GALVANOTAXIS OF SLIME MOLD

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INTRODUCTION

Since Verworn's (1896) observations that ameba is negatively galvanotactic, numerous investigators have suggested explanations for the phenomenon (see reviews by Mast, 1931, and Hahnert, 1932). These hypotheses include localized changes in surface tension, endosmotic streaming, anodal contraction, changes in the elastic strength of the plasma gel on either the anodal or cathodal side, cataphoresis, and changes in pH, but the precise mechanism of galvanotaxis is still unknown.

Negative galvanotaxis in several species of slime molds was reported by Watanabe *et al.* (1938). In the present work it was observed that the plasmodium of *Physarum polycephalum* also migrated toward the cathode. The movements and activities of this plasmodium have been described by Howard (1931), Seifriz (1937), Camp (1937), and Lewis (1942). The advancing edge of the plasmodium usually sends out numerous pseudopodia much in the manner of *Amoeba*. Groups of these pseudopodia may coalesce, giving a lobulate appearance to the advancing front; or the plasmodium may advance with a very uniform anterior margin behaving as though it were a large pseudopodium. Back of the advancing front the protoplasm thins into a sheet which in turn thins out into a network of veins in which the protoplasmic flow is periodically reversed. There is much variation in the duration of flow in any one vein and between different veins. The average duration of flow is longer in the direction of migration.

In a study of the effect of electricity on ameboid movement there are several advantages in using the plasmodium of *P. polycephalum:* (1) the yellow pigment of the mold is a natural pH indicator (Seifriz and Zetzmann, 1935); (2) the migration of the plasmodium is slow enough that an experiment can be run for several hours; (3) many transplants from the same organism can be used in one experiment; (4) macroscopic as well as microscopic observations are possible; (5) the size of the organism, *i.e.* the mass of the protoplasm, can be varied by using different sized transplants; and (6) because the flow of proto-

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plasm within the veins of the plasmodium is reversed at frequent intervals, a study of the effect of electric current on the periodicity of streaming is possible.

However, there are disadvantages: (1) the plasmodium is injured in making transfers; (2) it is very difficult to obtain successive transplants of equal size; and (3) there is considerable physiological variation depending upon age of culture (Seifriz and Zetzmann, 1935; Lewis, 1942) and upon nutrition (Cohen, 1939; Winer and Moore, 1941). Physiological variations were kept at a minimum by standardizing the technique of culturing and handling the mold.

Materials and Methods

The mold was cultured upon paper toweling in large finger bowls. Rolled oats were the food source. If the cultures were washed occasionally (about every 3rd day) with large amounts of water, a stock culture could be kept in good condition for several weeks. Only transplants from actively growing cultures were used.

When a large number of transplants were required, masses of plasmodium from the same area of a culture dish were transferred onto non-nutrient agar in Petri dishes. When these masses had migrated from the site of the transfer, small (5 to 10 $mm.^2$) blocks of agar were cut from the region of the advancing front and placed on agar strips with the plasmodial surface down. This method gave very satisfactory results and there was little, if any, variation in the behavior of such transplants.

Current from dry cells was passed through tap water agar strips of measured thickness and width. An ammeter was placed in the circuit so that total amperage could be read at any time and current densities calculated. In some experiments of short duration, lead or zinc electrodes embedded in agar blocks were laid directly on the agar strips at a distance from the transplants such that electrolytic products would not reach the mold during the experiment. Non-polarizable $Zn\text{-}ZnSO_4$ electrodes such as those used by Watanabe *et al.* (1938) were employed in a few experiments. However, the most successful and most frequently used electrode was a carbon rod in a beaker of tap water. A tap water agar bridge led from the beaker to a large agar block which was laid on the agar strip. The beaker of water, agar bridge, and agar block were replaced frequently to prevent electrolytic products from reaching the transplants on the agar strip.

The strips of agar were placed on a paraffin-coated glass plate in a box ($19 \times 16 \times 3$ inches) with a tight fitting glass lid to prevent desiccation. The strips were laid in the pattern deemed necessary for any particular experiment and were joined by placing a few drops of molten agar at the junctions. Continuous strips were made as long as 200 cm. For controls, agar strips not in the circuit were placed in the box.

Orientation was also obtained on paper toweling, filter paper, bisque (fired but unglazed potter's clay), and cellophane; however, agar was much more satisfactory for these experiments.

A photographic record was made of nearly all experiments.

Even if one is studying galvanotaxis in a form which is immersed in a medium—such as *Amoeba* in culture solution—one cannot know just how much of the current is going through the organism. It has been the practice to standardize conditions as

much as possible by measuring the current density in the medium (Bancroft, 1906 *a*; Luce, 1926; Hahnert, 1932; Mast, 1931 and others). In the present work the problem is even more difficult. The mold will spread but little under water; typical migration occurs only on a surface. Therefore, in these experiments the only way for the current to enter the mold is through the interface between the mold and the agar. The exact values for the amount of current going through the mold cannot be determined; but, as in the studies of galvanotaxis in *Amoeba* or *Paramecium*, one can standardize the amount of current going through the medium (in these experiments, agar). If the current density along the agar strip is constant and if transplants are of the same size, one can assume that the amount of current going through each transplant must be nearly the same.

EXPERIMENTAL

(*a*) *Threshold.*—It was found that at current densities of 1.0 to about 8 μa./mm.^{2} in tap water agar with a specific resistance of 1,770 ohms, all transplants migrated toward the cathode. Some orientation was obtained with current densities as low as 0.4 μa./mm.^{2} At about 8 to 12 μa./mm.^{2} protoplasmic streaming ceased; this effect appeared first at the anodal end and progressed toward the cathodal end.

(*b*) *Electrolytic Products.*—In all work on galvanotaxis or galvanotropism it is essential to rule out electrolytic products as the causative agency. Various experiments were performed which showed that the galvanotaxis of *P. polycephalum* is not caused by electrolytic products. In one of these, twelve transplants were placed upon an agar strip 200 cm. long. A current of 1.0 μa./mm.^{2} was passed. Transplants on two strips not in the circuit served as controls. All of the transplants in the circuit began migrating towards the cathode (Fig. 1). On the control strips the direction of migration was random. If the migration were caused by electrolytic products, one would expect the transplants nearest one or the other electrode to show galvanotaxis first and then each transplant along the strip to follow in succession. But transplants in the center of the strip began migrating as soon as those lying next to the electrodes.

Passing current through the 200 cm. strip for 24 hours *prior* to making transplants did not affect migration. This would indicate that no gradients were established in the agar strip and that there was no lasting "orientation" of the substrate.

In another series of experiments transplants on filter paper were placed on glass plates inclined at an angle of 30° from the horizontal. A flow of water (250 ml./hr.) was directed over the plates in such a manner that the flow was with, against, and perpendicularly to the direction of the electric current. The migration in each case was toward the cathode.

(*c*) *pH.*—A change in pH at the anodal and cathodal ends of *Amoeba proteus* has been offered by Mast (1931) as an explanation for the negative galvanotaxis of that organism. In the plasmodium there is a natural sulfur-yellow indicator.

If macerated, or if small pieces of plasmodium are shaken in buffers, a range of color from orange at pH 1.5 through golden and yellow to green at 7.5 can be obtained (Seifriz and Zetzmann, 1935). However, plasmodia spreading over agar buffered at pH 2.0 to 8.5 showed no change in color although optimum spreading occurs in the pH range of 4.5 to 7.0 (see Fig. 2).

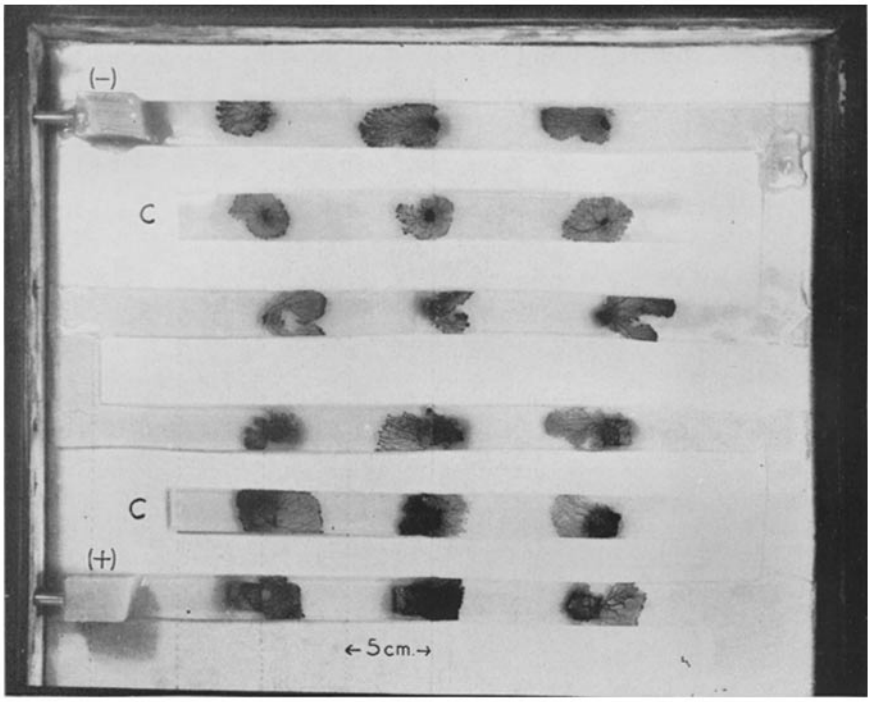

FIG. 1. Cathodal orientation of transplants placed on 200 cm. long strip of agar. Current density of 1.0 μa./mm.^{2} Time 5 hours. 2 control strips without current (*C*).

Seifriz (1935) found pH changes, as evidenced by color changes, when platinum micro electrodes were *inserted into* the plasmodium and a potential of 4 volts applied. In the experiments of the present work (with distant electrodes), no color change between the anodal and the cathodal portions of the plasmodia could be detected, at the relatively low current densities here used. To see whether currents greater than those necessary to stop protoplasmic streaming might cause a change in the color of the mold, two bare carbon electrodes were laid on the sides of a large mass of plasmodium (about 1 cm. in diameter and 0.5 cm. thick at the center). A potential of 180 volts was applied. Immediately there were color changes at the electrodes. But when agar blocks containing brom-thymol blue were placed between the electrodes and

the plasmodium, and a potential of 180 volts again applied, there was no change in the color of the mold until the changing color of the indicator in the agar blocks indicated that electrode products were reaching the mold.

When transplants were stained with neutral red there was no change in color in the cathodal and anodal portions, nor in the area between. When the polarity of the circuit was reversed there was no observable color change at either end. No color change was detected in the stained granules as they moved

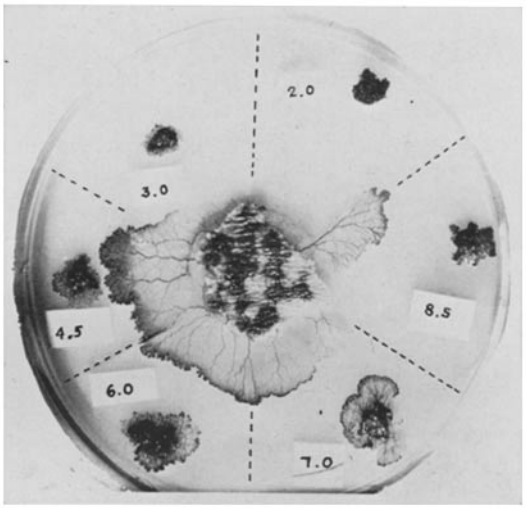

FIG. 2. Migration on buffered agar at 10 hours. Wedges of buffered agar were placed around a central unbuffered agar core upon which a large transplant was placed. Transplants were also placed at the periphery of each wedge. It can be seen that the spreading occurs at pH 4.5, 6.0, and 7.0 from both the center and peripheral transplants and that there is spreading from the center transplant along the contact line between pH 2.0 and 8.5 where there is a pH of about 5.0 because of diffusion.

toward or from either pole. The fact that there is no observed pH change associated with movement does not entirely exclude the possibility of a pH change, because the dye is taken up by the granules and vacuoles in much the same manner as in *Amoeba* (Mast, 1931). There could still be slight pH changes in the hyaloplasm insufficient to produce color changes in the stained granules and vacuoles and conceivably such slight change might be sufficient to influence migration. Effect of pH in the substratum was tested. Agar strips were prepared with equal specific resistances (1045 ohms) buffered with phosphate at pH 5.2 6.8, and 8.0. Twenty-five transplants on each of these strips showed typical reaction to direct current.

In summary, none of the experimental attempts to correlate galvanotaxis

in *P. polycephalum* with changes in pH was successful. The mold shows a wide pH tolerance, yet there is no color change in the natural indicator or introduced indicator associated with the passage of electric current *per se*. No evidence was obtained of any correlation of galvanotaxis with changes in pH in the substratum.

(*d*) *Cataphoresis.*—Cataphoresis of protoplasmic particles has been suggested as the cause of galvanotaxis in *Amoeba* (Heilbrunn and Daugherty, 1939). Cataphoresis of ultramicroscopic particles in the slime mold, *Stemonites*, has been reported by Taylor (1925). In the present work no ultramicroscopic observations were attempted, but numerous examinations at magnifications of 100 to 500 diameters were made on the plasmodium. There was no separation of large from small particles. There was an accumulation of protoplasm in the advancing tip, but this also occurs in plasmodia not in the electric current. There was no intensification of color at either the anodal or cathodal end, indicating there was no mass migration of the pigment-bearing granules.

TABLE I

Periodicity of Protoplasmic Streaming

Current density	Toward the front	Rest	Toward the rear	Rest
μa./mm.²	*sec.*	*sec.*	*sec.*	*sec.*
0.45	48	5	37	5.1
3.0	47	5	36	4
Control	48	5	38	5

Each figure is the average of 12 counts.

There was no significant difference in the periodicity of protoplasmic streaming between control and experimental transplants. Table I summarizes the results of observations on veins 0.1 to 0.2 mm. diameter 4 mm. from the advancing front in equal sized migrating transplants.

Another approach to the problem of whether cataphoresis may be a factor in galvanotaxis is to attempt to change the charge on protoplasmic particles. By placing *Amoeba proteus* in 0.0133 to 0.0166 M ammonium chloride, Heilbrunn and Daugherty (1939) were able to obtain anodal responses in 66 out of 153 cases, with the greatest percentage in 0.0166 M. They attributed the change from the normal cathodal response to reversal of the charge on the protoplasmic granules from positive to negative. In the present work indifferent response (no orientation) was obtained on agar containing 0.015 M ammonium chloride at current density of 2.0 μa./mm.² (Fig. 3, also Table II). But equally indifferent response was also obtained on agar containing 0.013 M sodium chloride (specific resistance equal to that of 0.015 M ammonium chloride) at a current density of 2.0 μa./mm.² There is no reason to believe that

sodium chloride would have changed the charge on the protoplasmic particles. When the current density was increased to 6.0 μa./mm.², all transplants showed

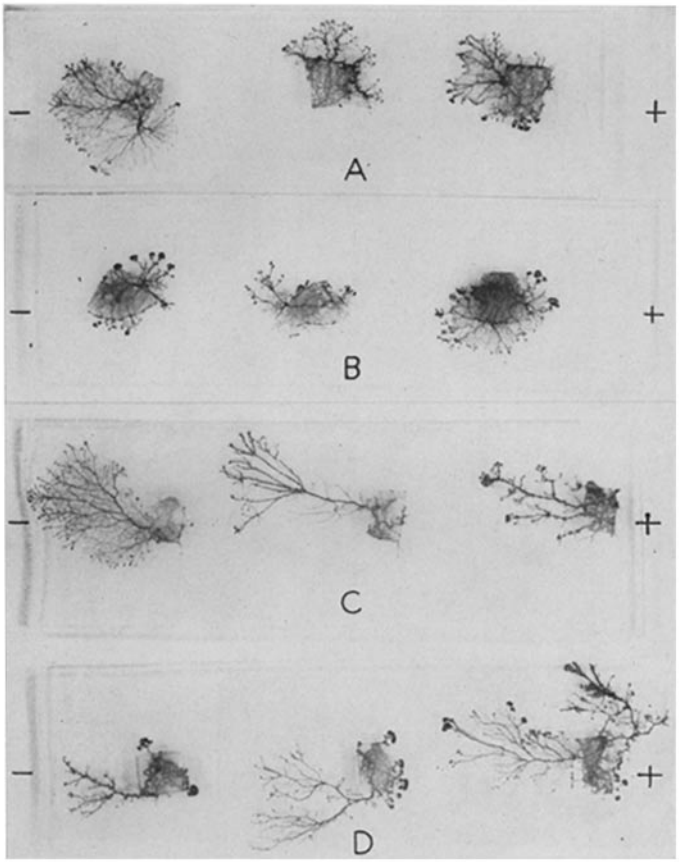

FIG. 3. Effect of ammonium chloride and sodium chloride upon galvanotaxis of *P. polycephalum*. Time 4 hours.

A. 0.013 M NaCl, 2.0 μa./mm.²

B. 0.015 M NH_4Cl, 2.0 μa./mm.²

C. 0.013 M NaCl, 6.0 μa./mm.²

D. 0.015 M NH_4Cl, 6.0 μa./mm.²

No orientation occurred with a current density of 2.0 μa./mm.², but orientation was pronounced at the higher current density. The lack of orientation at lower current densities was due to shunting effect of the salts in the agar.

orientation, both on the ammonium chloride and on the sodium chloride. It was concluded that the indifferent response obtained was due to shunting by the salt rather than to change of charge on the protoplasmic granules.

(*e*) *Effect of Salts.*—In the earlier works on the effect of salts upon galvanotaxis of ciliates a change in the response from cathodal to anodal could be obtained in some instances (Bancroft, 1905, 1906 *a*, and 1906 *b*; Coehn and Barratt, 1905). *P. polycephalum* has been shown to take up salts from its

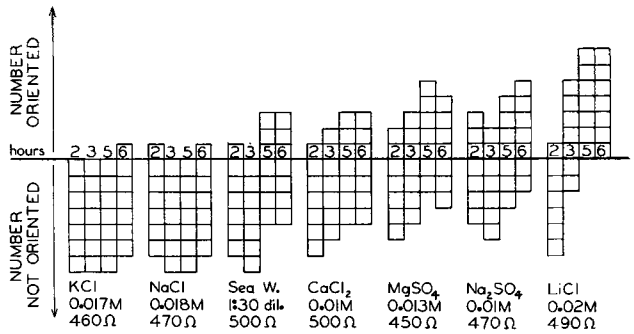

FIG. 4. Histogram of the effect of salts in the substratum upon orientation of *P. polycephalum*. Current density 3.0 μa./mm.^{2} Hours of exposure as indicated.

TABLE II

Effect of Ammonium Chloride and Sodium Chloride on Galvanotaxis of P. polycephalum at End of 4 Hours

No. of transplants	Current density	Salt	Response		
			Cathodal	Anodal	Indifferent
	μa./mm.^{2}				
12	3.0	0.001 M NH_4Cl	12	0	0
12	3.0	0.01 " "	12	0	0
7	2.0	0.015 " "	1	0	6
7	4.0	" " "	2	0	5
7	6.0	" " "	7	0	0
7	12.0	" " "	7	0	0
7	2.0	0.013 " NaCl	1	1	5
7	4.0	" " "	3	0	5
7	6.0	" " "	7	0	0
7	12.0	" " "	7	0	0

medium and to maintain an internal salt concentration at a constant increment above the concentration of the medium (Winer and Moore, 1941). Therefore, it was advisable to see whether salts in the agar would change the galvanotactic response. In one experiment a series of salts in agar was made with the specific resistances nearly equal, so that the difference in response due to shunting effect would be minimized. Fig. 4 summarizes the results and also gives the specific resistances and molarities of the salts used.

In the series: sea water, calcium chloride, magnesium sulfate, and lithium chloride there was a progressive increase in the number of transplants showing negative galvanotaxis as the experiment continued. Essentially no orientation took place on sodium chloride and potassium chloride, yet orientation occurred on lithium chloride. Because of the striking difference in the response, potassium chloride and lithium chloride were chosen for further experimentation. The results are presented graphically in Fig. 5. The thresholds for the galvanotactic response and for injury are higher on KCl-agar than on LiCl-agar. Specific resistance of plasmodia (macerated) which had been migrating on 0.017 M KCl-agar was 341 ± 11 ohms; on 0.02 M LiCl, 340 ± 18 ohms; and on tap water agar, 613 ± 80 ohms. It would appear that the difference in the

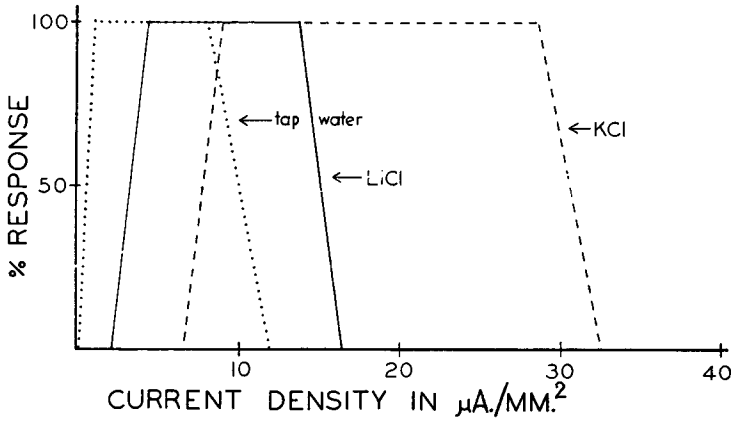

FIG. 5. Diagramatic representation of the effect of the K ion and Li ion in shifting the threshold for orientation and for cessation of protoplasmic flow.

action of LiCl and KCl is not due to difference in the amount of salt taken up by the mold, nor to difference in the internal resistance of the mold, hence in the amount of current going through it (assuming that there is no difference in the resistance of the plasma membrane between plasmodia grown on these two salts). Since the anion is common to the two salts, the difference in action must be due to the cation.

In all the experiments on the effects of salts in the agar on the galvanotactic response, it appears that the action of the salts used is not to change the galvanotactic response *per se* but to change threshold values for its appearance.

(*f*) *Rate of Migration.*—Hahnert (1932) found that the rate of locomotion in *Amoeba proteus* subjected to direct current increased slightly immediately after beginning of current flow and then progressively decreased with time. He also found that there was an inverse ratio between current density and rate of locomotion.

The rate of migration of *P. polycephalum* did not decrease as an experiment progressed, and, within rather wide limits, changing the current density did not alter the rate of migration. Equal sized and shaped transplants on tap water agar were subjected to current densities of 0.95 to 4.0 μa./mm.^{2} Since the specific resistance was the same in each strip, it was assumed that the

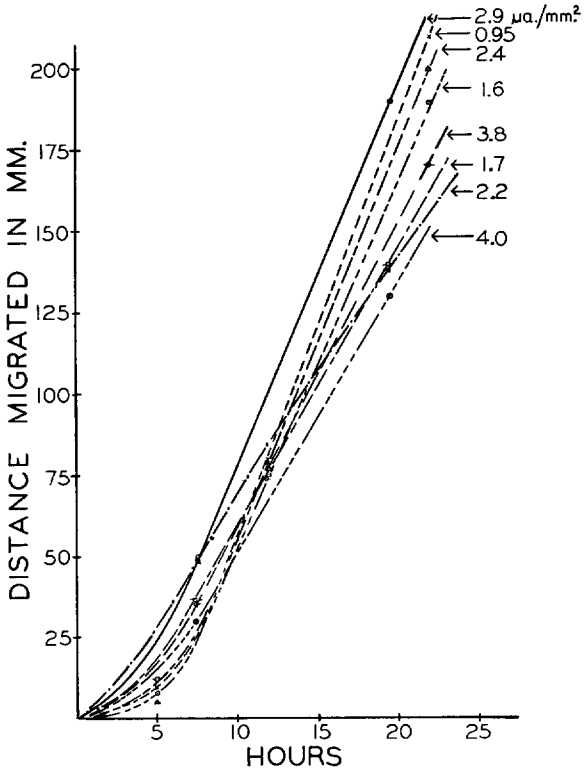

FIG. 6. Graph showing cathodal migration of equal sized transplants at different current densities. The slopes of the curves are not significantly different; there is no correlation between current density and rate.

amount of current going through the mold would be different for each current density. The distance migrated was plotted against time for plasmodia on each strip (Fig. 6). There was no appreciable difference in the rate of cathodal migration on these strips. *The action of these current densities was not to change the cathodal rate, but only to determine direction of migration; i.e., the current acts by inhibition of migration toward the anode.* This assumption is supported by the fact that when control transplants were placed on the *end* of an agar strip so that the migration was limited to one direction, the distance migrated was the same as for equal sized transplants on agar strips in the circuit (see Fig. 7).

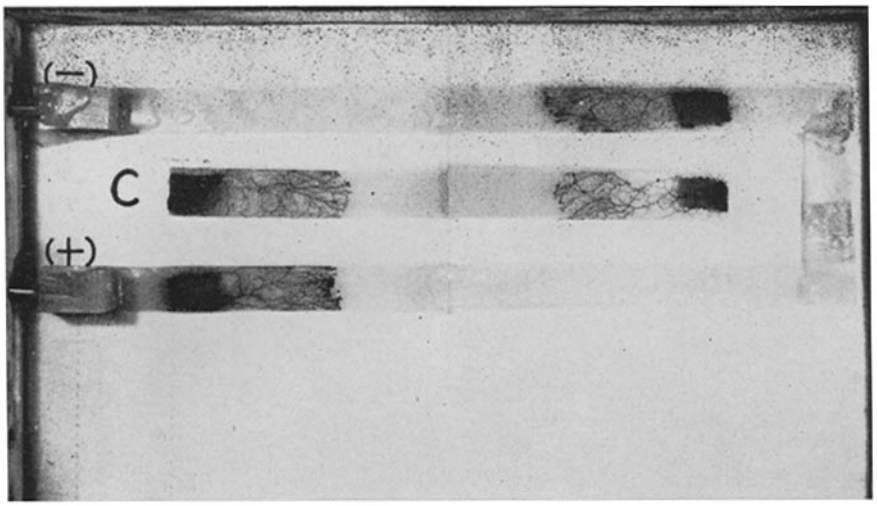

FIG. 7. Photograph showing that if a control transplant (*c*) is placed on the end of agar strip so that its migration must be unidirectional, the distance migrated is nearly equal to the migration of a transplant in direct current.

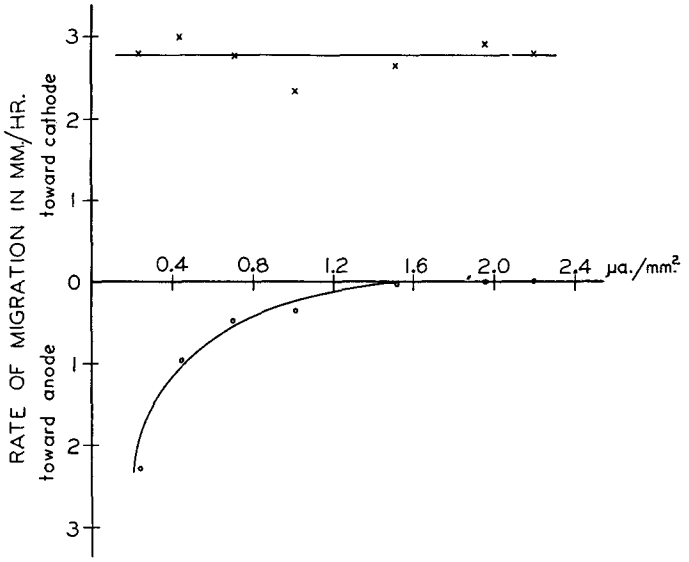

FIG. 8. Anodal inhibition as shown by the rate of anodal and the rate of cathodal migration at low current densities. Each point on the curve is average of rates of migration of seven transplants calculated at end of 1.5 hours. Rate of migration toward the cathode is not increased with increase in current density, but the rate of migration toward the anode is decreased as current density increases.

On the other hand there is a gradual decrease in the rate of migration to the *anode* as the current density is increased from zero (where the rate of migration is equal in both directions) to values which give complete anodal inhibition. For example, transplants were placed on agar strips with current densities of 0.22, 0.66, 1.1, 1.5, and 1.9 μa./mm.^{2} Seven transplants were placed on each strip. The rate of migration toward the cathode was not increased with increase in current density, but the rate of migration toward the anode was progressively less with increase in current density (Fig. 8).

These experiments show that fairly large current density has no effect on the rate of migration towards the *cathode*, but that the rate of migration towards the *anode* is rapidly decreased as the current density increases until there is complete anodal inhibition at fairly low densities. Therefore the galvanotactic response in *P. polycephalum* is due not to cathodal stimulation, but to anodal inhibition.

(*g*) *Effects of Alternating Current.*—Mast (1931) found that *Amoeba proteus* subjected to alternating current (60 cycle) oriented at right angles to the direction of the electrodes. Such orientation can be obtained in *P. polycephalum* using current densities of 4 μa./mm.^{2} The same effect can be obtained by reversing the polarity of direct current of the same density every minute. Orientation at right angles to the direction of the current is further evidence that the effect of the current is anodal inhibition, migration being blocked on both sides. Furthermore, the effect of periodic reversal of direct current indicates that the anodal inhibition can persist for at least 1 minute and is not counteracted when the anodal side of the transplant becomes the cathodal side.

DISCUSSION

Most investigators agree that the essential aspects of ameboid movement presented by Mast (1926) are correct although the precise physiological mechanisms are unknown (for review of the general problem, see De Bruyn, 1947). Any external agent, such as the electric current, which causes polarized ameboid movement, must exert an effect, directly or indirectly, upon one or more of these unknown mechanisms.

In the present work it has been demonstrated that electrode products are not the cause of galvanotaxis of *P. polycephalum.* Cathodal orientation occurred when a stream of water was flowing with, opposite, or perpendicularly to the direction of migration. Also, on an agar strip 200 cm. long all transplants oriented simultaneously.

There was no evidence of oriented configuration of the substratum such as Weiss (1946) found effective in directing the growth of nerve processes *in vitro.* First of all, one would have to assume that the electric current oriented all the different substrata (agar, cellophane, paper, bisque) in such a manner that the effect upon the migration of the mold was the same. If such orientation did occur, it disappeared upon opening the circuit since there was no orienta-

tion of transplants placed on agar through which current had previously flowed for 24 hours.

It has long been known (Clifford, 1897) that slime molds show positive rheotaxis. Since in the present work electric current passed through agar, it might be suggested that electroosmotic flow of water in the agar affected the migration. Two observations refute this: (1) the electroosmotic flow of water in the agar is towards the cathode, *i.e.* in the same direction as the galvanotaxis; and (2) migration to the cathode occurs when a stream of water is running either with, against, or perpendicularly to the direction of migration. Galvanotaxis supersedes rheotaxis.

The problem of electroosmotic flow of water within the organism itself is much more difficult. Pantin (1923) has suggested that loss of water accounts for the contractility of the posterior gel in *Amoeba*. Mast (1931) maintains that electroosmosis must play but a minor role in galvanotaxis since orientation of *Amoeba proteus* at right angles to the electrodes was obtained in alternating current. Similar orientation was obtained in the present work, and furthermore there was no enhanced movement toward the cathode with increase in current density. Since the amount of water moving by electroosmosis is directly proportional to the strength of the current (assuming no change of charge on the protoplasmic colloids), one would expect an increase of current density to either increase or decrease the rate if electroosmosis were a factor.

Cataphoresis is known to be a factor in the passive movement of microorganisms, or of cell components (Luce, 1926; Blinks, 1932; Rhodes, 1938). Heilbrunn and Daugherty (1939) suggested that galvanotaxis of *Amoeba proteus* may be due to cataphoretic movement of granules and particles pushing and perhaps liquefying the cortex. They found that exposure to ammonium chloride would change the galvanotactic response. In the present work, any changes in response on ammonium chloride–agar could be duplicated on sodium chloride–agar with equal specific resistance. Thus it would appear that the change in response observed was due to the shunting effect of salts in the substratum rather than to changing of charge upon protoplasmic granules. Furthermore, throughout these experiments, it was found that changes in current density between 1.0 and 4.0 μa. did not affect the rate of cathodal migration. If cataphoresis were an important factor, greater migration rates should have been obtained with the higher current densities.

The present work did not demonstrate a specific change in the solation-gelation mechanism of *P. polycephalum*. There was no microscopically visible evidence of a weakening of the tip of the plasmodium facing the cathode at the closing of the circuit. No significant changes occurred in the direction or duration of streaming in veins when the current was applied. No acid-base changes, as postulated by Mast (1931) as the cause of solation-gelation changes, could be demonstrated.

The negative galvanotactic response of the organism is due solely to inhibi-

tion of migration on the anodal side of the plasmodium; when complete, this inhibition limits the migration of the mold to a cathodal direction. When a plasmodium is placed on the end of an agar strip so that the migration can be in but one direction, it will migrate at the same rate as an equal sized plasmodium in the electric current. The rate of migration toward the cathode is *not* increased with increase in current density; neither is the rate decreased until injurious current densities ranging from 8.0 to 12 μa./mm.^{2} are reached. As the current density is increased from zero there is a progressive decrease in the rate of migration toward the anode until complete anodal inhibition occurs.

The mechanism of the anodal inhibition is not yet elucidated. It is possibly due to some effect on the contractile mechanism, perhaps inhibiting the unfolding of protein molecules as postulated by Goldacre and Lorch (1950) in *Amoeba*. However, no preferential uptake of neutral red such as they found could be detected in *Physarum*. There may be viscosity changes occurring only at the anodal region such as found by Tobias and Solomon (1950) in *Elodea*.

That orientation perpendicular to the electrodes, *i.e.* inhibition of the parts of the plasmodium facing the electrodes, occurs in alternating current indicates that anodal inhibition persists after reversal of the polarity of the current. The experiment on periodic reversal of direct current shows that the effect persists for at least 1 minute.

The author expresses his appreciation for the direction and encouragement given by Professor L. R. Blinks and for the suggestion and criticisms offered by numerous persons connected with the School of Biological Sciences and the Hopkins Marine Station, Stanford University.

SUMMARY

The plasmodium of *Physarum polycephalum* reacts to direct current by migration toward the cathode. Cathodal migration was obtained upon a variety of substrata such as baked clay, paper, cellophane, and agar with a current density in the substratum of 1.0 μa./mm.^{2} Injury was produced by current densities of 8.0 to 12.0 μa./mm.^{2} The negative galvanotactic response was not due to electrode products. Attempts to demonstrate that the response was due to gradients or orientation in the substratum, pH changes in the mold, cataphoresis, electroosmosis, or endosmosis were not successful. The addition of salts ($CaCl_2$, LiCl, NaCl, Na_2SO_4, $NaHCO_3$, KCl, $MgSO_4$, sodium citrate, and sea water) to agar indicated that change of cations had more effect than anions upon galvanotaxis and that the effect was upon threshold values. K ion (0.01 M KCl) increased the lower threshold value to 8.0 μa./mm.^{2} and the upper threshold value to 32.0 μa./mm.^{2}, whereas the Li ion (0.01 M LiCl) increased the lower threshold to only 4.0 μa./mm.^{2} and the upper threshold to only 16.0 μa./mm.^{2} The passage of electric current produced no increase in the rate of

cathodal migration; neither was there a decrease until injurious current densities were reached. With increase of subthreshold current densities there was a progressive decrease in rate of migration toward the anode until complete anodal inhibition occurred. There was orientation at right angles to the electrodes in alternating current (60 cycle) with current density of 4.0 μa./mm.^{2} and in direct current of 5.0 μa./mm.^{2} when polarity of current was reversed every minute. It is concluded that the negative galvanotactic response of *P. polycephalum* is due to inhibition of migration on the anodal side of the plasmodium and that this inhibition results in the limitation of the normal migration of the mold to a cathodal direction. The mechanism of the anodal inhibition has not been elucidated.

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