Potassium Loss during Galvanotaxis of Slime Mold

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ABSTRACT The posterior reticulated regions of the plasmodia of the slime mold, *Physarum polycephalum*, whose migration has been oriented by direct current (3.0 to 5.0 $\mu a/mm^2$ in the agar substrate), contain 30 per cent less potassium than the advancing non-reticulated region. The anterior regions have the same potassium concentration as that of the controls, approximately 32 meq/kg wet weight. Differences in potassium concentration between anterior and posterior regions of control plasmodia, not oriented by electric current, are less than 5 per cent. Sodium, in contrast to potassium, is generally less concentrated in the anterior than in the posterior regions of electrically oriented plasmodia, but sodium concentrations are extremely variable. No significant difference in protein concentration was found between oriented and control plasmodia. Thirty-five per cent of the total potassium, but none of the sodium, is found in acidified ethanol precipitates from plasmodial homogenates. Potassium, but not sodium, appears to be closely associated with processes which differentiate anterior from posterior in an oriented plasmodium.

INTRODUCTION

The migration of the plasmodium of *Physarum polycephalum* can be oriented or directed by electric current (Watanabe *et al.*, 1938; Anderson, 1951). Anderson reported that the effect of the current was to inhibit migration in the anodal direction rather than to stimulate movement in the cathodal direction and furthermore, that over a range of current densities in the substratum from about 1.0 to 8.0 $\mu a/mm^2$ the rate of migration was not appreciably affected.

Studies (Roter, 1953; Butkiewicz, 1953) undertaken to determine whether cataphoretic movement of the common cations sodium, potassium, and calcium could be detected, suggested that differences in the concentrations of these ions may be found between the advancing and the posterior regions of migrating plasmodia whether oriented by electric current or not. Calcium was higher in the anterior portion and sodium higher in the posterior for both experimental and control specimens. This was contrary to the logical

expectation that under normal conditions the shuttle type of protoplasmic streaming characteristic of this organism would equalize ion concentrations in all parts of the plasmodium. Furthermore, the studies indicated that potassium distribution was more affected than that of other cations by electric current; the anterior potassium concentration was always higher than that in the anodal or posterior portion of plasmodia oriented by current.

The present study was undertaken to pursue these preliminary findings further.

MATERIALS AND METHODS

Plasmodia of *P. polycephalum* were grown in axenic culture in the dark at 22°C on a defined medium slightly modified from that reported by Daniel and Rusch (1961). The modification consisted of substitution of phosphate buffer at pH 6.0 for $CaCO_3$, hemin (Kelley *et al.*, 1960) 10 mg/liter for chick embryo extract, and the addition of 2 per cent agar. In a few experiments plasmodia from a culture found to have extremely low sodium content were used. This culture was maintained on oatmeal by the method described by Camp (1936).

Migration was oriented by direct current using techniques previously described (Anderson, 1951). Current from dry cells was passed through agar strips (4 per cent in tap water) of measured width and thickness laid on a paraffin surface. In all experiments the voltage was adjusted to give a current density in the agar strip of 3.0 to 5.0 $\mu a/mm^2$ with a voltage gradient of 0.4 to 0.7 volt/cm.

Plasmodia placed on the end of an agar strip not in the circuit served as controls. The plasmodium will not migrate any appreciable distance over a paraffin surface. Therefore, a plasmodium placed on the end of an agar strip migrates unidirectionally from the site of transfer and has the same morphological appearance as plasmodia oriented by electric current (see Fig. 1). Time lapse photography did not reveal any obvious differences in pulsations and waves exhibited by experimental and control plasmodia.

Samples for analyses were taken when the leading edge of the plasmodium had migrated 3 or more cm from the site of transplantation. Samples were taken from the anterior and posterior regions (see Fig. 1) and weighed to the nearest 0.1 mg. In no instance were the slime and debris at the original site of transplantation included in the sample. The minimum sample size was 10 mg. In most instances only one anterior and one posterior sample could be obtained from any one plasmodium, but when large transplants were placed on wide agar strips, as shown in Fig. 1, multiple samples in both the anterior and posterior regions could be obtained from the same plasmodium.

We analyzed for potassium and sodium by standard flame photometric techniques using the internal standard method with a Baird-atomic KY instrument. Boiling the sample in 4 ml of water for 5 minutes released all sodium and potassium from the plasmodium.

Protein was determined by the method of Lowry *et al.* (1951).

RESULTS

The results of an experiment in which potassium, sodium, and protein were analyzed are presented in Table I. The potassium concentration is seen to be 30 per cent lower in the posterior than in the anterior regions of the plasmodia whose migration has been oriented by direct current. The corresponding difference between the two regions in the controls is only 4 per cent. The potassium concentration in the anterior portion of the experimental

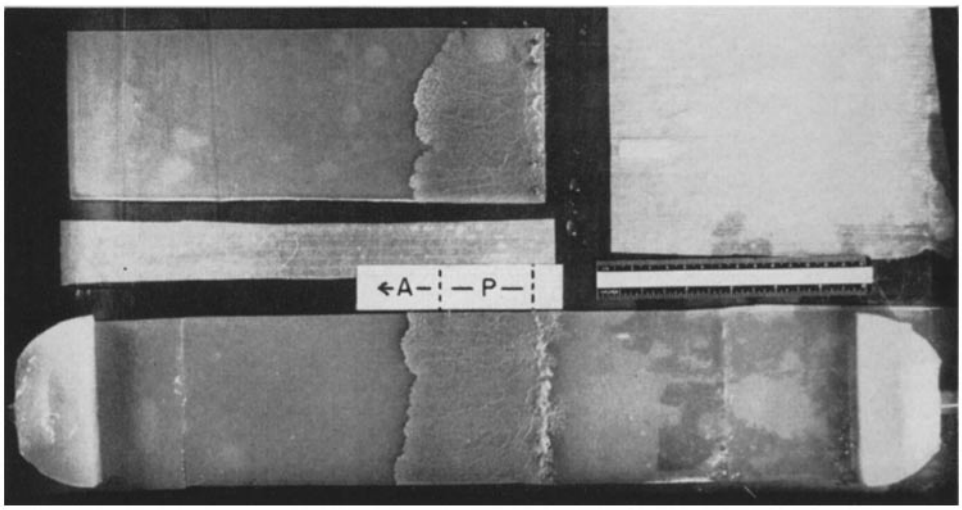

FIGURE 1. Experimental arrangement used for orienting migration of plasmodia. Current density, 3.0 $\mu a/mm^2$; voltage gradient, 0.4 volt/cm; time 5 hours. Experimental plasmodium is in lower part of figure; control plasmodium above. Cathode is to the left, anode to the right. Anterior samples were taken in area *A*, posterior samples in area *P*. Wet paper towels shown between the agar strips and in the upper right maintained a moist atmosphere.

plasmodia is slightly less, not more, than in the advancing portion of the controls. There is no significant difference in protein concentration between the anterior and posterior regions nor between experimental and control organisms. The ratio of potassium to protein therefore has the same value in the two regions for the controls and for the anterior region of the experimental plasmodia, but is lower for the posterior region of the experimental specimens.

This type of experiment, in which potassium has been measured along with something else, *e.g.* sodium, protein, carbohydrate, etc., has been repeated many times and although there is variation from experiment to experiment, the potassium analyses have consistently shown lower potassium

concentration in the posterior regions in the plasmodia oriented by current. Combining the potassium analyses from 6 separate experiments in which there were a total of 51 experimental and 40 control plasmodia, the average values are: experimental, anterior, 28 ± 2.8 meq/kg wet weight, posterior, 21.4 ± 3.1; control, anterior, 33.1 ± 3.1 and posterior 31.8 ± 2.1. The average of the per cent difference in potassium concentration between the anterior and the posterior of each of the above experimental plasmodia was 27 per cent, for the controls only 5 per cent.

Sodium, in contrast to potassium, is always less concentrated in the anterior portion of the plasmodia oriented by current. The controls either have nearly equal amounts in the two regions, or, as is more generally the

TABLE I

POTASSIUM, SODIUM, AND PROTEIN ANALYSES OF PLASMODIUM OF *P. POLYCEPHALUM*

Ten experimental and 9 control plasmodia. One sample taken from the anterior and posterior region of each plasmodium. Current density, 4.0 μa/mm^2; voltage gradient, 0.6 volt/cm.

		Anterior	Posterior
		Mean ± standard deviation	Mean ± standard deviation
K^+, *meq/kg*	Experimental	30.5 ± 3.1	22.0 ± 3.7
	Control	32.1 ± 3.1	30.6 ± 2.0
Na^+, *meq/kg*	Experimental	5.4 ± 2.0	8.8 ± 2.8
	Control	8.6 ± 2.1	8.6 + 3.2
Protein, *per cent*	Experimental	7.2 ± 2.4	6.7 ± 1.1
	Control	7.5 ± 2.0	7.1 ± 1.2
K^+/protein, *meq K* × 10^{-3}/*mg protein*	Experimental	0.43 ± 0.08	0.35 ± 0.09
	Control	0.44 ± 0.1	0.44 ± 0.08

case, slightly less in the anterior region. The sodium distribution is thus opposite to that for potassium in both experimental and control plasmodia. Moreover, the values obtained for sodium are more variable than those for potassium between individual plasmodia and between different cultures. In preliminary experiments we used an oatmeal-grown culture whose sodium content was extremely low. The sodium in the water used in maintaining the culture and in making the agar strips was 2.0 meq/liter, but in samples of the plasmodia it was never more than 0.5 meq/kg. However, these almost sodium-free plasmodia showed the usual galvanotactic response and had differences in potassium concentration between the anterior and posterior regions of individual plasmodia comparable to those reported for the cultures grown on a defined medium.

Although no significant difference in total protein was detected between anterior and posterior regions of either experimental or control plasmodia,

the mean protein content for individual experiments ranged from 5.4 to 9.7 per cent of wet weight. Thus the protein values vary more than the potassium values. While the protein content of individual plasmodia may vary, multiple samples taken from large plasmodia in either the anterior or posterior regions agreed within 5 per cent of the total value for protein.

In the course of making observations on the migration of plasmodia not subjected to electric current, the mold was placed on large sheets of non-nutrient agar and allowed to migrate for 3 days. After 8 to 12 hours these developed a consistent orientation, *i.e.* the trailing region did not produce an advancing front; any change of direction of migration originated at the existing front. One of these plasmodia had a distinct fan-like appearance

TABLE II

POTASSIUM AND SODIUM IN ALCOHOL PRECIPITATES OF PLASMODIA

Twenty mg plasmodia per 4 ml of 95 per cent ethanol. Heated to 78°C for 5 minutes. Washed with 2 ml of ethanol. All samples stirred vigorously with glass rod and shaken for 40 seconds on mechanical mixer before and after heating.

Treatment	Potassium per cent of total		Sodium per cent of total	
	Precipitate	Supernatant	Precipitate	Supernatant
Plasmodium introduced directly into ethanol	67	33	0	100
Plasmodium homogenized in ethanol	45	55	0	100
Plasmodium homogenized in ethanol acidified to pH 2 with HNO_3	35	65	0	100
Plasmodium homogenized in water (100 mg/1 ml). 0.2 ml of homogenate precipitated by alcohol	25	75	0	100

with a pronounced leading edge similar to those shown in Fig. 1. Another had a dendritic appearance. Two samples were taken in the anterior and posterior regions of each of these plasmodia. The potassium values obtained for the anterior of the plasmodium with the fan-like appearance were 32.4 and 33.0 meq/kg; for the posterior, 24.2 and 23.0. In the other plasmodium the values for the anterior were 30.1 and 29.6, and 25.8 and 28.6 for the posterior. This suggests that unequal distribution of potassium may be characteristic of migrating plasmodia which have established a definite orientation.

Several kinds of exploratory experiments were undertaken in an attempt to isolate a cytoplasmic component with high potassium content. Results using sucrose homogenization techniques have been inconclusive so far. However, precipitation in 95 per cent ethanol gave indications that potassium, but not sodium, is closely associated with some component of the plasmodium.

The data in Table II summarize the recovery of potassium under various conditions after precipitation of homogenized plasmodia by 95 per cent ethanol. In all instances potassium, but not sodium, was found in the alcohol-precipitable fraction. Even when the plasmodium was homogenized in 10 volumes of water prior to precipitation in alcohol, 25 per cent of the total potassium remained in the washed precipitate. Potassium added to the samples either before or after alcohol precipitation was completely recovered in the supernatant. Potassium added to 5 per cent egg albumin solutions was also completely recoverable in the supernatant.

DISCUSSION

The effect of low-level direct currents on migrating plasmodia is subtle. There is no stimulatory effect; the rate of locomotion is not enhanced (Anderson, 1951). The effect appears to be simply an inhibition of the migration anodally.

The vigorous shuttle type of protoplasmic streaming characteristic of this organism tempts one to assume that not only is any one portion of the plasmodium in communication with every other part, but also that there would likely be little chemical difference between one part of the plasmodium and another part. Yet the obvious gross morphological difference between the thin sheet-like anterior part of the plasmodium and the channeled posterior area implies chemical heterogeneity. Some support for this implication is given by finding in this study slight differences in potassium (about 4 per cent) between the anterior and posterior regions of the control plasmodia. This difference is more pronounced in plasmodia which have migrated freely for days over non-nutrient agar not in an electric field.

There is a far greater difference in potassium concentration between the anterior and posterior portion in plasmodia whose direction of migration has been oriented by direct current. The loss of potassium is on the anodal side where the action of the current has been shown to inhibit migration.

Whether the loss of potassium in the posterior portion of these plasmodia is the result of the current acting on the membrane or on some cytoplasmic constituent has not yet been elucidated. If the effect of the current were to alter the permeability of the membrane, one would expect the sodium content of those plasmodia having low sodium values to have increased somewhat, even if the content had not come up to values which would be in equilibrium with the sodium in the external substratum.

Electric currents have been reported to increase respiration in brain slices (McIlwain, 1951) and to decrease phosphorylation and to affect ATP formation in isolated brain mitochondria (Abood and Gerard, 1953; Abood, 1954). However, the voltage gradients used to produce these effects were more than

one order of magnitude larger than those necessary to orient the migration of slime mold plasmodia.

Although potassium has been reported to be necessary in several enzyme systems and to form complexes with adenosinetriphosphate, in general univalent cations are thought to be weakly bound with no particular discrimination between sodium and potassium (for review of this problem see Rothstein, 1959). Both sodium and potassium have been reported in erythrocyte lipid fractions (Kirschner, 1958) and in the solvents after lipid extraction (Solomon *et al.*, 1956), but one ion has not been found to the exclusion of the other. Therefore, it was surprising to find considerable amounts of potassium, but no sodium, in the precipitate of mold boiled in alcohol. This clearly indicates that potassium is much more closely associated with some cytoplasmic constituent or compound than sodium. Although ethanol reduces the dissociation constant of carboxyl groups (Lichtenstein, 1939), the acidification of ethanol to pH 2 should have released any potassium associated with carboxyl groups. Furthermore, finding that potassium added to the sample either before or after precipitation in alcohol is completely recoverable in the supernatant strengthens the hypothesis that the potassium in slime mold plasmodia is not completely free. This is in contrast to sodium which does appear to be completely free.

What specific role potassium plays in the migrating phenomenon cannot be ascertained from the present study, but the evidence on sodium is clear: normal movement and galvanotactic response by a plasmodium which was essentially sodium-free, and the absence of sodium in any alcohol-precipitable fraction, imply that sodium plays no essential role in the migrating phenomenon, and that it may not be essential for the function of any normal cytoplasmic constituent.

At the present time it is not clear how the electric current prohibits movement anodally. The effects of prolonged exposure of the plasmodium to low-level direct currents are probably multiple and complex. The fact that there is demonstrable loss of potassium does not necessarily implicate the loss *per se* as the cause of the anodal inhibition. However, the loss occurs on the side of the plasmodium which is inhibited from migrating. This suggests that normal potassium concentration is either necessary for, or very closely associated with, the normal processes occurring at the advancing front of a migrating plasmodium.

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