changes in the steady current density normal to the membrane in both regions. For each measurement the current density was averaged over a 30-s interval and plotted as a point in the figure (bars indicate rms noise). These points were then connected by straight lines.

most wrinkled end. This is an interesting observation because it indicates that the steady current is not just a byproduct of the streaming itself, and also argues against the simple notion that the steady current directly drives streaming. However it is quite compatible with the idea that the current somehow controls a streaming process driven in some other way and determines the allowed streaming direction.

Ion Dependence of the Steady Current

In an effort to determine which ions might be carrying the steady current we made a preliminary study of the effects of various ion changes on the magnitude of the current. Since the current density can vary over a 10-fold range from

ameba to ameba, we limited our study to a comparison of the average steady current in the same ameba over a 10-min period before and after each medium change. These results are summarized in Table I.

When Ca^{++} was replaced by Mg^{++} and buffered to less than 10^{-8} M free Ca^{++}, there was a three- to fivefold reversible fall in the steady current. This suggests that Ca^{++} influx may be a substantial component of the inward steady current. However, when H^+ was increased 100-fold to pH 5.2 at the same time, this fall did not occur. Perhaps in ultra-low Ca^{++}, H^+ influx can act as a substitute, or the pH change can increase the permeability of some other ion. However, after 15–20 min in this 10^{-8} M Ca^{++} medium most amebas stop streaming entirely while

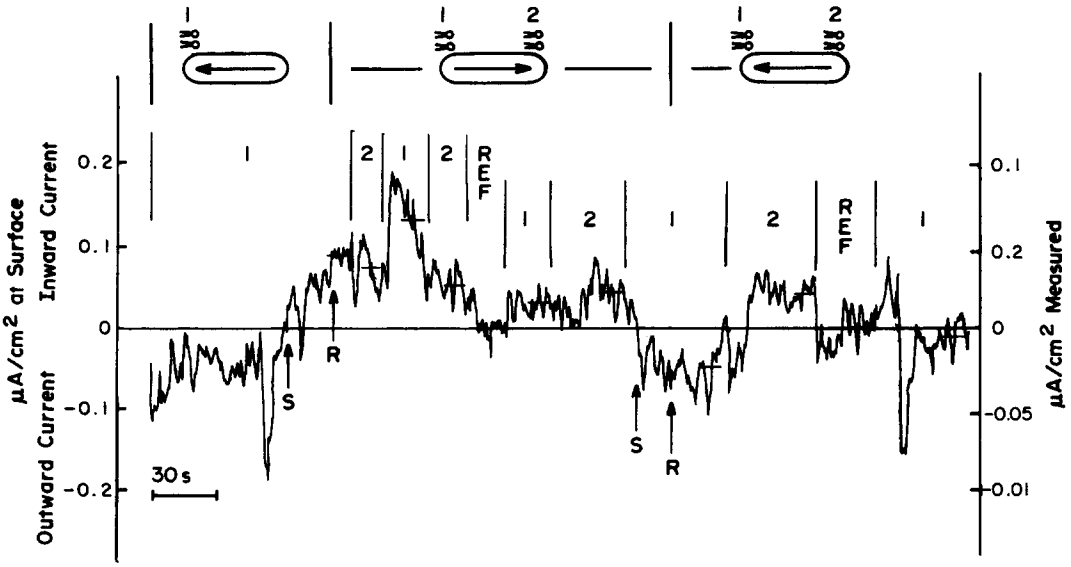

FIGURE 5. Representative recording of the current densities at the positions indicated during two streaming reversals. Abscissa: time; ordinate: current density. *S*, streaming stops; *R*, reversed streaming begins. The reference postion (Ref) was 1 mm away from the ameba. Horizontal lines drawn in represent the average value over the last 10 s in each position.

the reduced steady current continues. This suggests that Ca^{++} influx is indeed necessary for normal streaming although other ions are also apparently carrying some of the steady current.

On the other hand, Mg^{++} does not appear to be a component of the steady current since the change to Mg^{++}-free medium actually resulted in a small increase in the current. Nor does H^+ appear to be a major carrier of the inward current under normal conditions. A 50-fold decrease in the H^+ concentration actually resulted in a 2.5-fold increase in the steady current.

The ameba's membrane is very nearly a K^+ electrode since the membrane potential depolarizes by 45 mV per 10-fold increase in external K^+ (Bruce and Marshall, 1965). Thus, we could study the effect of membrane potential changes on the steady current by varying external K^+. When the external K^+ was raised

10-fold and 25-fold by adding K_2SO_4, the steady current fell. When both K^+ and Cl^- were increased by raising KCl 50-fold an actual reversal of the current was observed in two of three animals studied. This reversal can probably be attributed to Cl^- entry since the membrane potential is reduced to about 0 mV in this medium (Bruce and Marshall, 1965), and the normally outward driving force on Cl^- is reversed at 0 mV and 25 mM external Cl^-.

These ion changes give little information concerning the ions carrying the outward current. However, K^+ is likely to be an outward current carrier since it

TABLE I

IONIC DEPENDENCE OF THE STEADY TAIL CURRENT*

Ion changed	pH	Resistivity	Medium	$\mu A/cm^2 \pm SE$ at membrane	No. measurements	Comments
		Ω-cm				
1a. Ca^{++} & Mg^{++}	5.1	5,600	Ca^{++}-free	0.02+0.01	2	Ca^{++} removal and replacement by Mg^{++} reduces the steady current three- to fivefold.
	5.1	6,000	Marshall's	0.10±0.01	2	
1b. Ca^{++} & Mg^{++}	5.1	6,000	Marshall's	0.21±0.03	2	
	5.1	5,600	Ca^{++}-free	0.08±0.04	3	
2. Mg^{++}	4.8	6,000	Marshall's	0.06±0.01	2	Mg^{++} removal has litde effect.
	4.8	5,200	Mg^{++}-free	0.10±0.01	7	
	4.8	6,000	Marshall's	0.09±0.01	3	
3. H^+	5.5	6,000	Marshall's	0.13±0.05	3	A 50-fold decrease in H^+ results in a 2.5-fold increase in the steady current.
	7.2	6,000	Marshall's	0.33±0.01	2	
4. Ca^{++}, Mg^{++}, H^+	7.2	6,000	Marshall's	0.33±0.01	2	Ca^{++} replacement by Mg^{++} does not change the steady current if H^+ is increased 100-fold.
	5.2	5,600	Ca^{++}-free	0.30±0.01	3	
5. K^+ & SO_4^-	4.8	6,000	Marshall's	0.05±0.01	3	10×, 25×, and 50× K^+ stop or reduce the current
	4.8	1,300	10× K_2SO_4	0.02±0.02	4	
	4.8	780	25× K_2SO_4	−0.02±0.02	4	
	4.8	430	50× K_2SO_4	0.04±0.05	5	
	4.8	6,000	Marshall's	0.03±0.01	2	
6. K^+ & Cl^-	5.5	6,000	Marshall's	0.07±0.01	2	50× KCl stops or reverses the current
	5.5	220	50× KCl	−0.20±0.02	4	
	5.5	6,000	Marshall's	0.09±0.02	2	

* In each case a single ameba was studied during the media changes indicated and in the order indicated.

is by far the most abundant internal cation and has the highest permeability. Any fall in membrane potential resulting from the inward tail current would drive out K^+.

Spontaneous Pulses

By far the most striking feature of the extracellular current is the spontaneous pulsing.[2] The average peak amplitude of these pulses is on the order of 10-fold

[2] We refer to these pulses as spontaneous to distinguish them from pulses stimulated by the vibrating probe. Unlike the stimulated pulses, the spontaneous pulse frequency is independent of the distance between the probe and the ameba's surface.

larger than the average steady current, and peak pulse current densities of 4 μA/cm^2 are fairly common. Unlike the steady current, the pulses may enter or leave the tail. Some typical pulses can be seen in Fig. 6. They always rise faster than they fall and are about 6 s long. The average rise time to half of their peak amplitude is 0.6 s. Although most pulses are monophasic, á few percent seem to be biphasic (as seen in Fig. 6 A). It is very unlikely that these represent traveling waves because their initial phase can be either outward or inward current. Moreover, we estimate the length constant of *Chaos* to be about 1 cm, a value about 10 times longer than the usual animal.[3] A biphasic pulse is probably a chance combination of two independent pulses which happen to occur one after the other and have widely separated inward regions.

We could not directly determine the spatial pattern of the pulse current since we had only one vibrating probe and could not scan the ameba within the pulse time of a few seconds. However, the average pulse amplitudes shown in Table II indicate that inward pulse current densities are two- to threefold larger than the outward pulse currents, suggesting that the inward current area is about one-third of the outward current area. Therefore, an inward pulse at the tail would probably have a current pattern similar to that of the steady current shown in Fig. 3 C. Because of this similarity we have used the same extrapolation factor of 2 to calculate the surface current densities.

Spontaneous Pulses—Relation with Morphological Changes

Here we describe only the most obvious relations between pulsing and morphological changes. (*a*) Amebas with many pseudopods tend to drive far more pulses through themselves than do monopodial forms. (*b*) When many pseudopods are forming and streaming outward, most pulses enter the pod(s); when most pseudopods are retracting, pulses enter the tail. (*c*) Specifically, we observed 20 cases in which pseudopod retraction occurred while we were measuring the current normal to a pseudopod's side. In every case, the beginning of retraction was accompanied or immediately preceded by an outward pulse. The delay between a preceding outward pulse and retraction never exceeded 10–15 sec. (*d*) It was also our impresion that with a sudden cessation of streaming (an event which may be followed by retraction or by renewed advance) as well as a sudden acceleration of a pseudopod's advance, both of these events were often immediately preceded by a pulse of current into this pseudopod's side, i.e. so frequently and so immediately as to preclude mere chance as an explanation. (*e*) We have occasionally observed rapid secretion of a membrane-bound globule about 50 μm wide from the tail of *Chaos*. Such an event is always accompanied by an inward tail pulse of the usual duration but of about twice the average amplitude.

[3] This length constant L (which is the distance over which a locally produced membrane potential change is attenuated by e-fold) is given by:

$$L = r\,R_m/2\,R_i$$

r, the typical animal's radius is about 0.02 cm. R_m, the membrane's specific resistance has been measured to be 20 kΩ-cm^2 in Marshall's medium (Bruce and Marshall, 1965, Fig. 3). R_i, the cytoplasm's resistivity, has been measured as 250 Ω-cm in this medium (Bruce and Marshall, 1965, p. 161).

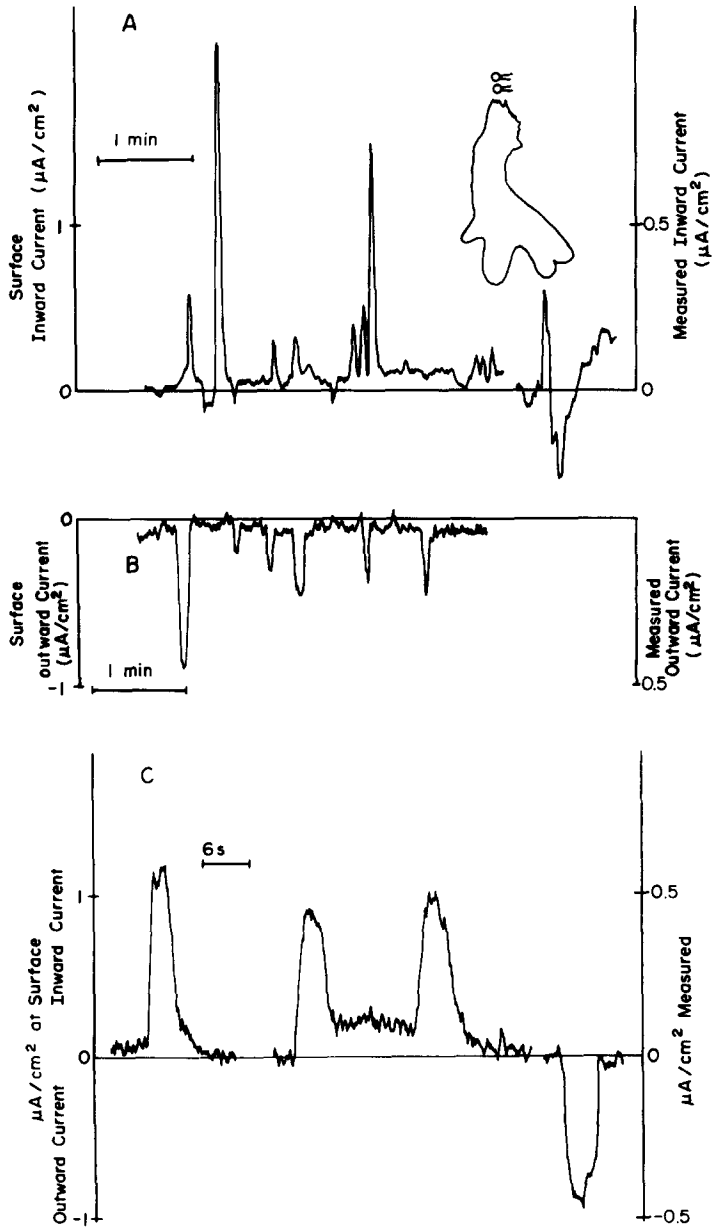

FIGURE 6. Spontaneous current pulses. Abscissa: time; ordinate: current density. A, A series of inward spontaneous current pulses recorded during a period of pseudopod retraction with the probe vibrating normal to the tail as shown in the drawing. B, A series of outward current pulses recorded during a period of active pseudopod formation with the probe in the same position shown in A. A 1-s time constant was used in both A and B. C, Representative recordings of spontaneous current pulses on a faster time scale with a 0.1-s time constant. The first and last pulses were recorded while vibrating normal to the side of a pseudopod, while the second and third pulses were measured normal to the tail.

Stimulated Pulses

An occasional pseudopod, perhaps 1 in 10, responds to an approaching vibrating probe (5–10 μm away) with a characteristically small local inward current pulse. Such a stimulated pulse is usually followed by the formation of a hyaline bulge under the probe. This, in turn, usually becomes a new pseudopod which advances towards the vibrating ball as if it were prospective prey. This striking phenomenon is illustrated in Fig. 7 and the results of its systematic study are summarized in Tables III and IV.

A responsive pseudopod typically has several streams of advancing endoplasm instead of one. In some cases, such a pod has responded a second time or even a third time to a probe approaching a second or third part of it. A response typically begins about 5–10 s after a probe has reached a position about 5–10 μm from the pod's surface. The measured amplitude of such a stimulated inward pod pulse averaged only one-seventh the size of a spontaneous inward pod pulse (see Fig. 7 B); so the two types could hardly ever be confused.

TABLE II

SPONTANEOUS PULSE CHARACTERISTICS

Pulse location	Current direction	Peak surface current density	No. of pulses	Frequency*	Comment
		$\mu A/cm^2 \pm SE$		min^{-1}	
End of tail	Inward	1.2±0.3	32	0.4–4	Pseudopod retraction
Side of pseudopod	Outward	−0.6±0.1	34	0.3–1.2	dominant
Side of pseudopod	Inward	1.0±0.2	12	0.2–1.2	Pseudopod formation
End of tail	Outward	−0.4±0.1	12	0.4–4	dominant

* The frequency varies a good deal and is higher in amebas with many pseudopods and many streaming velocity changes within the pseudopods.

In addition to observing 46 stimulated inward pulses followed by a local stimulated hyaline bulge or active pseudopod, we also observed five apparently stimulated outward pulses. These, too, were characteristically small, so they could hardly be confused with spontaneous pulses. Moreover, in all five of these cases, the outward pulse was followed within 5–10 s by the formation of a hyaline bulge and then a new pod centered 30–50 μm to the side of the probe rather than just under it. We interpret this as indicating that stimulated pulses traverse a relatively localized region as shown in Fig. 7 C. the region of current entry should be only 30–60 μm wide to readily explain the outward pulses. Moreover the surrounding ring of returning, outward current must be of comparable area to balance the inward flow. Such localization of the inward current is easily explained by the local stimulation, but localization of the return current is harder to explain. Perhaps a responsive part of the animal's membrane has a relatively high conductance before it is stimulated.

The stimulated current dipole is apparently much smaller than the steady one, so the current density should change relatively rapidly with distance from the cell membrane. We estimate that the apparent center of the stimulated current dipole is only about 50 μm below the membrane. Since the center of the

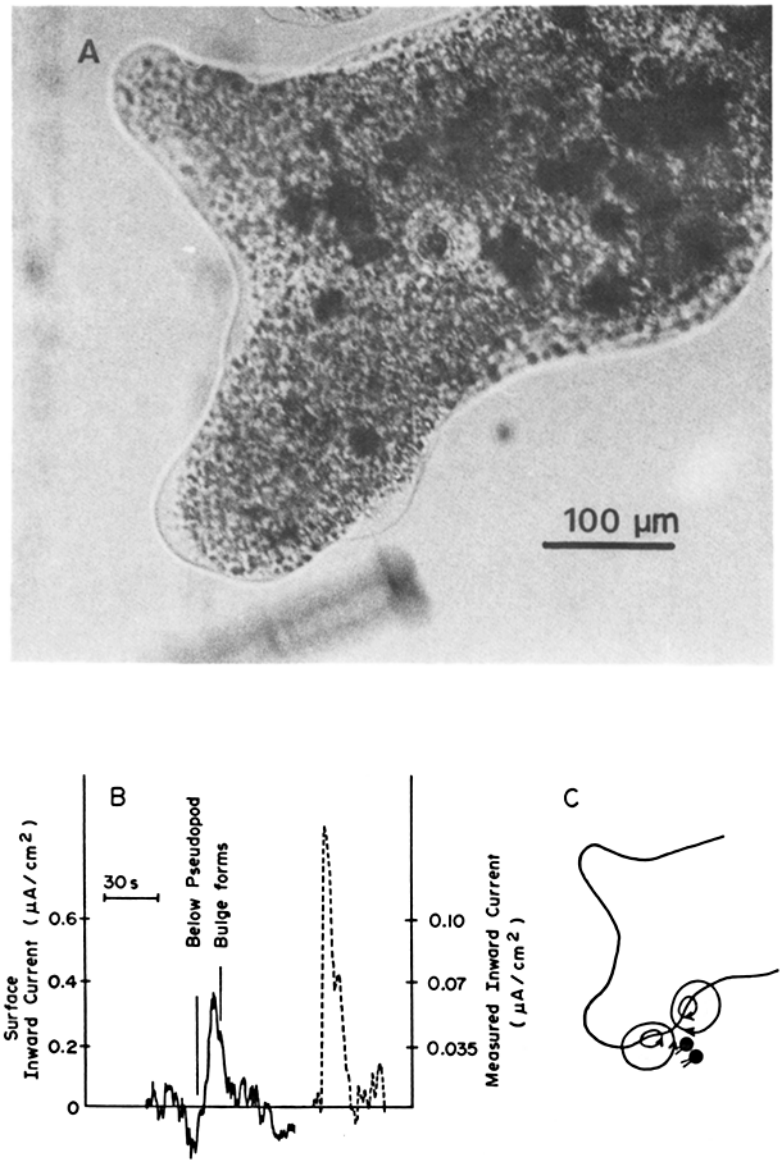

FIGURE 7. A stimulated current pulse. A, Photomicrograph of a large pseudopod with a new pseudopod forming near the vibrating probe. B, Recording of the stimulated current pulse which entered the region near the probe a few seconds after the probe was moved to that position below the pseudopod side. Just after the peak of the inward pulse the hyaline bulge formed and the photograph shown in A was taken. The dotted pulse on the right is one of the smallest spontaneous pulses measured and is included to illustrate the large amplitude difference between the two pulse types. The extrapolated current density scale on the left applies only to the stimulated pulse. C, The local stimulated pulse current pattern inferred from all the inward and outward stimulated pulses.

vibrating probe during pulse measurements is about 40 μm outside of the membrane, the current density at the membrane should exceed the measured one by about $((40 + 50)/50)^3 = 6$. This is the extrapolation factor used to estimate the surface current density in Fig. 7 and Table IV.

In addition to the 46 stimulated inward pulses and 5 stimulated outward pulses associated with a stimulated response, we observed 10 cases in which a new pseudopod formed near the probe without first registering a stimulated pulse. In some of these cases the probe may have been in the region between the

TABLE III

SUMMARY OF STIMULATED PULSES AND ACCOMPANYING PSEUDOPOD FORMATION

Event	No. of observations
Inward pulse without stimulated hyaline cap	17
Inward pulse precedes new hyaline cap only	10
Inward pulse precedes new hyaline cap and pseudopod	36
Outward pulse precedes a new pseudopod, ~40 μm away	5
Stimulated active pod under probe without measured pulse	10

TABLE IV

STIMULATED PULSES IN VARIOUS MEDIA

Current direction	Medium*	No. pulses	Average amplitude		Relative stimulation frequency‡
			Measured	At surface	
			$\mu A/cm^2 \pm SE$		min^{-1}
Inward	Marshall's	28	0.07±0.003	0.40±0.02	1
	Mg^{++}-free	11	0.07±0.003	0.41±0.02	1
	[Mg^{++}, Cl^-]-free	20	0.06±0.003	0.34±0.02	1
	Ca^{++}-free	4	0.08±0.005	0.45±0.03	0.06
Outward	Marshall's	3	−0.07±0.008	−0.40±0.05	–
	Mg^{++}-free	2	−0.09±0.015	−0.54±0.09	–

* All media at pH 5.5

‡ Pulses were first stimulated in normal Marshall's and the same ameba was then studied in the indicated medium. The relative stimulation frequency indicates the number of stimulus pulses per unit time in the new medium compared with Marshall's.

inward and outward currents (where the current direction was roughly tangential), and in others the pulse may have been stimulated to occur before the probe had approached close enough to the surface to detect it. On the basis of the same extrapolation formula used above, we calculate that if a pulse occurred while the probe was only 20-μm farther away, its measured size would have been halved, reducing it to the peak-to-peak noise level of 30 nA/cm^2. This last possibility appears even more likely when one considers that some pseudopods were probably initiated spontaneously during probe approach and not in response to mechanical stimulation by the probe.

Ion Dependence of Stimulated Pulses

The amplitudes of the stimulated pulses averaged about 0.4 μA/cm^2 and were independent of the presence or absence of Mg^{++}, Ca^{++}, or Cl^- in the medium

(Table IV). On the other hand, while their frequency was also independent of the presence or absence of Mg^{++} or Cl^-, it was highly dependent upon the presence of Ca^{++}. The frequency of mechanically stimulated pulses in calcium-free medium was only about 6% of the frequency in Marshall's medium. We studied three amebas for a combined total of 80 min in Ca^{++}-free medium and could only stimulate 4 pulses compared with 10 pulses stimulated in 13 min in normal Marshall's with one of the same amebas.

Considered together, these facts suggest that Ca^{++} ions are a normal component of such mechanically stimulated pulses, while Mg^{++} ions are not (however they imply little about Cl^- since so little Cl^- should move in against 70 mV, even in the presence of the usual 1 mM outside).

Ion Dependence of Morphological Changes

As we have already noted, spontaneous streaming shows a somewhat delayed requirement for external Ca^{++} since it does not stop entirely in most animals until 15–20 min after exposure to calcium-free (i.e. about 10^{-8} M Ca^{++}) medium. However, there is no such dependence upon any of the other major ions, namely Mg^{++}, Cl^-, or H^+. Spontaneous streaming continues for hours in media free of deliberately added Mg^{++} or Cl^-, and at pHs of up to 7.5 (cf Käppner, 1961). We could also stimulate new pseudopod formation by the close approach of the vibrating probe as easily in these media as in normal Marshall's. However we could only rarely stimulate pod formation in Ca^{++}-free medium. Only five pseudopods were apparently stimulated by the probe during the 80 min compared to an expected 60–80 stimulated pods if the amebas were in normal Marshall's. Some or all of the five observed stimulated pseudopods may have been coincidental, and to the extent that they were stimulated by the probe it should be kept in mind that the membrane sensitivity and architecture in Ca^{++}-free medium is likely to be abnormal. Perhaps mechanical stimulation in this medium can release Ca^{++} from internal stores.

Increases in external calcium, on the other hand, stimulate pod formation. When an ameba is transferred from Ca^{++}-free to Ca^{++}-only medium (0.7 mM $CaCl_2$), many clear hyaline bulges form over its entire surface. This transfer probably results in a relatively modest increase in free calcium under the cell membrane. A grosser increase may be induced by actually touching the surface of a pseudopod with the probe in Marshall's medium. In any case, such actual contact induces a striking and immediate lurch of granules towards the probe. The responding granules are seen to move as though tied together, and this lurch is usually followed by a brief cessation of normal streaming movement in the entire pseudopod. Such freezing is in turn often followed by a reversal of streaming. However, contact in Ca^{++}-free media induces none of these responses.

DISCUSSION

These extracellular electrical current measurements have revealed that changes in the ameba's streaming pattern are reflected in, and in some cases preceded by changes in the natural transcellular currents. This striking relation between ameboid movement and membrane-controlled electrical events is the most important result of this investigation. Since these electrical events fall into three

distinct categories, we will discuss each separately. Our discussion is informed by the idea of control by local cation, particularly calcium entry (cf. Jaffe et al., 1974; Jaffe and Nuccitelli, 1977).

Steady Current

The tail or uroid of an ameba may be defined as the region showing relatively steady retraction; while its pseudopods may be defined as generally advancing regions. We find a relatively steady current of about 0.1 $\mu A/cm^2$ entering the tail; while retraction of a pseudopod—which if continued long enough indicates its conversion into the tail—is generally preceded by entry of a comparable current. These facts suggest some causal relationship between local retraction and steady current entry.

The tail is also indicated by the relatively wrinkled character of its membrane. In quiescent amebas, showing little or no internal streaming, a comparable current continues to enter the tail defined as the wrinkled region. This indicates that the steady current is not produced by streaming, and also suggests the possibility that local current entry somehow induces local retraction.

When three of the four cations in the artificial pondwater used (namely Ca^{++}, Mg^{++}, and H^+) are reduced one at a time, only calcium reduction reduces the steady inward current. Specifically, when Ca^{++} is reduced from the usual 5×10^{-4} M to 10^{-8} M (and replaced by Mg^{++}), the steady inward current is immediately reduced three- to fivefold. This suggests that calcium carries much of the steady inward current. However, we cannot exclude the possibility that this external calcium decrease is decreasing the permeability of another ion such as Cl^-. Therefore it is difficult to determine what fraction of the inward current is actually carried by Ca^{++} without tracer experiments. The fourth cation, K^+, was not reduced because this change would hyperpolarize the cell, increasing the force driving all cations in, and the result would be difficult to interpret.

If, indeed, calcium ions are a substantial component of the tail current, it could act to produce and maintain a considerably higher concentration of free calcium under the tail membrane than elsewhere. If 0.1 $\mu A/cm^2$ were carried entirely by Ca^{++} for 100 s (the approximate time for the cytoplasm to complete one cycle within the ameba), 50 $pmol/cm^2$ would be carried in. Spread over a layer 100 μm deep, this would raise the total local calcium concentration by 5 μM.

A recent study of *Physarum* strands provides some further evidence that free calcium is high in the tail region during ameboid streaming. The endoplasm within these strands is squeezed back and forth (with a period of about 120 s) by alternating contractions of the strand's two ends. Ridgway and Durham (1976) have observed by the aequorin method that free calcium rises where and when contraction occurs. These contraction-associated oscillations of Ca^{++}-mediated luminescence were very small, indicating very low Ca^{++} control levels. While this oscillatory streaming is not quite the same as the motion of *Chaos*[4] or *A. proteus*,

[4] An attempt to detect spontaneous aequorin luminescence in *Chaos* by Taylor et al. (1975) was unsuccessful. However, by imposing a microscope objective between the ameba and the photomultiplier they could gather only about 4% of the luminescence which Ridgway and Durham (1976) could, and at the same time were injecting only 1–10% as much aequorin due to the smaller volume of *Chaos*.

there is an obvious similarity between the tail regions of these amebas and the contractile end of a *Physarum* strand, a similarity which suggests similar calcium gradients. In addition to the obvious morphological similarity, it should be noted that a given volume of cytoplasm may go through a contractile cycle with a period of minutes in amebas as well as in *Physarum*. At least in a monopodial ameba this occurs as the cytoplasm streams in the fountain pattern around the animal rather than oscillating.

What might be the effect of an increased free calcium level in the tail? There is good evidence that relatively small increases in local free calcium can favor local contraction within amebas. Taylor (1976) reports that injection anywhere inside *Chaos* of fluid buffered at only 0.7 μM Ca^{++} (so-called flare solution) induces local contraction. Conversely, lowering the Ca^{++} level with 5 mM EDTA injection results in relaxation.

Altogether, the above evidence and reasoning again confirms the existence of tail contractions in amebas (cf. Komnick et al., 1973, p. 179), and more important, perhaps, suggests that a local Ca^{++} leak helps to establish and maintain the tail. Several other observations support or are at least consistent with this inference:

(*a*) Pantin observed long ago that the movement of marine amebas is stopped within minutes by transfer to an artificial seawater without added Ca^{++} (Pantin, 1926). When one considers the inevitable impurities of the other salts used, it seems very likely that Ca^{++} in these inhibitory media was lower than 10 μM. He concluded that Ca^{++} was the only external cation essential for movement. We ourselves have observed that 15–20 min after the medium's Ca^{++} concentration is reduced to 10^{-8} M with EGTA, most specimens of *Chaos* stop streaming entirely. No other ion reduction stops movement in *Chaos*. Furthermore, this Ca^{++} appears to be needed within the cell because Hawkes and Holberton (1973) have observed that treatment of *A. discoides* with La^{3+} stops the animals' movement within a few minutes. La^{3+} at similar concentrations is known to block Ca^{++} influx in some other systems, e.g., cultured rat heart cells (Langer and Frank, 1972).

(*b*) Hahnert long ago observed the immediate effects of relatively small fields on the tail of *A. proteus*. When a current density as small as 100 $\mu A/cm^2$ was passed through the medium with the positive pole at the tail, an 8% increase in the rate of retraction was seen within 15 s (Hahnert, 1932, Table IV; see also Korohoda and Kurowska, 1970). We find the resistivity of Hahnert's salt solution to be 9 kΩ-cm and estimate the length of his test animals (from his Fig. 2) to have averaged 0.6 mm. We calculate from these data that the normal membrane potential of -70 mV (Braatz-Schade and Haberey, 1975) was raised to -100 mV at the tail end of his animals. This would increase the force driving cations into the tail by 40% at the just accelerating field. Since Ca^{++} is the only cation whose influx is necessary for contraction, it is likely that this field effect acts by driving more Ca^{++} in at the tail.

(*c*) In their study of free Ca^{++} oscillations in *Physarum* strands, Ridgway and Durham also observed oscillations in the externally measured potential difference between the strand's ends. At or slightly after the time of peak free Ca^{++} in

a strand end, it became more electronegative. This indicates current entry during the rise in Ca^{++} and suggests a local Ca^{++} leak.

Finally, we should point out that the direction of the steady current is opposite to the prediction of Bingley and Thompson (1962) on the basis of intracellular microelectrode measurements (see also Bingley, 1966). However, these authors' use of large, 3 MΩ electrodes could have caused a greater injury current into the pseudopod tip where the membrane was relatively stretched, resulting in the smaller membrane potentials observed there as suggested by Batueva (1964).

Spontaneous Pulses

Current pulses through *Chaos* of duration and shape similar to those we see were previously reported by Tasaki and Kamiya (1964) using conventional electrodes and animals partially squeezed into a fine tube. They also found that the pulses cross an animal in either direction and, using intracellular electrodes, observed concurrent episodes of membrane depolarization having peak changes of 30–80 mV. Episodes of membrane depolarization having shape and duration similar to our current pulses were also observed in *A. proteus* by Josefsson (1966). In unstimulated animals these depolarization episodes were invariably associated with emptying of the contractile vacuole and occurred every few minutes. More frequent episodes could be stimulated by treatment with alcian blue, an inducer of pinocytosis.

We have observed that the beginning of pseudopod retraction is invariably accompanied or immediately preceded by a current pulse leaving this pseudopod's side. This clear association, as well as some more tenuous ones reported in the Results should be useful leads towards analyzing membrane control of movement in amebas.

Stimulated Pulses

An occasional pseudopod responds to the mild mechanical stimulus produced by the approach of a vibrating probe towards its flank. It generates a characteristic 5–10-s long localized pulse of inward current which is usually followed by a hyaline cap and then pseudopod formation. Ion substitution experiments suggest that these stimulated current pulses are carried inwards by calcium ions. If they are, then such a pulse could act to induce local contraction under the reacting patch of membrane. We estimate the average integrated value of the total charge density which enters during a pulse as 2.3×10^{-6} C/cm^2; and this would correspond to about 10 pmol/cm^2 of Ca^{++}. Spread over a layer 10-μm deep, this would raise the Ca^{++} concentration there by 10 μM. Moreover, the literature shows two observations that may be direct indicators of high calcium within the hyaline cap. First, Pollack (1928, p. 540) has observed that in amebas injected with the Ca^{++} precipitator alizarin (which forms purple crystals of Ca-alizarinate), "if the amoeba attempts to put forth a pseudopod as evidenced by a slight lifting of the membrane a shower of these purplish red granules are seen to appear in this area and the pseudopod formation is immediately stopped." He interpreted this as indicating a local increase in free Ca^{++} which was precipitated as insoluble calcium alizarinate. Second, Taylor et al. (1975) observed that after

very strong electrical stimulation of aequorin-injected *Chaos* specimens, 5-s long episodes of relatively weak (calcium-mediated) luminescence were sometimes seen at the advancing, cathodally directed tips.

In any case, our 10 μM estimate may again be compared with Taylor's report that injection of fluid buffered at 0.7 μM Ca^{++} induces local contraction inside *Chaos* (Taylor, 1976). If we combine this comparison with evidence that hyaline caps consist of fluid squeezed out of contracting cytoplasm under the membrane (Allen, 1973) it is reasonable to infer that mild mechanical stimulation induces hyaline cap initiation via a local pulse of calcium entry. It appears that the hyaline cap, in turn, somewhow induces the advance of a new pseudopod. There is substantial evidence that this advance is due to oriented contraction within a ring, the so-called fountain zone, within the advancing pseudopod (Taylor et al., 1973); however, this first study of currents through amebas has provided no evidence on its control.

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